Phosphorus-Containing Inhibitors of HMG-CoA Reductase. 2.[1] Synthesis and Biological Activities of a Series of Substituted Pyridines Containing a Hydroxyphosphinyl Moiety[2]

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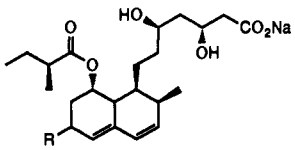
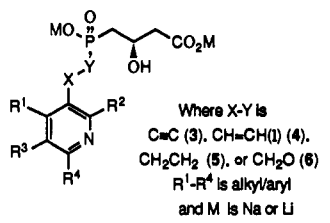
A series of 2,3,4,(5),6-substituted pyridines containing a hydroxyphosphinyl functionality have been prepared and were evaluated for their ability to inhibit the enzyme HMG-CoA reductase. Systematic substitution of both R^1–R^4 and X–Y led to compounds of type 3–6 with in vitro potency greater than that of mevinolin (Na salt).

Introduction

High serum cholesterol levels have been linked to the development of atherosclerosis and coronary heart disease (CHD).[3] A major constituent of serum cholesterol, low density lipoprotein (LDL), is widely believed to be atherogenic upon oxidative modification in vivo,[4] and therefore methods to reduce circulating levels of LDL are highly desirable. Mevinolin (1) and pravastatin (2), two closely



1. R = α-Me (Mevinolin Na)
2. R = β-OH (Pravastatin)

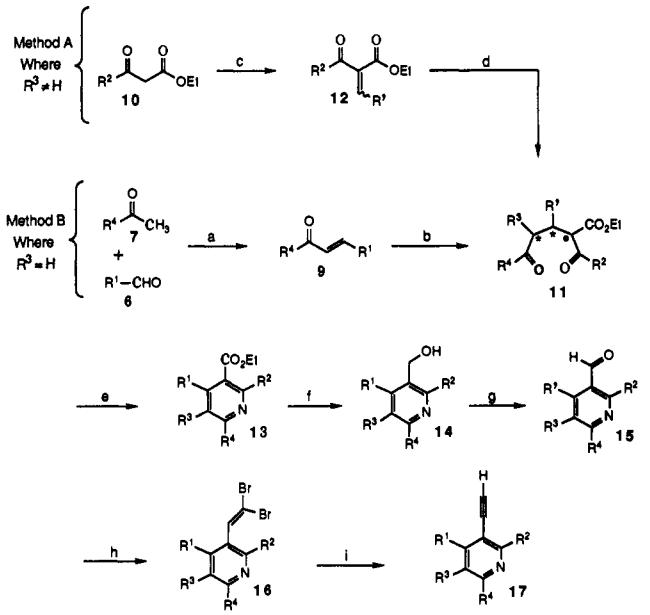


Where X-Y is C=C (3), CH=CH(1) (4), CH_2CH_2 (5), or CH_2O (6). R^1-R^4 is alkyl/aryl and M is Na or Li

related natural products, are currently finding use as therapeutic agents in the treatment of hypercholesterolemia.[5] These compounds act as HMG-CoA reductase (HMGR) inhibitors. Through a complex sequence of regulatory mechanisms, they serve to increase hepatic LDL receptor levels, thereby lowering LDL concentration in the plasma.[6] Inhibition of HMGR, the rate-limiting enzyme in the biosynthesis of cholesterol, is therefore a proven approach to the treatment of hypercholesterolemia.

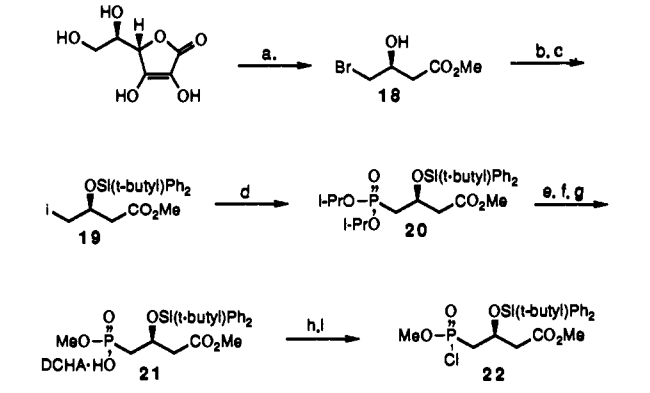
In an attempt to design better, more potent reductase inhibitors, much effort has been expended on replacing the complex decalin portion of the mevinic acids (i.e. 1 or 2) with structurally simpler, achiral aromatic surrogates.[7] In most cases, the 3,5-dihydroxyheptanoic acid portion of the molecule, the pharmacophore that interacts with the 3-

Scheme I[a]



[a] (a) EtONa, EtOH, room temperature; (b) 10, EtONa, EtOH, room temperature; (c) 8, piperidine, HOAc, PhH, reflux, $-H_2O$; (d) $R^4COCH_2R^3$, $LiN(TMS)_2$, THF, −78 °C; (e) NH_4OAc, $Cu(OAc)_2$, HOAc, reflux; (f) $LiAlH_4$, THF; (g) Dess-Martin periodinane, *tert*-butyl alcohol, CH_2Cl_2, room temperature, *or* $(CO)_2Cl_2$, DMSO, CH_2Cl_2, −78 °C then TEA *or* TPAP, 4-methylmorpholine *N*-oxide, 4A molecular sieves, CH_2Cl_2, room temperature; (h) CBr_4, PPh_3, $CH_2Cl_2(CH_3CN)$; (i) *n*-BuLi (2.2 equiv), THF, −78 °C, then saturated NH_4Cl quench.

Scheme II[a]



[a] (a) See ref 12; (b) $(t\text{-Bu})Ph_2SiCl$, DMAP, imidazole, DMF; (c) NaI, MEK, reflux; (d) $(i\text{-PrO})_3P$, 160 °C; (e) TMSBr, BSTFA, CH_2Cl_2; (f) MeOH, DCC, pyridine; (g) dicyclohexylamine, Et_2O; (h) 5% $KHSO_4$, then TMSDEA, CH_2Cl_2; (i) $(CO)_2Cl_2$, catalytic DMF, CH_2Cl_2.

(1) For part 1 in this series, see: Karanewsky, D. S.; Badia, M. C.; Ciosek, C. P., Jr.; Robl, J. A.; Sofia, M. J.; Simpkins, L. M.; DeLange, B.; Harrity, T. W.; Biller, S. A.; Gordon, E. M. Phosphorus-Containing Inhibitors of HMG-CoA Reductase. 1. 4-[(2-Arylethyl)hydroxyphosphinyl]-3-hydroxybutanoic Acids: A New Class of Cell Selective Inhibitors of Cholesterol Biosynthesis. *J. Med. Chem.* **1990**, *33*, 2952–2956.

(2) Presented in part at the 199th Meeting of the American Chemical Society, Boston, MA, April 1990, Abstract MEDI 128.

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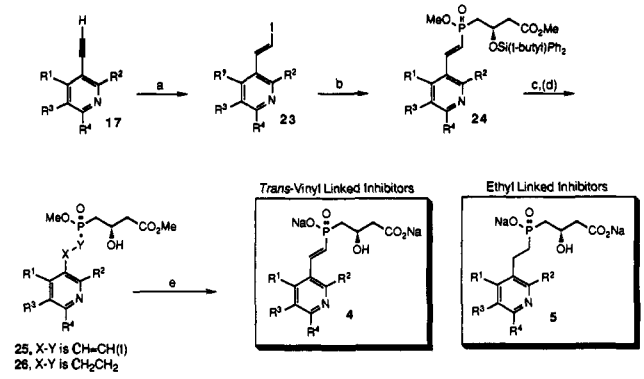
hydroxy-3-methylglutaryl (HMG) binding domain of the enzyme,[8] has been retained. In our previous communication,[1] we described a rationale for the design of a new class of HMGR inhibitors that utilizes a hydroxyphosphinyl functionality in place of the commonly exploited C-5 hydroxy functionality present in the 3,5-dihydroxyheptanoic acid pharmacophore. The hydroxyphosphinyl group was designed to bind to the protonated form of the catalytic group, which serves to activate substrate carbonyl groups toward delivery of a hydride ion in the enzymatic reduction of HMG-CoA to mevalonic acid.

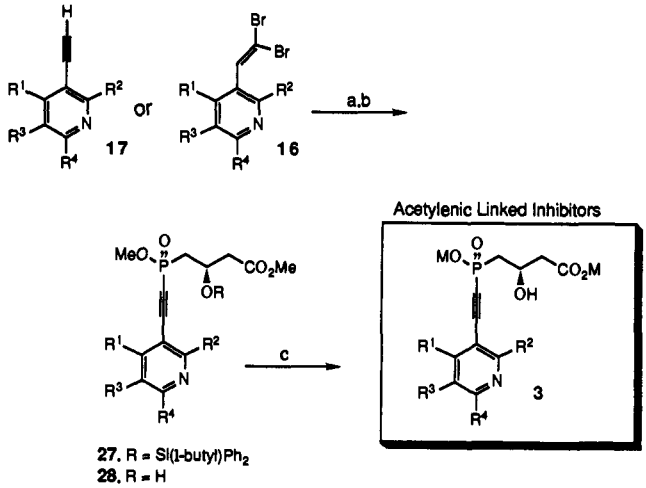
We have prepared hydroxyphosphinyl-containing HMGR inhibitors utilizing a wide variety of aromatic hydrophobic binding domain surrogates. In this paper, we

Scheme III[a]



[a] (a) Bu_3SnH, cat. AIBN, 140 °C, then I_2, Et_2O; (b) *t*-BuLi, THF, −78 °C, then **22**, THF, −100 °C; (c) TBAF, HOAc, THF; (d) H_2, Pd/C, MeOH; (e) NaOH, H_2O, dioxane, Δ.

Scheme IV[a]



[a] (a) *n*-BuLi (1.1 equiv for **17**, 2.2 equiv for **16**), THF, −78 °C, then **22**, THF, −78 °C; (b) TBAF, HOAc, THF, then CH_2N_2, Et_2O; (c) NaOH or LiOH, H_2O, dioxane, 50 °C.

describe the utilization of substituted pyridines[2,9] in the synthesis of hydroxyphosphinyl containing inhibitors **3–6**, in which both the "linker" portion (X–Y) of the molecule and the substituents on the pyridine "anchor" have been widely varied.

Chemistry

Methods for the synthesis of the requisite pyridine nuclei are depicted in Scheme I. Claisen–Schmidt condensation of methyl ketone **7** with aldehyde **8** provided *trans*-enone **9**. Ethoxide-catalyzed Michael addition of β-keto ester **10** to **9** gave the desired adducts **11**, usually as a 1:1 mixture of diastereomers. Method B provides **11**

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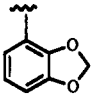
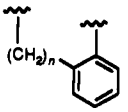
(9) During the course of this work, others have disclosed their efforts on pyridine based 3,5-dihydroxyheptanoic acid containing HMGR inhibitors; see (a) Beck, G.; Kesseler, K.; Baader, E.; Bartmann, W.; Bergmann, A.; Granzer, E.; Jendralla, H.; Kerekjarto, B. v.; Krause, R.; Paulus, E.; Schubert, W.; 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. (b) Angerbauer, R.; Fey, P.; Hubsch, W.; Phillips, T.; Bischoff, H.; Petzinna, D.; Schmidt, D.; Thomas, G. European Patent Application EP-A-0325130. (c) Chucholowski, A. W.; Roth, B. D.; Creswell, M. W.; Sliskovic, D. R. European Patent Application EP-A-0306929.

Table I. Pyridyl Alcohols 14

no.	R^1	R^2	R^3	R^4	mp, °C	% yield[a] (method)	formula	anal.[b]
14a	4-FC_6H_4	i-C_3H_7	H	C_6H_5	167–169	82 (B)	$C_{21}H_{20}FNO$	C, H, N
14b	4-FC_6H_4	i-C_3H_7	H	2-MeC_6H_4	114–115	65 (B)	$C_{22}H_{22}FNO$	C, H, F, N
14c	4-FC_6H_4	i-C_3H_7	H	2-$(C_6H_5CH_2)C_6H_4$	122–124	40 (B)	$C_{28}H_{26}FNO$	C, H, F, N
14d	4-FC_6H_4	i-C_3H_7	H	1-naphthyl	73–75	30 (B)	$C_{25}H_{22}FNO$	C, H, F, N
14e	4-FC_6H_4	i-C_3H_7	H	2,3,5,6-$(F)_4C_6H_1$	130–132	60 (B)	$C_{21}H_{15}F_4NO$	c
14f	4-FC_6H_4	i-C_3H_7	H	2-thienyl	151–153	37 (B)	$C_{19}H_{18}FNOS$	C, H, F, N, S
14g	4-FC_6H_4	i-C_3H_7	H	CH_3	154–155	22 (B)	$C_{16}H_{18}FNO$	C, H, F, N
14h	4-FC_6H_4	i-C_3H_7	H	i-C_3H_7	88–90	57 (B)	$C_{18}H_{22}FNO$	C, H, F, N
14i	4-FC_6H_4	i-C_3H_7	H	c-C_3H_5	94–95	24 (B)	$C_{18}H_{20}FNO$	C, H, F, N
14j	4-FC_6H_4	i-C_3H_7	H	$(C_6H_5)_2CH$	139–140	13 (B)	$C_{28}H_{26}FNO$	C, H, F, N
14k	4-FC_6H_4	i-C_3H_7	H	t-C_4H_9	112–113	49 (B)	$C_{19}H_{24}FNO$	C, H, F, N
14l	4-FC_6H_4	i-C_3H_7	H	c-C_6H_{11}	101–104	40 (B)	$C_{21}H_{26}FNO$	C, H, F, N
14m	4-FC_6H_4	i-C_3H_7	H	1-adamantyl	143–145	56 (B)	$C_{25}H_{30}FNO$	C, H, F, N
14n	4-FC_6H_4	i-C_3H_7	H		114–115	42 (B)	$C_{22}H_{20}FNO_3$	C, H, F, N
14o	4-FC_6H_4	i-C_3H_7	CH_3	C_6H_5	182–184	68 (A)	$C_{22}H_{22}FNO$	C, H, F, N
14p	4-FC_6H_4	i-C_3H_7	CH_2CH_3	C_6H_5	228–230	53 (A)	$C_{23}H_{24}FNO$	C, H, F, N
14q	4-FC_6H_4	i-C_3H_7	i-C_3H_7	C_6H_5	244–246	21 (A)	$C_{24}H_{26}FNO$	C, H, F, N
14r	4-FC_6H_4	i-C_3H_7	C_6H_5	C_6H_5	169–171	52 (A)	$C_{27}H_{24}FNO$	C, H, F, N
14s	4-FC_6H_4	i-C_3H_7	F	C_6H_5	163–165	8 (A)	$C_{21}H_{15}F_2NO$	c
14t	4-FC_6H_4	i-C_3H_7	$n = 1$		166–167	13 (A)	$C_{22}H_{20}FNO$[d]	C, H, F, N
14u	4-FC_6H_4	i-C_3H_7	$n = 2$		138–139	41 (A)	$C_{23}H_{22}FNO$	C, H, F, N
14v	4-FC_6H_4	i-C_3H_7	$n = 3$		161–162	68 (A)	$C_{24}H_{24}FNO$	C, H, F, N
14w	4-FC_6H_4	t-C_4H_9	H	C_6H_5	oil	20 (B)	$C_{23}H_{24}FNO$	c
14x	4-FC_6H_4	c-C_3H_5	H	C_6H_5	176–177	62 (B)	$C_{21}H_{18}FNO$	C, H, F, N
14y	4-FC_6H_4	c-C_3H_5	CH_3	C_6H_5	140–142	71 (A)	$C_{22}H_{20}FNO$	C, H, F, N
14z	4-FC_6H_4	C_2H_5	CH_3	C_6H_5	180–181	61 (A)	$C_{21}H_{20}FNO$	c
14aa	4-FC_6H_4	CH_3	CH_3	C_6H_5	178–180	72 (A)	$C_{20}H_{18}FNO$	C, H, F, N
14bb	i-C_3H_7	4-FC_6H_4	H	C_6H_5	172–173	31 (B)	$C_{21}H_{20}FNO$	C, H, F, N
14cc	4-F-3-MeC_6H_3	i-C_3H_7	H	C_6H_5	159–160	60 (B)	$C_{22}H_{22}FNO$	C, H, F, N
14dd	4-F-2-MeC_6H_3	i-C_3H_7	H	C_6H_5	134–135	66 (B)	$C_{22}H_{22}FNO$	C, H, F, N

[a] Represents overall yield from 12 (method A) or from 9 (method B). [b] Analytical results were within ±0.4% of the theoretical value. [c] Microanalysis was not performed. Compound possessed ^1H NMR and MS in accord with assigned structure. [d] Anal. Calcd: C, 79.25. Found: C, 78.74.

in generally good yields in the cases where R^3 = H but was unsatisfactory in cases where R^3 was alkyl or aryl. In these cases, introduction of the R^3 substituent was best carried out utilizing method A. β-Keto α,β-unsaturated ester **12**, generated by Knoevenagel condensation of β-keto ester **10** with aldehyde **8**, readily underwent Michael addition with lithium enolate $R^4C(OLi)=CHR^3$ to give **11** as a complex mixture of diastereomers. Treatment of 1,5-diketone **11** with NH_4OAc in hot HOAc afforded the intermediate dihydropyridine, which underwent $Cu(OAc)_2$ oxidation[10] in situ, affording pyridyl ester **13**. Utilization of either method A or method B allowed for the rapid and convenient generation of tetra- and pentasubstituted pyridines **13**, in which the substituents R^1–R^4 could be independently selected from a variety of alkyl or aryl groups. Simple $LiAlH_4$ reduction of **13** gave pyridyl alcohols **14** (Table I). Alcohols **14** provided an entry to phosphonic acid based inhibitors **6** (see Scheme V), but a one-carbon homologation was necessary for generation of the phosphinic acid class of compounds (see Schemes III and IV). Oxidation of **14** could be effected under a variety of conditions to give the corresponding aldehydes **15**. Reaction of **15** with CBr_4/PPh_3 provided the vinyl dibromides[11] (Table II) in generally excellent yields. Treatment of **15** with *n*-BuLi in THF at −78 °C generated the corresponding acetylenic anions in situ. The anions could be utilized in carbon–phosphorus bond formation directly or quenched with a proton source to give acetylenes **17**.

The routes we have developed[1] for the synthesis of both the phosphinic and phosphonic acid based inhibitors utilize phosphonochloridate **22** as a synthon for the introduction of the 3-hydroxy-4-(hydroxyphosphinyl)butanoic side chain. The *S* enantiomer of compound **22** was prepared by a multistep route (outlined in Scheme II) from isoascorbic acid via known[12] bromohydrin ester **18**. Silylation of **18** followed by Finkelstein reaction on the silylated bromide provided **19** in 74% overall yield. Arbuzov reaction of **19** was best effected with triisopropyl phosphite to give **20** in 75% yield. Phosphorus deesterification with TMSBr followed by reesterification with MeOH/DCC in pyridine gave the corresponding phosphonic acid monomethyl ester, which was conveniently isolated and stored in stable form as its dicyclohexylamine salt **21**. Regeneration of the free acid followed by subsequent treatment with TMSDEA and oxalyl chloride thus provided phos-

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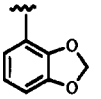
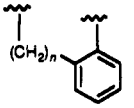
(12) (a) Bock, K.; Lundt, I.; Pedersen, C. Synthesis of (*S*)- and (*R*)-4-Amino-3-hydroxybutyric Acid (GABOB) and (*S*)- and (*R*)-Carnitine from Arabinose or *Ascorbic Acid. *Acta Chem. Scand., B* **1983**, *37*, 341–344. (b) Isbell, H. S.; Frush, H. L. Oxidation of L-ascorbic acid by hydrogen peroxide: preparation of L-threonic acid. *Carbohydr. Res.* **1979**, *72*, 301–304.

Table II. Pyridyl Vinyl Dibromides **16**

no.[a]	R^1	R^2	R^3	R^4	mp, °C	% yield[b] (method)[c]
16a	4-FC_6H_4	i-C_3H_7	H	C_6H_5	oil	88 (C)
16b	4-FC_6H_4	i-C_3H_7	H	2-MeC_6H_4	108–110	68 (D)
16c	4-FC_6H_4	i-C_3H_7	H	2-$(C_6H_5CH_2)C_6H_4$	foam	62 (D)
16d	4-FC_6H_4	i-C_3H_7	H	1-naphthyl	foam	74 (D)
16e	4-FC_6H_4	i-C_3H_7	H	2,3,5,6-$(F)_4C_6H_1$	77	62 (D)
16f	4-FC_6H_4	i-C_3H_7	H	2-thienyl	107–108	86 (D)
16g	4-FC_6H_4	i-C_3H_7	H	CH_3	oil	94 (D)
16h	4-FC_6H_4	i-C_3H_7	H	i-C_3H_7	52–53	71 (D)
16i	4-FC_6H_4	i-C_3H_7	H	c-C_3H_5	oil	71 (D)
16j	4-FC_6H_4	i-C_3H_7	H	$(C_6H_5)_2CH$	141–142	86 (D)
16k	4-FC_6H_4	i-C_3H_7	H	t-C_4H_9	98–100	68 (D)
16l	4-FC_6H_4	i-C_3H_7	H	c-C_6H_{11}	98–100	72 (D)
16m	4-FC_6H_4	i-C_3H_7	H	1-adamantyl	176–177	69 (D)
16n	4-FC_6H_4	i-C_3H_7	H		129–131	74 (D)
16o	4-FC_6H_4	i-C_3H_7	CH_3	C_6H_5	169–170	85 (D)
16p	4-FC_6H_4	i-C_3H_7	CH_2CH_3	C_6H_5	155–157	82 (D)
16q	4-FC_6H_4	i-C_3H_7	i-C_3H_7	C_6H_5	foam	83 (D)
16r	4-FC_6H_4	i-C_3H_7	C_6H_5	C_6H_5	155–158	88 (D)
16s	4-FC_6H_4	i-C_3H_7	F	C_6H_5	105–107	76 (D)
16t	4-FC_6H_4	i-C_3H_7	n = 1		foam	58 (D)[d]
16u	4-FC_6H_4	i-C_3H_7	n = 2		121–122	76 (E)
16v	4-FC_6H_4	i-C_3H_7	n = 3		173–175	83 (D)
16w	4-FC_6H_4	t-C_4H_9	H	C_6H_5	oil	58 (D)
16x	4-FC_6H_4	c-C_3H_5	H	C_6H_5	170–172	69 (E)
16y	4-FC_6H_4	c-C_3H_5	CH_3	C_6H_5	155–157	77 (E)
16z	4-FC_6H_4	C_2H_5	CH_3	C_6H_5	137–138	72 (E)
16aa	4-FC_6H_4	CH_3	CH_3	C_6H_5	141–143	73 (E)
16bb	i-C_3H_7	4-FC_6H_4	H	C_6H_5	124–126	84 (C)
16cc	4-F-3-MeC_6H_3	i-C_3H_7	H	C_6H_5	102–104	89 (D)
16dd	4-F-2-MeC_6H_3	i-C_3H_7	H	C_6H_5	128–129	75 (D)

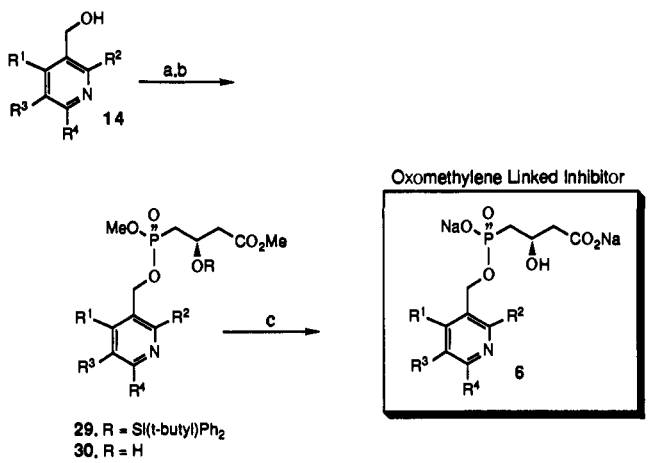
[a] All spectral data were consistent with assigned structures. [b] Represents overall yield from **14**. [c] Represents method of oxidation. Method C: Dess–Martin periodinane, *tert*-butyl alcohol, CH_2Cl_2, room temperature. Method D: $(CO)_2Cl_2$, DMSO, CH_2Cl_2, −78 °C, then TEA. Method E: TPAP, 4-methylmorpholine *N*-oxide, 4A molecular sieves, CH_2Cl_2, room temperature. [d] CH_3CN used as solvent in the formation of **16w** from **15w**.

phonochloridate **22**. Silylation of the free acid of **21** prior to treatment with oxalyl chloride generates TMSCl rather than HCl as a byproduct of the reaction, allowing the *tert*-butyldiphenylsilyl protecting group to remain intact.

Scheme III outlines the route developed for the synthesis of *trans*-vinyl (X–Y = CH=CH(t)) and ethyl (X–Y = CH_2CH_2) linked inhibitors **4** and **5**. Hydrostannylation of acetylene **17** with tributyltin hydride under free-radical conditions[13] followed by treatment of the intermediate *trans*-vinylstannane with iodine stereospecifically provided the *trans*-vinyl iodides **23** in good yields. Metallation of **23** with *tert*-butyllithium generated the corresponding vinyl anion, which was subsequently coupled with phosphonochloridate **22** at −100 °C to give **24** in yields averaging 55%. Higher reaction temperatures led to a substantial diminution in product yield. Desilylation with buffered fluoride provided **25**, which was saponified to give *trans*-vinyl-linked inhibitors **4**, or, was subjected to catalytic hydrogenation followed by saponification to give ethyl linked inhibitors **5**.

Synthesis of ethynyl (X–Y = C≡C) linked inhibitors **3** was, in general, more straight forward (Scheme IV). The

Scheme V[a]



[a] (a) **22**, pyridine, 4 °C; (b) TBAF, HOAc, THF; (c) NaOH, H_2O, dioxane, 55 °C.

lithium anion of **17**, generated by the reaction of either **16** or **17** with *n*-butyllithium, smoothly underwent coupling with phosphonochloridate **22** at −78 °C to give **27**, usually in 65–80% yields. Desilylation followed by saponification thus provided diacids **3**. In the case of the ethynyl-linked compounds, cleavage of the silyl ether of **27** with fluoride ion also led to partial deesterification at the methyl phosphinate ester. Reesterification with diazomethane was

(13) Tolstilkov, G. A.; Miftakhov, M. S.; Danilova, N. A.; Vel'der, Y. L. Regio- and Stereoselective Hydrostannylation of 3-Hydroxy-4-phenoxy-1-butyne: Effective Approach to Intermediates in the Total Synthesis of ω-Aryloxyprostaglandins. *Synthesis* **1986**, 496–499.

necessary in order to obtain the desired products, 28, in consistently good yields.

Phosphonic acid based inhibitors 6 were generated as shown in Scheme V. Reaction of pyridyl alcohols 14 with phosphonochloridate 22 in pyridine gave 29, which were subsequently desilylated and saponified to give inhibitors of type 6. Treatment of diesters 30 with base led to a mixture of both 6 and 14, resulting from competing hydrolysis of the methyl and pyridylmethyl phosphonic esters.

Biological Results

Compounds 3–6 were tested for inhibition of the conversion of ^{14}C-HMG-CoA to [^{14}C]mevalonic acid by partially purified HMG-CoA reductase (Table III). Activities are expressed as concentration of drug producing 50% inhibition of the enzyme (I_{50} value). The I_{50}'s of the sodium salts of mevinolin (1) and pravastatin (2) are shown for comparison. Structure–activity relationships were studied by (i) varying the nature of the substituents ortho to the binding domain pharmacophore, (ii) varying the substituents at carbons C-5 and C-6 (R^3 and R^4) on the pyridine ring, (iii) varying the nature of the "linking" group X–Y, and (iv) fusing the C-5 and C-6 positions of the pyridine ring with cycloalkylbenzo substituents.

Workers at Merck had previously shown[7b] in a dihydroxyheptanoic acid based inhibitor series that, for optimal inhibitory potency, an aryl and an alkyl group must flank the HMGR binding domain pharmacophore. Early in our studies, we found that placement of the alkyl substituent (preferably isopropyl) at R^2 and the aryl substituent (preferably 4-fluorophenyl) at R^1 lead to compounds of higher potency relative to their regioisomers (compare 3a and 4a with 3bb and 4bb). Subsequent studies were carried out utilizing this substitution pattern. It is apparent that the enzyme is able to accommodate a wide variety of substituents at C-6 (R^4) of the pyridine nucleus. Very large groups such as naphthyl (3d), 2-benzylphenyl (3c), and adamantyl (3m) are well tolerated. In general, the presence of sterically demanding groups such as diphenylmethyl (3j) and *tert*-butyl (3k) is preferred over smaller substituents such as methyl and isopropyl. A notable exception is seen in the case where R^4 is cyclopropyl (3i). This compound was found to be 20-fold more active than its isopropyl counterpart (3h).

Substitution at C-5 (R^3) of the pyridine nucleus with an alkyl or aryl group dramatically increases intrinsic potency (compare compounds 3o–r with 3a). The effect is greatest with methyl and decreases with increasing steric bulk (i.e. for R^3, methyl > ethyl > isopropyl > phenyl) with R^4 as phenyl. It is believed that this effect is due to a favorable skewing of the R^4 phenyl group out of the plane of the pyridine ring. In order to test this hypothesis, a series of conformationally restricted cycloalkylbenzo-fused pyridines were evaluated (compounds 3t–v). Cyclopentyl- and cyclohexylbenzo-fused pyridines 3t and 3u were essentially equipotent to their nonfused counterpart 3a, whereas cycloheptylbenzo-fused pyridine 3v was 4–5-fold more active. The propylene bridge in 3v necessarily holds the fused phenyl group out of the plane with the pyridine ring.[14] The converse is true with methylene or ethylene bridging units. As proposed above, deviation of planarity of the R^4 phenyl substituent leads to optimal inhibitory potency.

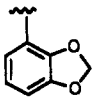
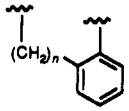
In order to study the relationship between activity, the linker group X–Y, and the alkyl substituent at R^2, a variety of inhibitors were synthesized in which the R^2 group (R^2 = methyl, ethyl, cyclopropyl, and isopropyl) as well as the linker X–Y (C≡C, CH=CH(t), CH_2CH_2, and CH_2O) were varied. These studies show there is a strong interdependence between R^2 and X–Y. Where R^2 is isopropyl or cyclopropyl (e.g 3–6a,k,o,p,v,y), the general order of activity with respect to X–Y is CH=CH(t) > CH_2O ≥ C≡C > CH_2CH_2. In general, compounds possessing the *trans*-vinyl group are 2–32-fold more active than their acetylenic or methylene ether counterparts and 5–95-fold more potent than their ethyl-linked counterparts. A reversal in activity occurs when R^2 is methyl. In this case (e.g. 3aa, 5aa, and 6aa), the order of activity is CH_2CH_2 ≫ C≡C ≈ CH_2O. As expected, ethyl substitution at R^2 (e.g. 3–6z) exhibits activity that is intermediate between that of isopropyl and methyl substitution (i.e. CH_2CH_2 ≈ C≡C for X–Y). In essentially all cases studied, the *trans*-vinyl group was found to be the superior linking functionality, regardless of the substitution pattern at R^1 and R^2. The SAR of the phosphonic acid based inhibitors 6 (X–Y is CH_2O) more closely parallels that of the inhibitors possessing the acetylenic or *trans*-vinyl linkers, rather than the isosteric ethylene linkers. These data indicate that the alkyl R^2 group must be tailored to the appropriate linker X–Y in order to optimize inhibitory potency. On the basis of these SAR, the most potent compounds possess either an isopropyl or a cyclopropyl group at R^2, a *trans*-vinyl or oxomethylene linker for X–Y, a 4-fluorophenyl group at R^1, and substitution at both R^3 and R^4. Indeed, most of the compounds that possess low to subnanomolar activity against HMGR (i.e. 4o, 4p, 4v, 6v, and 6y) fulfill these criteria.

Since the main site of both LDL synthesis and expression of LDL receptors is in the liver, inhibition of cholesterol biosynthesis in extrahepatic tissue may lead to undesirable side effects. We therefore felt it would be advantageous to develop HMGR inhibitors that would be selective for hepatic cells over extrahepatic cells.[15] Consequently, the phosphorus-based inhibitors were evaluated for their ability to inhibit cholesterol synthesis from [^{14}C]acetate in both hepatic and nonhepatic cells (Table IV). For comparison, mevinolin (1) and pravastatin (2) were also evaluated. One striking difference between pravastatin and mevinolin is exhibited in their ability to inhibit cholesterol synthesis in whole cells. Pravastatin shows inhibition in freshly isolated rat hepatocytes com-

(14) For a study on the conformational analysis of bridged biphenyls and 2,2′-bipyridines, see: Jaime, C.; Font, J. Conformational Analysis of Bridged Biphenyls and 2,2′-Bipyridines Empirical Force Field Calculations (MM2-V4). *J. Org. Chem.* 1990. *55*. 2637–2644.

(15) For papers concerning cell and tissue selectivity of HMGR inhibitors, see: (a) Tsujita, Y.; Kuroda, M.; Shimada, Y.; Tanzawa, K.; Arai, M.; Kaneko, I.; Tanaka, M.; Masuda, H.; Tarumi, C.; Watanabe, Y.; Fiji, S. CS-514, A Competitive Inhibitor of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase: Tissue Selective Inhibition of Sterol Synthesis and Hypolipidemic Effect on Various Animal Species. *Biochim. Biophys. Acta* 1986, *877*, 50–60. (b) Reference 7e. (c) Germershausen, J. I.; Hunt, V. M.; Bostedor, R. G.; Bailey, P. J.; Karkas, J. D.; Alberts, A. W. Tissue Selectivity of the Cholesterol-Lowering Agents Lovastatin, Simvastatin, and Pravastatin in Rats in Vivo. *Biochem. Biophys. Res. Commun.* 1989, *158*, 667–675. (d) Roth, B. D.; Bocan, T. M. A.; Blankley, C. J.; Chucholowski, A. W.; Creger, P. L.; Creswell, M. W.; Ferguson, E.; Newton, R. S.; O'Brien, P.; Picard, J. A.; Roark, W. H.; Sekerke, C. S.; Sliskovic, D. R.; Wilson, M. W. Relationship between Tissue Selectivity and Lipophilicity for Inhibitors of HMG-CoA Reductase. *J. Med. Chem.* 1991, *34*, 463–466. (e) 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.

Table III. Inhibition of HMG-CoA Reductase in Vitro for Compounds 1–6

no.	R^1	R^2	R^3	R^4	X–Y	M	$[\alpha]_D$, deg (c, MeOH)	% yield[a] (method)[b]	formula[c]	I_{50},[d] nM
1[e]	–	–	–	–	–	–	–	–	–	4.0
2	–	–	–	–	–	–	–	–	–	24.0
3a	4-FC_6H_4	i-C_3H_7	H	C_6H_5	C≡C	Li	+4.8 (0.72)	59 (I)	$C_{26}H_{23}FNO_5PLi_2 \cdot 0.80H_2O$	62.0
4a	4-FC_6H_4	i-C_3H_7	H	C_6H_5	CH=CH(t)	Li	+6.2 (0.56)	36 (F)	$C_{28}H_{25}FNO_5PLi_2 \cdot 1.30H_2O$[f]	1.9
5a	4-FC_6H_4	i-C_3H_7	H	C_6H_5	CH_2CH_2	Li	−3.3 (0.45)	10[g]	$C_{28}H_{27}FNO_5PLi_2 \cdot 1.76H_2O$	181
6a	4-FC_6H_4	i-C_3H_7	H	C_6H_5	CH_2O	Na	−2.4 (0.47)	34 (J)	$C_{25}H_{25}FNO_6PNa_2 \cdot H_2O$	12.7
3b	4-FC_6H_4	i-C_3H_7	H	2-MeC_6H_4	C≡C	Li	+4.0 (0.59)	15 (H)	$C_{27}H_{25}FNO_5PLi_2 \cdot 1.10H_2O$	32.6
3c	4-FC_6H_4	i-C_3H_7	H	2-$(C_6H_5CH_2)C_6H_4$	C≡C	Na	+3.4 (0.53)	55 (H)	$C_{33}H_{29}FNO_5PNa_2 \cdot 1.24H_2O$[h]	62.6
3d	4-FC_6H_4	i-C_3H_7	H	1-naphthyl	C≡C	Na	+4.9 (0.80)	33 (H)	$C_{30}H_{25}FNO_5PNa_2 \cdot 1.22H_2O$	28.7
3e	4-FC_6H_4	i-C_3H_7	H	2,3,4,5-$F_4C_6H_1$	C≡C	Na	+2.7 (0.48)	28 (H)	$C_{26}H_{19}F_5NO_5PNa_2 \cdot 4.60H_2O$[i]	8.9
3f	4-FC_6H_4	i-C_3H_7	H	2-thienyl	C≡C	Na	+7.6 (0.95)	33 (H)	$C_{24}H_{21}FNO_5PSNa_2 \cdot 1.55H_2O$	14.6
3g	4-FC_6H_4	i-C_3H_7	H	CH_3	C≡C	Na	+4.7 (0.62)	22 (H)	$C_{21}H_{21}FNO_5PNa_2 \cdot 3.0H_2O$	231
3h	4-FC_6H_4	i-C_3H_7	H	i-C_3H_7	C≡C	Na	+5.6 (0.78)	16 (H)	$C_{23}H_{25}FNO_5PNa_2 \cdot 1.40H_2O$	80.9
3i	4-FC_6H_4	i-C_3H_7	H	c-C_5H_5	C≡C	Na	+5.1 (0.74)	28 (H)	$C_{23}H_{23}FNO_5PNa_2 \cdot 2.50H_2O$	4.2
3j	4-FC_6H_4	i-C_3H_7	H	$(C_6H_5)_2CH$	C≡C	Na	+4.2 (0.38)	17 (I)	$C_{33}H_{29}FNO_5PNa_2 \cdot 2.0H_2O$	14.9
3k	4-FC_6H_4	i-C_3H_7	H	t-C_4H_9	C≡C	Na	+6.5 (0.77)	33 (H)	$C_{24}H_{27}FNO_5PNa_2 \cdot 1.17H_2O$	6.1
4k	4-FC_6H_4	i-C_3H_7	H	t-C_4H_9	CH=CH(t)	Na	+3.3 (0.60)	16 (F)	$C_{24}H_{29}FNO_5PNa_2 \cdot 2.10H_2O$	3.2
5k	4-FC_6H_4	i-C_3H_7	H	t-C_4H_9	CH_2CH_2	Na	+0.9 (0.68)	28 (G)	$C_{24}H_{31}FNO_5PNa_2 \cdot 2.0H_2O$	17.2
6k	4-FC_6H_4	i-C_3H_7	H	t-C_4H_9	CH_2O	Na	−1.6 (0.43)	32 (J)	$C_{23}H_{29}FNO_6PNa_2 \cdot 1.50H_2O$	1.4
3l	4-FC_6H_4	i-C_3H_7	H	c-C_6H_{11}	C≡C	Na	+4.4 (0.45)	34 (H)	$C_{29}H_{29}FNO_5PNa_2 \cdot 1.60H_2O$	48.4
3m	4-FC_6H_4	i-C_3H_7	H	1-adamantyl	C≡C	Na	+5.8 (0.72)	15 (H)	$C_{30}H_{33}FNO_5PNa_2 \cdot 2.0H_2O$	43.8
3n	4-FC_6H_4	i-C_3H_7	H		C≡C	Li	+6.9 (0.72)	13 (H)	$C_{27}H_{23}FNO_7PLi_2 \cdot 1.40H_2O$	84.6
3o	4-FC_6H_4	i-C_3H_7	CH_3	C_6H_5	C≡C	Li	+9.4 (0.36)	46 (H)	$C_{27}H_{25}FNO_5PLi_2 \cdot 1.06H_2O$	4.5
4o	4-FC_6H_4	i-C_3H_7	CH_3	C_6H_5	CH=CH(t)	Na	+10.0 (0.50)	47 (F)	$C_{27}H_{27}FNO_5PNa_2 \cdot 1.2H_2O$	1.2
5o	4-FC_6H_4	i-C_3H_7	CH_3	C_6H_5	CH_2CH_2	Na	+0.8 (0.49)	65 (G)	$C_{27}H_{29}FNO_5PNa_2 \cdot 3.69H_2O$	9.2
6o	4-FC_6H_4	i-C_3H_7	CH_3	C_6H_5	CH_2O	Na	−2.0 (0.50)	34 (J)	$C_{26}H_{27}FNO_6PNa_2 \cdot 0.94H_2O$	5.1
3p	4-FC_6H_4	i-C_3H_7	CH_2CH_3	C_6H_5	C≡C	Na	+11.1 (0.45)	53 (H)	$C_{28}H_{27}FNO_5PNa_2 \cdot 1.89H_2O$	5.6
4p	4-FC_6H_4	i-C_3H_7	CH_2CH_3	C_6H_5	CH=CH(t)	Na	+10.9 (0.52)	36 (F)	$C_{28}H_{29}FNO_5PNa_2 \cdot 3.85H_2O$	0.55
5p	4-FC_6H_4	i-C_3H_7	CH_2CH_3	C_6H_5	CH_2CH_2	Na	+1.0 (0.48)	68 (G)	$C_{28}H_{31}FNO_5PNa_2 \cdot 3.33H_2O$	19.1
6p	4-FC_6H_4	i-C_3H_7	CH_2CH_3	C_6H_5	CH_2O	Na	−0.1 (0.82)	33 (J)	$C_{27}H_{29}FNO_6PNa_2 \cdot H_2O$	2.5
3q	4-FC_6H_4	i-C_3H_7	i-C_3H_7	C_6H_5	C≡C	Na	+11.0 (0.39)	40 (H)	$C_{29}H_{29}FNO_5PNa_2 \cdot 2.78H_2O$	9.8
3r	4-FC_6H_4	i-C_3H_7	C_6H_5	C_6H_5	C≡C	Na	+12.1 (0.52)	49 (H)	$C_{32}H_{27}FNO_5PNa_2 \cdot 1.22H_2O$	15.6
3s	4-FC_6H_4	i-C_3H_7	F	C_6H_5	C≡C	Na	+5.8 (0.48)	40 (H)	$C_{28}H_{22}FNO_5PNa_2 \cdot 1.79H_2O$	44.9
3t	4-FC_6H_4	i-C_3H_7	$n = 1$		C≡C	Li	+14.1 (0.46)	18 (H)	$C_{27}H_{23}FNO_5PLi_2 \cdot 1.31H_2O$	75.6
3u	4-FC_6H_4	i-C_3H_7	$n = 2$		C≡C	Na	+11.4 (0.40)	18 (H)	$C_{28}H_{25}FNO_5PNa_2 \cdot 2.25H_2O$	52.5
3v	4-FC_6H_4	i-C_3H_7	$n = 3$		C≡C	Na	+11.2 (0.60)	66 (H)	$C_{29}H_{27}FNO_5PNa_2 \cdot 0.80H_2O$	14.4
4v	4-FC_6H_4	i-C_3H_7	$n = 3$		CH=CH(t)	Na	+12.2 (0.45)	21 (F)	$C_{29}H_{29}FNO_5PNa_2 \cdot 2.50H_2O$	1.3
5v	4-FC_6H_4	i-C_3H_7	$n = 3$		CH_2CH_2	Na	+1.3 (0.38)	80 (G)	$C_{29}H_{31}FNO_5PNa_2 \cdot 2.04H_2O$	18.6
6v	4-FC_6H_4	i-C_3H_7	$n = 3$		CH_2O	Na	−0.3 (0.34)	28 (J)	$C_{28}H_{29}FNO_6PNa_2 \cdot 2.0H_2O$	1.2
3w	4-FC_6H_4	t-C_4H_9	H	C_6H_5	C≡C	Li	+13.1 (0.42)	17 (H)	$C_{27}H_{25}FNO_5PLi_2 \cdot 1.01H_2O$	68.4
3x	4-FC_6H_4	c-C_3H_5	H	C_6H_5	C≡C	Na	+5.6 (0.81)	59 (H)	$C_{26}H_{21}FNO_5PNa_2 \cdot 2.0H_2O$	90.9
6x	4-FC_6H_4	c-C_3H_5	H	C_6H_5	CH_2O	Na	−1.2 (0.50)	35 (J)	$C_{25}H_{23}FNO_6PNa_2 \cdot 2.50H_2O$	29
3y	4-FC_6H_4	c-C_3H_5	CH_3	C_6H_5	C≡C	Na	+8.8 (0.62)	57 (H)	$C_{27}H_{23}FNO_5PNa_2 \cdot 1.25H_2O$	4.6
4y	4-FC_6H_4	c-C_3H_5	CH_3	C_6H_5	CH=CH(t)	Na	+6.7 (0.50)	30 (F)	$C_{27}H_{25}FNO_5PNa_2 \cdot 1.33H_2O$	2.5
5y	4-FC_6H_4	c-C_3H_5	CH_3	C_6H_5	CH_2CH_2	Na	−0.3 (0.32)	76 (G)	$C_{27}H_{27}FNO_5PNa_2 \cdot 1.25H_2O$	9.2
6y	4-FC_6H_4	c-C_3H_5	CH_3	C_6H_5	CH_2O	Na	−2.4 (0.41)	32 (J)	$C_{26}H_{25}FNO_6PNa_2 \cdot 1.75H_2O$	1.3
3z	4-FC_6H_4	C_2H_5	CH_3	C_6H_5	C≡C	Na	+8.1 (0.35)	21 (H)	$C_{29}H_{23}FNO_5PNa_2 \cdot 2.0H_2O$	109.9
4z	4-FC_6H_4	C_2H_5	CH_3	C_6H_5	CH=CH(t)	Na	+11.8 (0.48)	13 (F)	$C_{29}H_{25}FNO_5PNa_2 \cdot 1.75H_2O$	4.7
5z	4-FC_6H_4	C_2H_5	CH_3	C_6H_5	CH_2CH_2	Na	0 (0.33)	46 (G)	$C_{29}H_{27}FNO_5PNa_2 \cdot 1.25H_2O$	79.8
6z	4-FC_6H_4	C_2H_5	CH_3	C_6H_5	CH_2O	Na	−0.7 (0.45)	22 (J)	$C_{25}H_{25}FNO_6PNa_2 \cdot 3.13H_2O$	19.4
3aa	4-FC_6H_4	CH_3	CH_3	C_6H_5	C≡C	Na	+7.5 (0.4)	19 (H)	$C_{25}H_{21}FNO_5PNa_2 \cdot 2.75H_2O$	1300
5aa	4-FC_6H_4	CH_3	CH_3	C_6H_5	CH_2CH_2	Na	0 (0.44)[j]	44[g]	$C_{25}H_{25}FNO_5PNa_2 \cdot 2.05H_2O$	235
6aa	4-FC_6H_4	CH_3	CH_3	C_6H_5	CH_2O	Na	−0.7 (0.58)	29 (J)	$C_{24}H_{23}FNO_6PNa_2 \cdot 1.84H_2O$	1300
3bb	i-C_3H_7	4-FC_6H_4	H	C_6H_5	C≡C	Li	+7.5 (0.85)	41 (I)	$C_{26}H_{23}FNO_5PLi_2 \cdot H_2O$	420
4bb	i-C_3H_7	4-FC_6H_4	H	C_6H_5	CH=CH(t)	Li	+1.8 (0.45)	12 (F)	$C_{29}H_{25}FNO_5PLi_2 \cdot 2.25H_2O$	45.3
3cc	4-F-3-MeC_6H_3	i-C_3H_7	H	C_6H_5	C≡C	Li	+9.5 (0.78)	53 (H)	$C_{27}H_{25}FNO_5PLi_2 \cdot 1.11H_2O$	22.0
3dd	4-F-2-MeC_6H_3	i-C_3H_7	H	C_6H_5	C≡C	Na	+8.8 (0.38)	33 (I)	$C_{27}H_{25}FNO_5PNa_2 \cdot 3.0H_2O$	156
4dd	4-F-2-MeC_6H_3	i-C_3H_7	H	C_6H_5	CH=CH(t)	Na	+1.6 (0.41)	12 (F)	$C_{27}H_{27}FNO_5PNa_2 \cdot 2.2H_2O$	3.9
5dd	4-F-2-MeC_6H_3	i-C_3H_7	H	C_6H_5	CH_2CH_2	Na	−1.9 (0.43)	63 (G)	$C_{27}H_{29}FNO_5PNa_2 \cdot 1.75H_2O$	115

[a] Represents overall yield from vinyl dibromide 16 or acetylene 17. [b] Method F (Scheme III, steps a–c, e). Method G (Scheme III, steps d, e from 25). Method H (Scheme IV, steps a–c from 16). Method I (Scheme IV, steps a–c from 17). Method J (Scheme V, steps a–c). [c] Analyzed for C, H, N, F, and P. Results were within ±0.4% of theory unless otherwise noted. [d] Compounds were assayed against rat microsomal reductase using 100 μM R,S-HMG-CoA and 2.7 mM NADPH. See the Experimental Section for a description of this assay. Reductase enzyme I_{50} values and 95% confidence intervals were calculated from the linear segments of composite log dose versus percent inhibition regressions from 2–5 experiments. The average 95% confidence interval for I_{50} values reported was ±18.4% with a range of 8–40%. [e] Dihydroxy acid form, sodium salt. [f] Anal. Calcd: H, 5.36. Found: H, 5.82. [g] Obtained via hydrogenation of 27 rather than 25. [h] Not analyzed for phosphorus. [i] Anal. Calcd: H, 4.18. Found: H, 3.68. [j] $[\alpha]^{Hg}_{365} = -3.9°$ (MeOH, $c = 0.44$).

parable to mevinolin but is a much weaker inhibitor in human skin fibroblasts (31-fold). In fact, mevinolin is 7.7-fold more potent in fibroblasts than in hepatocytes. In contrast, our phosphorus-containing inhibitors exhibit a 4–140-fold selectivity for inhibition in hepatocytes versus fibroblasts, with 3o being the most selective. This selectivity is directly related to the presence of the phosphinic acid functionality. The corresponding dihydroxyheptanoic

Table IV. Inhibition of Cholesterol Synthesis from [^{14}C]Acetate in Hepatocytes and Fibroblasts and Inhibition of Cholesterol Biosynthesis from [^{14}C]Acetate in Rats on Intravenous (iv) and Oral (po) Administration[a]

no.	reductase (I_{50}, nM)	hepatocytes (I_{50}, nM)	fibroblasts[b] (I_{50}, nM)	selectivity[c]	in vivo testing (ED_{50}, mpk) iv	po
1[d]	4.0	146	18.8	0.13	0.033	0.40[e]
2	24.0	100	3080	31	0.053	0.75
3a	59	197	9300	47	0.47	3.9
4a	1.9	77	2000	26	0.22	21.4
3o	4.5	81	11300	140	0.13	3.1
3k	6.1	556	2400	4.3	0.7	3.5
4o	1.2	260	2000	7.7	0.1	0.46
3p	5.6	519	6750	13	ND[f]	4.5
4p	0.55	241	4700	19.5	0.2	>10

[a] The average 95% confidence intervals for the reported reductase, hepatocyte, and fibroblast I_{50} values were ±18.4, 40.9, and 56.9%, respectively. The average 95% confidence intervals for the iv and po ED_{50} values were 33.8 and 37.6%, respectively. All compounds were tested in 2–5 experiments. [b] Human skin fibroblasts. [c] Selectivity is measured as a ratio of I_{50} fibroblasts/I_{50} hepatocytes. [d] Tested as the dihydroxy acid form, sodium salt. [e] Tested po as the corresponding δ-lactone form. [f] Not determined.

acid[16] of 4a (where the P(O)OH group in 4a is replaced by (S)-OH) is 69-fold more potent in fibroblast (I_{50} = 2.6 nM) than in hepatocytes (I_{50} = 180 nM). These and other examples[1] indicate that hepatocyte selectivity is a general phenomenon in the phosphinic and phosphonic acid class of reductase inhibitors.

Also listed in Table IV are data obtained for the inhibition of cholesterol biosynthesis from [^{14}C]acetate in rats for a selected number of inhibitors. In general, these phosphinic acids are not as effective as the mevinic acids 1 and 2 upon intravenous (iv) or oral (po) administration. An exception is compound 4o, which shows in vivo activity comparable to that of both 1 and 2. The oral activity of these phosphorus-containing HMGR inhibitors shows no direct correlation with either in vivo reductase inhibitory potency or with in vivo activity after intravenous administration. However, there does appear to be a correlation between iv in vivo activity and activity in isolated rat hepatocytes. For example, despite the fact that 3o and 3k are nearly equipotent against HMGR, 3k is a 7-fold weaker inhibitor of cholesterol biosynthesis in hepatocytes. This is mirrored in a 5-fold loss in potency relative to 3o upon iv administration. However, 3o is still 4-fold less active than mevinolin (1) on iv administration despite equivalent intrinsic potency against reductase. This suggests that the poor in vivo activity of these compounds may be due in part to poor bioavailability to the liver, the target organ. Differences in oral activity (e.g., compare 3o and 4o) are probably due to poor oral absorption. The reasons for the lack of correlation between the in vitro and in vivo potencies of these compounds are currently under investigation.

Conclusion

A potent series of phosphorus-containing reductase inhibitors has been synthesized based on the utilization of highly substituted pyridine nuclei as hydrophobic anchor groups. By proper selection of both the pyridine anchor group and linker X–Y, compounds with enzyme inhibitory activities comparable to or greater than mevinolin (Na salt) have been attained. As determined with rat hepatocytes and human skin fibroblasts, these compounds also show a degree of hepatocyte selectivity not generally exhibited in the dihydroxyheptanoic acid class of inhibitors. In these studies, compound 4o exhibited acute in vivo activity in rats comparable to the clinically proven agents 1 and 2. Inhibitor 4o has been studied for cholesterol-lowering activity in other animal species such as rabbits, dogs, and monkeys. The results of these studies will be presented separately. In addition, an extension of this work to other aromatic and heteroaromatic hydrophobic anchor systems will also be the subject of future disclosures.

Experimental Section

All reactions were carried out under a static atmosphere of argon and stirred magnetically unless otherwise noted. All reagents used were of commercial quality and were obtained from Aldrich Chemical Co. Dry THF and Et_2O were obtained by distillation from the sodium ketyl of benzophenone under nitrogen. Dry CH_2Cl_2 was obtained by distillation from CaH_2 under nitrogen. Pyridine and dioxane were obtained from American Burdick and Jackson and were stored over 4A molecular sieves. Boiling points are uncorrected. Melting points were obtained on a Hoover Uni-melt melting point apparatus and are uncorrected. Infrared spectra were recorded on a Mattson Sirius 100-FTIR spectrophotometer. ^{1}H NMR spectra were recorded on a JEOL JNM-GX270 spectrometer using Me_4Si as an internal standard. Optical rotations were measured in a 1-dm cell on a Perkin-Elmer 241 polarimeter and c is expressed in g/100 mL. All flash chromatographic separations were performed using E. Merck silica gel (60, particle size, 0.040–0.063 mm). MCI Gel CHP-20P is a highly porous polystyrene–divinylbenzene copolymer resin (75–150 μM) supplied by Mitsubishi Chemical Industries Ltd. Reactions were monitored by TLC using 0.25 mm E. Merck silica gel plates (60 F_{254}) and were visualized with UV light, 5% phosphomolybdic acid in 95% EtOH, or *p*-anisaldehyde in EtOH/H_2SO_4/HOAc.

General Procedure for the Synthesis of 1,5-Diketones 11. Method A. 2-[(4-Fluorophenyl)methylene]-4-methyl-3-oxopentanoic Acid, Ethyl Ester (12, R^1 = 4-FC_6H_4, R^2 = i-C_3H_7). A mixture of 4-fluorobenzaldehyde (3.00 g, 24 mmol), ethyl isobutyrylacetate (3.82 g, 24 mmol), piperidine (240 μL), and HOAc (42 μL) was refluxed in benzene (15 mL) with removal of water (Dean–Stark trap) for 22 h. The cooled mixture was diluted with Et_2O, washed successively with 2% HCl, saturated $NaHCO_3$, H_2O, and brine, dried (Na_2SO_4), filtered, and stripped to yield an oil. Distillation of the oil (bp 110–113 °C (0.25 mmHg)) afforded 12 (R^1 = 4-FC_6H_4, R^2 = i-C_3H_7, 5.32 g, 83%) as a pale yellow liquid. The compound was obtained as a 1:1 mixture of *E* and *Z* isomers (a and b): TLC R_f 0.35 (20% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$) δ 1.07 (d, *J* = 7.2 Hz, 6 H_a), 1.18 (d, *J* = 7.2 Hz, 6 H_b), 1.25–1.35 (m, 6 $H_{a\&b}$), 2.70 (m, 1 H_a), 3.14 (m, 1 H_b), 4.25–4.37 (m, 4 $H_{a\&b}$), 7.01–7.09 (m, 4 $H_{a\&b}$), 7.34–7.49 (m, 4 $H_{a\&b}$), 7.53 (s, 1 H_b), 7.72 (s, 1 H_a); IR (neat) 1722, 1699, 1605, 1510, 1239 cm^{-1}. Anal. ($C_{15}H_{17}FO_3$) C, H, F. In the same manner, ethyl 3-cyclopropyl-3-oxopropionate[17] (R^2 = c-C_3H_5), methyl propionylacetate (R^2 = CH_2CH_3), and ethyl acetoacetate (R^2 = CH_3) were reacted with 4-fluorobenzaldehyde to give the corresponding Knoevenagel condensation products 12 in 82%, 70%, and 68% yields, respectively.

(16) The corresponding dihydroxyheptanoic acid (Li salt) of 4a was prepared in racemic form from 15a utilizing methods similar to that described in ref 7c.

(17) Jackman, M.; Bergman, A. J.; Archer, S. The Preparation of Some 6-Substituted-2-thiouracils. *J. Am. Chem. Soc.* **1948**, *70*, 497–500.

β-(4-Fluorophenyl)-α-(2-methyl-1-oxopropyl)-δ-oxobenzenepentanoic Acid, Ethyl Ester (11o). A −78 °C solution of $LiN(TMS)_2$ (1.0 M in THF, 14.1 mL, 14.1 mmol) in dry THF (15 mL) was treated with a solution of propiophenone (1.900 g, 14.2 mmol) in THF (1.5 mL) over a 5-min period. After 1 h, a solution of compound 12 (R^1 = 4-FC_6H_4, R^2 = i-C_3H_7, 3.717 g, 14.1 mmol) in THF (3 mL) was added dropwise to the above solution. After 1.5 h, the mixture was quenched with saturated NH_4Cl and warmed to room temperature. The mixture was diluted with H_2O and subsequently extracted twice with Et_2O. The combined Et_2O extracts were washed with brine, dried (Na_2SO_4), filtered, and stripped to give an oil. Flash chromatography (15% EtOAc in hexane as eluant) afforded Michael adduct 11o (4.755 g, 85%) as a complex mixture of three diastereomers. The mixture was used directly in the next reaction: TLC R_f 0.34–0.31 (20% EtOAc in hexanes); IR ($CHCl_3$) 2974. 1740, 1713, 1682, 1510, 1224 cm^{-1}. In most cases, an excess of ketone $R^4COCH_2R^3$ (1.2 equiv) and $LiN(TMS)_2$ (1.2 equiv) relative to 12 were used for the formation of compound 11. The crude adducts were used directly in the next reaction prior to removal of the volatiles by vacuum distillation (0.2 mmHg at 80 °C).

Method B. 3-(4-Fluoro-3-methylphenyl)-1-phenyl-2-propen-1-one (9, R^1 = 4-F, 3-MeC_6H_3, R^4 = C_6H_5). A mixture of 4-fluoro-3-methylbenzaldehyde 8 (16.000 g, 115.8 mmol) and acetophenone (13.920 g, 115.8 mmol) in absolute EtOH (120 mL) was treated with a solution of EtONa in EtOH (21% wt solution, 4.3 mL, 11.6 mmol). A precipitate soon fell out of solution. After stirring at room temperature for 16 h, the mixture was cooled to −10 °C and the precipitate was collected by filtration. The solid was washed with cold EtOH and dried in vacuo to yield enone 9 (R^1 = 4-F, 3-MeC_6H_3, R^4 = C_6H_5, 23.560 g, 85%) as a pale yellow solid: mp 100–101 °C; TLC R_f 0.42 (20% EtOAc in hexane); ^1H NMR ($CDCl_3$) δ 2.32 (s, 3 H), 7.04 (t, J = 8.8 Hz, 1 H), 7.40–7.62 (m, 6 H), 7.75 (d, J = 15.8 Hz, 1 H), 7.97–8.06 (m, 2 H); IR (KBr) 1659, 1600, 1587, 1501, 1247 cm^{-1}. Anal. ($C_{16}H_{13}FO$) C, H, F.

β-(4-Fluoro-3-methylphenyl)-α-(2-methyl-1-oxopropyl)-δ-oxo-δ-phenylpentanoic Acid, Ethyl Ester (11cc). A slurry of enone 9 (R^1 = 4-F, 3-MeC_6H_3, R^4 = C_6H_5, 23.165 g, 96.5 mmol) and ethyl isobutyrylacetate (22.88 g, 144.6 mmol) in absolute EtOH (400 mL) was treated with a solution of EtONa in EtOH (21% wt solution, 5.4 mL, 14.5 mmol). After being stirred at room temperature for 4.5 h, the solution was concentrated to 200 mL and partitioned between 50% saturated NH_4Cl and EtOAc. The layers were separated, and the EtOAc layer was washed with H_2O (2×) and brine (2×), dried (Na_2SO_4), filtered, and stripped to yield an oil. The oil was taken up in warm hexane and cooled to produce a solid. The solid was boiled in hexanes and cooled to give Michael adduct 11cc (30.815 g, 80%), a 1:1 mixture of diastereomers, as a white amorphous solid: TLC R_f 0.34 and 0.30 (20% EtOAc in hexanes); ^1H NMR ($CDCl_3$, 270 MHz, integration values are relative) δ 0.70 (d, J = 6.6 Hz, 3 H), 0.94–1.05 (m, 6 H), 1.07–1.13 (m, 6 H), 1.24 (t, J = 7.2 Hz, 3 H), 2.18 (s, 6 H), 2.39 (m, 1 H), 2.76 (m, 1 H), 3.20–3.52 (m, 4 H), 3.93 (q, J = 7.2 Hz, 2 H), 4.06–4.23 (m, 6 H), 6.83 (pseudo t, 2 H), 7.01 (m, 4 H), 7.38–7.57 (m, 6 H), 7.87 (m, 4 H); IR (KBr) 1738, 1711, 1683, 1503, 1245 cm^{-1}. Anal. ($C_{24}H_{27}FO_4$) C, H, F.

General Procedure for the Synthesis of Pyridyl Alcohols 14 (Table I). 4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinecarboxylic Acid, Ethyl Ester (13o). A mixture of 11o (4.730 g, 11.87 mmol), NH_4OAc (2.745 g, 35.6 mmol), and $Cu(OAc)_2$ (5.935 g, 29.7 mmol) in glacial HOAc (30 mL) was gently refluxed for 24 h. The solution was cooled to room temperature and subsequently poured into an ice-cold mixture of concentrated NH_4OH (50 mL) in H_2O (100 mL). The mixture was extracted twice with Et_2O, and the pooled Et_2O extracts were washed with H_2O and brine, dried (Na_2SO_4), filtered, and stripped to yield an oil. The oil was flash chromatographed (20% EtOAc in hexanes as eluant) to give pyridyl ester 13o as an oil (3.916 g, 87%), which slowly solidified on standing: mp 84–88 °C; TLC R_f 0.47 (20% EtOAc in hexanes); ^1H NMR ($CDCl_3$) δ 1.00 (t, J = 7.0 Hz, 3 H), 1.33 (d, J = 6.5 Hz, 6 H), 2.04 (s, 3 H), 3.12 (m, 1 H), 4.01 (q, J = 7.0 Hz, 2 H), 7.05–7.59 (m, 9 H); IR (KBr) 1718, 1510, 1270 cm^{-1}. Anal. ($C_{24}H_{24}FNO_2$) C, H, F, N.

4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinemethanol (14o). An ice-cold slurry of $LiAlH_4$ (1.49 g, 39.3 mmol) in dry THF (50 mL) was treated with a solution of ester 13o (4.571 g, 12.11 mmol) in dry THF (20 mL). Ten minutes after the addition, the cooling bath was removed and the mixture was stirred at room temperature for 4 h. Additional $LiAlH_4$ (500 mg) was added, and stirring was continued for 2 more h. The solution was recooled to 0 °C and quenched in succession with H_2O (2 mL), 10% NaOH (2.5 mL), and H_2O (6 mL). The solution was filtered, and the salts were washed with EtOAc. The filtrate was washed with H_2O and brine and then dried (Na_2SO_4). Filtration and removal of the solvent afforded a solid. The solid was recrystallized from EtOAc/hexane to provide compound 14o (3.729 g, 92%) as white crystals: mp 182–184 °C; TLC R_f 0.20 (20% EtOAc in hexanes); ^1H NMR ($CDCl_3$) δ 1.29 (t, J = 5.3 Hz, 1 H, OH), 1.36 (d, J = 7.0 Hz, 6 H), 1.96 (s, 3 H), 3.50 (m, 1 H), 4.44 (d, J = 5.3 Hz, 2 H), 7.12–7.26 (m, 4 H), 7.33–7.47 (m, 3 H), 7.54–7.60 (m, 2 H); IR (KBr) 3420, 1509, 1218 cm^{-1}. Anal. ($C_{22}H_{22}FNO$) C, H, N, F.

General Procedure for the Synthesis of Pyridyl Vinyl Dibromides 16 (Table II). Oxidation with Dess-Martin Periodinane.[18] 4-(4-Fluorophenyl)-2-(1-methylethyl)-6-phenyl-3-pyridinecarboxaldehyde (15a). A slurry of Dess-Martin periodinane (8.60 g, 20.3 mmol) in CH_2Cl_2 (100 mL) was treated with *tert*-butyl alcohol (1.9 mL, 1.49 g, 20.2 mmol), and the mixture was stirred at room temperature for 15 min. A solution of alcohol 14a (5.011 g, 15.6 mmol) in CH_2Cl_2 (85 mL) was then added over a 5-min period. After 30 min, the mixture was diluted with Et_2O and 1 N NaOH and stirred rapidly for 10 min. The organic layer was separated and washed in succession with 1 N NaOH, H_2O, and brine, dried (Na_2SO_4), filtered, and stripped. The solid residue was flash chromatographed (10% EtOAc in hexanes as eluant) to give aldehyde 15a (4.314 g, 87%) as a white solid: mp 105–107 °C (hexane); TLC R_f (0.50 (20% EtOAc in hexanes); ^1H NMR ($CDCl_3$) δ 1.41 (d, J = 6.6 Hz, 6 H), 3.98 (m, 1 H), 7.16 (m, 2 H), 7.33–7.53 (m, 5 H), 7.57 (s, 1 H), 8.17 (m, 2 H), 10.07 (s, 1 H); IR (KBr) 1688, 1573, 1508, 1233 cm^{-1}. Anal. ($C_{21}H_{18}FNO$) C, H, F, N.

Oxidation with TPAP/NMO.[19] 6-(Cyclopropyl)-4-(4-fluorophenyl)-5-methyl-2-(1-methylethyl)-3-pyridinecarboxaldehyde (15y). A solution of 4-methylmorpholine *N*-oxide (4.002 g, 34.2 mmol) in CH_2Cl_2 (130 mL) was dried over $MgSO_4$ for 15 min. The solution was filtered directly into a 500-mL flask, using approximately 30 mL of CH_2Cl_2 to effect the transfer. The flask was then charged with dry 4A molecular sieves (16 g), alcohol 14y (5.686 g, 17.05 mmol), and tetrapropylammonium perruthenate (TPAP, 301 mg, 0.86 mmol). After being stirred at room temperature for 30 min, the black solution was diluted with Et_2O (200 mL), stirred for 5 min, and then filtered through a plug of silica gel (65 × 30 mm), washing with Et_2O. The filtrate was stripped to give a pale yellow solid. The solid was recrystallized from EtOAc/hexane to give aldehyde 15y (3.982 g) as white crystals. Flash chromatography of the mother liquor (20% EtOAc in hexane as eluant) gave additional product, which was recrystallized from hexane (499 mg). Total pooled solids, 4.481 g (79%): mp 137–139 °C; TLC R_f 0.50 (20% EtOAc in hexane); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.00 (m, 2 H), 1.24 (m, 2 H), 2.00 (s, 3 H), 3.16 (m, 1 H), 7.14–7.26 (m, 4 H), 7.39–7.58 (m, 5 H), 9.88 (s, 1 H); IR (KBr) 1686, 1545, 1508, 1223 cm^{-1}. Anal. ($C_{22}H_{18}FNO$) C, H, F, N.

Oxidation with Oxalyl Chloride/DMSO.[20] 4-(4-Fluorophenyl)-6,7-dihydro-2-(1-methylethyl)benzo[6,7]cyclohepta[1,2-*b*]pyridine-3-carboxaldehyde (15v). A −78 °C solution of oxalyl chloride (630 μL, 917 mg, 7.2 mmol) in CH_2Cl_2 (40 mL) was treated dropwise with a solution of dry DMSO (1.10 mL, 1.21 g, 15.5 mmol) in CH_2Cl_2 (1 mL). After 10 min, a solution

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of alcohol 14v (2.000 g, 5.5 mmol) in THF (5 mL) was added dropwise to the above mixture. Fifteen minutes after the addition, TEA (4.6 mL) was added and the mixture was stirred at −78 °C for 5 min and then warmed to room temperature. The mixture was diluted with Et_2O and washed twice with H_2O and once with brine. The organic layer was dried (Na_2SO_4), filtered, and stripped to give a yellow oil, which produced a solid upon cooling to −78 °C in hexane. The mixture was crystallized from hexane to give aldehyde 15v (1.775 g, 89%) as white needles: mp 132–134 °C; TLC R_f 0.54 (20% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.37 (d, J = 7.0 Hz, 6 H), 2.06 (m, 2 H), 2.18 (m, 2 H), 2.62 (m, 2 H), 3.96 (m, 1 H), 7.11–7.48 (m, 7 H), 7.89 (d, J = 8.0 Hz, 1 H), 9.90 (s, 1 H); IR (KBr) 1693, 1546, 1507, 1223 cm^{-1}. Anal. ($C_{24}H_{22}FNO$) H, F, N; C: calcd 80.20, found 79.58.

3-(2,2-Dibromoethenyl)-4-(4-fluorophenyl)-6,7-dihydro-2-(1-methylethyl)benzo[6,7]cyclohepta[1,2-*b*]pyridine (16v). A solution of carbon tetrabromide (2.336 g, 7.0 mmol) in CH_2Cl_2 (6 mL) was added over a 7-min period to a cold (0 °C) solution of aldehyde 15v (1.688 g, 4.7 mmol) and triphenylphosphine (3.698 g, 14.1 mmol) in CH_2Cl_2 (20 mL). After the addition was complete, the cooling bath was removed and the mixture was stirred at room temperature for 25 min. The solution was quenched with saturated $NaHCO_3$ and extracted twice with CH_2Cl_2. The organic layers were dried (Na_2SO_4), filtered, and concentrated. The concentrate was flash chromatographed (40% CH_2Cl_2 in hexane as eluant) to give vinyl dibromide 16v as a solid. Recrystallization of the material from EtOAc/hexane provided pure 16v (2.257 g, 93%) as a white solid: mp 173–175 °C; TLC R_f 0.44 (10% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.33 (broad, 6 H), 2.06 (m, 2 H), 2.18 (m, 2 H), 2.61 (m, 2 H), 3.19 (m, 1 H), 7.03–7.43 (m, 8 H), 7.84 (d, J = 8.4 Hz, 1 H); IR (KBr) 2950, 2920, 1603, 1508, 1222 cm^{-1}. Anal. ($C_{25}H_{22}Br_2FN$) C, H, Br, F, N.

(*S*)-4-Iodo-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic Acid, Methyl Ester (19). A solution of bromohydrin 18 (4.00 g, 20.4 mmol), imidazole (6.94 g, 102 mmol), and DMAP (12 mg) in dry DMF (40 mL) was treated with *tert*-butylchlorodiphenylsilane (5.84 mL, 6.17 g, 22.5 mmol), and the homogeneous mixture was stirred at room temperature overnight. The mixture was partitioned between 5% $KHSO_4$ and EtOAc, and the organic phase was washed with H_2O and brine, dried (Na_2SO_4), filtered, and stripped to give 9.32 g (100%) of the crude silyl ether (TLC R_f 0.75 (25% EtOAc in hexanes)). A solution of the silyl ether (9.32 g, 20.1 mmol) in dry methyl ethyl ketone (MEK, 60 mL) was treated with sodium iodide (15.06 g, 100.5 mmol), and the yellow suspension was refluxed for 5 h. The mixture was cooled, diluted with EtOAc, and filtered, and the filtrate was washed with dilute $NaHSO_3$ and brine. The organic layer was dried (Na_2SO_4), filtered, and stripped to give a yellow oil. Flash chromatography (25% CH_2Cl_2 in hexanes as eluant) afforded iodide 19 (7.69 g, 74% from 18) as a colorless oil: TLC R_f 0.75 (25% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.05 (s, 9 H), 2.67 (m, 2 H), 3.20 (m, 2 H), 3.58 (s, 3 H), 3.95 (m, 1 H), 7.28–7.72 (m, 10 H).

(*S*)-4-[Bis(isopropyloxy)phosphinyl]-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic Acid, Methyl Ester (20). Freshly distilled triisopropyl phosphite (113.4 mL, 93.92 gm, 451 mmol) was added in one portion to iodide 19 (21.70 g, 45.1 mmol), and the mixture was heated at 155 °C for 16.5 h. The mixture was cooled to room temperature, and the excess triisopropyl phosphite and volatile reaction products were removed by short path distillation (10 mmHg) followed by Kugelrohr distillation (100 °C, 8 h at 0.5 mmHg). The product was further purified by flash chromatography (6:3:1 hexanes–acetone–toluene as eluant) to afford 20 (17.68 g, 75%) as a clear viscous oil: TLC R_f 0.32 (6:3:1 hexanes–acetone–toluene); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.01 (s, 9 H), 1.12 and 1.19 (2 d, J = 6.3 Hz each, 12 H), 1.87–2.24 (m, 2 H), 2.60 and 2.65 (2 d, J = 7.4 Hz each, 1 H), 2.88 and 2.94 (2 d, J = 3.7 Hz each, 1 H), 3.59 (s, 3 H), 4.44–4.57 (m, 3 H), 7.35–7.45 (m, 6 H), 7.65–7.70 (m, 4 H).

(*S*)-4-(Hydroxymethoxyphosphinyl)-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic Acid, Methyl Ester, Dicyclohexylamine (1:1) Salt (21). A solution of compound 20 (10.66 g, 30.5 mmol) in dry CH_2Cl_2 (80 mL) was treated dropwise (5 minutes) with bis(trimethylsilyl)trifluoroacetamide (BSTFA, 8.71 mL, 8.44 g, 32.8 mmol), followed by dropwise addition (10 min) of trimethylsilyl bromide (TMSBr, 6.75 mL, 7.84 g, 51.3 mmol). After stirring at room temperature for 20 h, the reaction mixture was quenched with 200 mL of 5% $KHSO_4$ and stirred vigorously for 15 min. The aqueous layer was extract with EtOAc (3×), and the pooled organic layers were washed with brine, dried (Na_2SO_4), filtered, and stripped. The residue was azeotroped twice with 50 mL of toluene. The precipitate that formed was suspended in toluene and removed by filtration. The filtrate was concentrated, and the azeotrope/filter process was repeated to give a viscous, clear oil. The oil was dissolved in pyridine (50 mL) and subsequently treated with dicyclohexylcarbodiimide (DCC, 4.65 g, 22.6 mmol) followed by methanol (1.67 mL, 1.31 g, 41 mmol). After being stirred at room temperature for 20 h, the mixture was filtered through a pad of Celite, which was subsequently washed with EtOAc. The filtrate was stripped, redissolved in EtOAc, and washed with 5% $KHSO_4$ (2×) and brine. The EtOAc solution was dried (Na_2SO_4), filtered, and stripped, and the residue was azeotroped twice with toluene. The residue was suspended in toluene and filtered. The filtrate was again concentrated, taken up in toluene, filtered, stripped, and placed under high vacuum to give the corresponding phosphonate monoester (10.2 g, >100%, TLC R_f 0.50 (7:2:1 *n*-PrOH–NH_4OH–H_2O)) as a clear, viscous oil. The monoester (1.16 g, 2.57 mmol) was dissolved in dry Et_2O (10 mL) and treated with dicyclohexylamine (0.528 mL, 0.481 g, 2.65 mmol). The resulting homogeneous solution was stored at room temperature for 7 h and at −20 °C for 16 h. The solid/liquid suspension was warmed to room temperature and filtered, and the solid was washed with cold Et_2O and dried in vacuo to give 21 (1.25 g, 77% yield) as a white powdery solid: mp 155–156 °C; TLC R_f 0.57 (20% MeOH in CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.00 (s, 9 H), 1.08–1.92 (m, 22 H), 2.56–2.62 (m, 1 H), 2.64–2.77 (m, 2 H), 3.11 (d, J = 11.0 Hz, 3 H), 3.22 and 3.28 (2 m, 1 H), 3.52 (s, 3 H), 4.02 (m, 1 H), 7.32–7.40 (m, 6 H), 7.65–7.71 (m, 4 H); IR (KBr) 1736 cm^{-1}; $[\alpha]_D$ = −16.0° (MeOH, c = 3.57). Anal. ($C_{22}H_{31}O_6PSi \cdot C_{12}H_{23}N$) C, H, N.

General Procedure for the Synthesis of Acetylenic Linked Phosphinic Acids 3. (*S*)-4-[[[4-(4-Fluorophenyl)-2-(1-methylethyl)benzo[6,7]cyclohepta[1,2-*b*]pyridin-3-yl]-ethynyl]methoxyphosphinyl]-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic Acid, Methyl Ester (27v). DCHA salt 21 (3.682 g, 5.83 mmol) was partitioned between EtOAc and 5% $KHSO_4$. The EtOAc layer was washed three times with 5% $KHSO_4$ and then with brine, dried (Na_2SO_4), filtered, and stripped to give a colorless oil (phosphonic acid monomethyl ester). The oil was dissolved in dry CH_2Cl_2 (10 mL) and treated with diethyl(trimethylsilyl)amine (2.10 mL, 1.61 g, 11.1 mmol). After the mixture was stirred at room temperature for 1 h, the solvent was removed in vacuo and the residue was azeotroped with dry toluene (15 mL). The residue was redissolved in dry CH_2Cl_2 (15 mL), cooled to 0 °C, and treated with 2 drops of DMF and oxalyl chloride (620 μL, 902 mg, 7.1 mmol). After 15 min, the solution was warmed to room temperature and stirred for an additional 45 min. The solvent was stripped, and the yellow residue (phosphonochloridate 22) was azeotroped with toluene (15 mL) and dried in vacuo (oil pump) for 1 h.

Meanwhile, a solution of vinyl dibromide 16v (2.000 g, 3.88 mmol) in THF (10 mL) at −78 °C was treated with *n*-BuLi (2.5 M in hexane, 3.3 mL, 8.2 mmol) over a 1-min period, and the resulting clear green solution was stirred at −78 °C for 50 min. The acetylenic anion solution was added dropwise via canula over a 10-min period to a −78 °C solution of the above prepared phosphonochloridate 22 in THF (12 mL). The resulting mixture was stirred at −78 °C for 30 min and then quenched with 50% saturated NH_4Cl. The solution was warmed to 0 °C and poured into saturated $NaHCO_3$. The aqueous phase was extracted once with Et_2O. The Et_2O layer was washed with brine, dried (Na_2SO_4), filtered, and stripped to give an oil. The residue was flash chromatographed (40% EtOAc in hexanes as eluant) to afford compound 27v, a mixture of diastereomers, as a colorless foam (2.517 g, 82%): TLC R_f 0.31 (40% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.02 (s, 9 H), 1.31 and 1.35 (2 d, J = 6.6 Hz each, 6 H), 2.00–2.38 (m, 6 H), 2.47–2.81 (m, 4 H), 3.30 and 3.37 (2 d, $J_{H,P}$ = 12.6 Hz each, 3 H), 3.54 (m, 1 H), 3.58 (s, 3 H), 4.51 (m, 1 H), 6.99–7.46 (m, 13 H), 7.58–7.72 (m, 4 H), 7.83 (d, J = 7.2 Hz, 1 H); IR (KBr) 2168, 1740, 1508, 1224, 1036 cm^{-1}. In the case where acetylene 17 is used in the coupling reaction, 1.1 equiv

of *n*-BuLi is added to a solution of the acetylene in **17** in THF at −78 °C. After 20 min, the acetylenic anion solution is then coupled to **22** as described above.

(*S*)-4-[[[4-(4-Fluorophenyl)-2-(1-methylethyl)benzo[6,7]cyclohepta[1,2-*b*]pyridin-3-yl]ethynyl]methoxyphosphinyl]-3-hydroxybutanoic Acid, Methyl Ester (28v). A mixture of compound **27v** (2.487 g, 3.15 mmol) and HOAc (810 μL, 850 mg, 14.1 mmol) in THF (40 mL) was treated with tetra-*n*-butylammonium fluoride (1.0 M in THF, 11.0 mL, 11.0 mmol). After stirring at room temperature for 18 h, the solution was diluted with EtOAc and washed with 5% $KHSO_4$ (3×) and once with brine. The EtOAc layer was dried (Na_2SO_4), filtered, and stripped to afford a yellow oil. The oil was dissolved in Et_2O, cooled to 0 °C, and treated with excess diazomethane for 10 min. The excess diazomethane was destroyed by the addition of HOAc, and the solvent was removed in vacuo. The residue was flash chromatographed (40% acetone in hexanes as eluant) to afford compound **28v** (1.534 g, 89%) as a colorless foam: TLC R_f 0.38 (1:1 acetone–hexanes); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.40 (d, *J* = 6.6 Hz, 6 H), 1.94–2.15 (m, 4 H), 2.15–2.28 (m, 2 H), 3.53–3.67 (m, 4 H), 3.59 (d, $J_{H,P}$ = 12.6 Hz, 3 H), 3.57–3.70 (m, 2 H, C*H*(CH_3)$_2$ and O*H*), 3.73 (s, 3 H), 4.36 (m, 1 H), 7.12–7.48 (m, 7 H), 7.85 (d, *J* = 6.6 Hz, 1 H); IR (KBr) 2170, 1737, 1508, 1223, 1035 cm^{-1}.

(*S*)-4-[[[4-(4-Fluorophenyl)-2-(1-methylethyl)benzo[6,7]cyclohepta[1,2-*b*]pyridin-3-yl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic Acid, Disodium Salt (3v). A solution of compound **27v** (780 mg, 1.42 mmol) in dioxane (7 mL) was treated with 1 N NaOH (5.0 mL, 5.0 mmol), and the mixture was stirred at room temperature for 18 h. The solvent was evaporated, and the residue was chromatographed on CHP-20P (25 mm × 90 mm), eluting in succession with H_2O (200 mL), 50% MeOH in H_2O (200 mL), and MeOH (100 mL). The desired fractions were pooled and evaporated, and the residue was taken up in H_2O and lyophilized to give **3v** (744 mg, 90%) as a white solid: TLC R_f 0.17 (8:1:1 CH_2Cl_2–HOAc–MeOH); ^1H NMR (CD_3OD, 270 MHz) δ 1.36 (d, *J* = 7.0 Hz, 6 H), 1.55–1.72 (m, 2 H), 2.01–2.20 (m, 4 H), 2.26 (dd, *J* = 7.8, 15.0 Hz, 1 H), 2.40 (dd, *J* = 4.2, 15.0 Hz, 1 H), 2.59 (m, 2 H), 3.83 (m, 1 H), 4.19 (m, 1 H), 7.16–7.42 (m, 7 H), 7.72 (m, 1 H); IR (KBr) 2164, 1634, 1508, 1213, 1184, 1058 cm^{-1}. Anal. ($C_{29}H_{27}FNNa_2O_5P\cdot0.80H_2O$) C, H, F, N, P.

General Procedure for the Synthesis of *trans*-Vinyl- and Ethyl-Linked Phosphinic Acids 4 and 5. 3-(1-Ethynyl)-4-(4-fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenylpyridine (17o). To a solution of *n*-BuLi (2.5 M in hexanes, 4.00 mL, 10.0 mmol) in dry THF (8 mL) at −78 °C was added a solution of vinyl dibromide **16o** (2.267 g, 4.63 mmol) in dry THF (8 mL) over a 5-min period. After being stirred at −78 °C for 1 h, the pale green solution was quenched with saturated NH_4Cl and warmed to room temperature. The mixture was diluted with H_2O and extracted with Et_2O, and the Et_2O extract was washed with brine, dried (Na_2SO_4), filtered, and stripped to yield a solid. The residue was recrystallized from EtOAc/hexane to afford acetylene **17o** (1.420 g, 93%, 2 crops) as a white solid: mp 178.0–178.5 °C; TLC R_f 0.43 (10% EtOAc in hexanes); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.34 (d, *J* = 7.0 Hz, 6 H), 2.04 (s, 3 H), 3.18 (s, 1 H), 3.69 (m, 1 H), 7.15 (m, 2 H), 7.27 (m, 2 H), 7.36–7.48 (m, 3 H), 7.60 (m, 2 H); IR (KBr) 3165, 2099, 1509, 1213 cm^{-1}. Anal. ($C_{23}H_{20}FN$) C, H, F, N.

(*E*)-4-(4-Fluorophenyl)-3-(2-iodoethenyl)-5-methyl-2-(1-methylethyl)-6-phenylpyridine (23o). A mixture of acetylene **17o** (1.355 g, 4.1 mmol) and AIBN (20 mg) in tri-*n*-butyltin hydride (2.0 mL) was rapidly heated to 120 °C. After 4 min of heating, the mixture was treated with additional Bu_3SnH (0.6 mL) and the temperature of the reaction was raised to 140 °C. Approximately 20 mg of AIBN was added to the reaction mixture 1 and 2 h after heating was initiated. After 3 h, the mixture was cooled to room temperature, diluted with Et_2O (50 mL), and treated with solid I_2 (3.50 g, 13.8 mmol). The dark reaction mixture was stirred for 45 min and then poured into a 50% saturated $NaHCO_3$ solution containing 6.7 g of $Na_2S_2O_3$. The layers were shaken and separated. The ethereal layer was washed successively with H_2O, 1.7 M NH_4OH, and brine, dried (Na_2SO_4), filtered, and stripped to yield a wet solid. The solid was taken up in Et_2O, filtered through Celite, and stripped. The residue was recrystallized from hexane to give compound **23o** (1.335 g) as white crystals. The mother liquor was flash chromatographed (5% EtOAc in hexanes as eluant), and the desired fractions were pooled, stripped, recrystallized, and pooled with the above solid to give a total of 1.637 g (87%) of *trans*-vinyl iodide **23o**: mp 148.5–150.0 °C; TLC R_f 0.13 (2% EtOAc in hexanes); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.29 (d, *J* = 7.0 Hz, 6 H), 2.00 (s, 3 H), 3.31 (m, 1 H), 6.03 (d, *J* = 15.2 Hz, 1 H), 7.05–7.22 (m, 5 H), 7.34–7.49 (m, 3 H), 7.59 (m, 2 H); IR (KBr) 2961, 1508, 1221, 841 cm^{-1}. Anal. ($C_{23}H_{21}FIN$) C, H, F, I, N.

(*E*),(*S*)-4-[[2-[4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinyl]ethenyl]methoxyphosphinyl]-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic Acid, Methyl Ester (24o). A solution of *trans*-vinyl iodide **23o** (1.400 g, 3.06 mmol) in THF (6 mL) was added over a 5-min period to a −100 °C solution of fresh *tert*-butyllithium (1.7 M in pentane, 3.70 mL, 6.3 mmol) in THF (8 mL). The resulting deep red solution was stirred at −100 °C for 25 min and then added via canula over an 8-min period to a −100 °C solution of phosphonochloridate **22** (prepared as in the example for compound **27v** from 3.288 g **21**) in THF (15 mL). The resulting yellow mixture was stirred at −100 °C for 5 min and at −78 °C for 25 min and then quenched with 50% saturated NH_4Cl. The solution was warmed to room temperature, diluted with H_2O, and poured into saturated $NaHCO_3$. The aqueous phase was extracted twice with Et_2O. The combined Et_2O layers were washed with brine, dried (Na_2SO_4), filtered, and stripped. The resulting yellow oil was flash chromatographed (50% EtOAc in hexanes as eluant) to afford adduct **24o**, a 1:1 mixture of diastereomers, as an off-white foam (1.541 g, 66%): TLC R_f 0.22 (40% EtOAc in hexanes); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.01 and 1.03 (2 s, 9 H), 1.20–1.31 (m, 7 H), 1.78 (m, 1 H), 1.98 and 2.00 (2 s, 3 H), 2.56 (m, 1 H), 2.81 (m, 1 H), 3.19 (pseudo t, $J_{H,P}$ = 11.5 Hz, 3 H), 3.21 (m, 1 H), 3.59 and 3.61 (2 s, 3 H), 4.38 and 4.52 (2 m, 1 H), 5.01 (dd, *J* = 17.9, 24.8 Hz, 0.5 H), 5.26 (dd, *J* = 17.9, 24.3 Hz, 0.5 H), 6.89–7.72 (m, 20 H); IR ($CHCl_3$) 2959, 1740, 1605, 1508, 1223, 1036 cm^{-1}.

(*E*),(*S*)-4-[[2-[4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinyl]ethenyl]methoxyphosphinyl]-3-hydroxybutanoic Acid, Methyl Ester (25o). A solution of compound **24o** (1.519 g, 1.98 mmol) in THF (15 mL) was treated with HOAc (640 μL, 671 mg, 11.2 mmol) followed by tetra-*n*-butylammonium fluoride (1.0 M in THF, 10.0 mL, 10.0 mmol). After being stirred at room temperature for 19 h, the solution was poured into saturated $NaHCO_3$ and extracted with EtOAc. The EtOAc extract was washed with brine, dried (Na_2SO_4), filtered, and stripped to give an oil that was subsequently flash chromatographed (40–60% acetone in hexanes as eluant). Compound **25o** (978 mg, 94%) was obtained as a white foam: TLC R_f 0.34 (1:1 acetone–hexanes); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.30 (d, *J* = 7.0 Hz, 6 H), 1.68–1.93 (m, 2 H), 2.00 (s, 3 H), 2.57 (m, 2 H), 3.30 (m, 1 H), 3.43 and 3.47 (2 d, $J_{H,P}$ = 4.7 and 4.1 Hz, 3 H), 3.66 and 3.79 (2 d, *J* = 2.4 Hz each, 1 H, O*H*), 3.72 (s, 3 H), 4.19 and 4.31 (2 m, 1 H), 5.51 (dd, *J* = 17.6, 24.6 Hz, 0.5 H), 5.52 (dd, *J* = 17.6, 24.3 Hz, 0.5 H), 7.10–7.65 (m, 10 H); IR ($CHCl_3$) 2961, 1736, 1605, 1510, 1221, 1034 cm^{-1}.

(*S*)-4-[[2-[4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinyl]ethyl]methoxyphosphinyl]-3-hydroxybutanoic Acid, Methyl Ester (26o). A mixture of compound **25o** (494 mg, 0.94 mmol) and 10% Pd on carbon (110 mg) in MeOH (20 mL) was shaken under 50 psi of H_2 for 3 days. The solution was filtered through Celite, stripped, and flash chromatographed (50% acetone in hexanes) to give compound **26o** (419 mg, 85%) as a colorless oil: TLC R_f 0.36 (1:1 acetone–hexanes); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.33 (d, *J* = 6.6 Hz, 6 H), 1.57–1.91 (m, 4 H), 1.92 (s, 3 H), 2.42–2.59 (m, 2 H), 2.60–2.74 (m, 2 H), 3.25 (m, 1 H), 3.55 and 3.57 (2 d, $J_{H,P}$ = 10.8 Hz each, 3 H), 3.72 (s, 3 H), 3.78 and 3.87 (2 d, *J* = 3.0 Hz each, 1 H, O*H*), 4.25 and 4.40 (2 m, 1 H), 7.11–7.25 (m, 4 H), 7.33–7.47 (m, 3 H), 7.56 (m, 2 H); IR ($CHCl_3$) 1734, 1509, 1221, 1179, 1040 cm^{-1}.

(*S*)-4-[[2-[4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinyl]ethenyl]hydroxyphosphinyl]-3-hydroxybutanoic Acid, Disodium Salt (4o). A solution of compound **25o** (461 mg, 0.88 mmol) in dioxane (5 mL) was treated with 1 N NaOH (3.2 mL, 3.2 mmol), and the mixture was stirred at 60 °C for 1.5 h. The solvent was evaporated, and the residue was dissolved in H_2O and chromatographed on CHP-20P (25 mm

× 80 mm), eluting in succession with H_2O (150 mL) and 50% MeOH in H_2O (200 mL). The desired fractions were pooled and evaporated, and the residue was taken up in H_2O and lyophilized to give 4o (430 mg, 87%) as a white solid: TLC R_f 0.10 (8:1:1 CH_2Cl_2–HOAc–MeOH); ^1H NMR (CD_3OD, 400 MHz) δ 1.27 (d, J = 7.0 Hz, 6 H), 1.54 (dd, J = 7.2, 14.5 Hz, 2 H), 1.93 (s, 3 H), 2.33 (m, 2 H), 3.57 (m, 1 H), 4.10 (m, 1 H), 5.85 (dd, J = 18.0, 19.8 Hz, 1 H), 7.07 (pseudo t, J = 18.0 Hz, 1 H), 7.19 (d, J = 7.0 Hz, 4 H), 7.37–7.54 (m, 5 H); MS (FAB) $[M - 2\ Na + 3\ H]^+$ 498. Anal. ($C_{27}H_{27}FNNa_2O_5P \cdot 1.2H_2O$) C, H, F, N, P.

(*S*)-4-[[2-[4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinyl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic Acid, Disodium Salt (5o). Saponification of ethyl linked phosphinate **26o** was similar to that of *trans*-vinyl-linked phosphinate **25o** to give **5o** in 77% yield: TLC R_f 0.10 (8:1:1 CH_2Cl_2–HOAc–MeOH); ^1H NMR (CD_3OD, 270 MHz) δ 1.41 (d, J = 7.0 Hz, 6 H), 1.49 (dd, J = 6.0, 12.6 Hz, 2 H), 1.71 (m, 2 H), 1.93 (s, 3 H), 2.35 (m, 2 H), 2.78 (m, 2 H), 3.58 (m, 1 H), 4.25 (m, 1 H). 7.20–7.60 (m, 9 H); IR (KBr) 2961, 1579, 1509, 1405, 1157 cm^{-1}. Anal. ($C_{27}H_{29}FNNa_2O_5P \cdot 3.69H_2O$) C, H, F, N, P.

General Procedure for the Synthesis of Phosphonic Monoesters 6. (*S*)-4-[[[5-Ethyl-4-(4-fluorophenyl)-2-(1-methylethyl)-6-phenyl-3-pyridinyl]methoxy]methoxyphosphinyl]-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic Acid, Methyl Ester (29p). A 0 °C solution of phosphonochloridate **22** (from 2.89 g, 4.57 mmol DCHA salt **21**) in pyridine (20 mL) was treated with a solution of alcohol **14p** (888 mg, 2.54 mmol) in dry pyridine (7.0 mL). The resulting mixture was stirred at 0 °C for 16 h, diluted with EtOAc, and washed with 50% saturated NH_4Cl. The organic layer was then washed with H_2O followed by brine, dried (Na_2SO_4), filtered, and stripped. The amber residue was subject to flash chromatography (30% EtOAc in hexane) to give adduct **29p** (1.104 gm, 56%) as a yellow oil: TLC R_f 0.53 (45% EtOAc in hexanes); ^1H NMR ($CDCl_3$, 270 MHz) δ 0.70 (m, 3 H), 1.00 (s, 9 H), 1.22–1.38 (m, 8 H), 1.90 and 2.12 (2 m, 1 H), 2.37 (m, 2 H), 2.55 and 2.81 (2 m, 1 H), 3.29–3.39 (m, 4 H), 3.58 (s, 3 H), 4.43 (m, 1 H), 4.59 and 4.71 (2 m, 2 H), 7.02–7.70 (m, 9 H); IR (CH_2Cl_2) 2954, 1740, 1511, 1223, 1015 cm^{-1}.

(*S*)-4-[[[5-Ethyl-4-(4-fluorophenyl)-2-(1-methylethyl)-6-phenyl-3-pyridinyl]methoxy]methoxyphosphinyl]-3-hydroxybutanoic Acid, Methyl Ester (30p). The silyl protecting group on **29p** was removed via the same procedure as that described for compound **24o** to give **30p** in 90% yield: TLC R_f 0.59 (1:1 acetone–hexanes); ^1H NMR ($CDCl_3$, 270 MHz) δ 0.70 (t, J = 6.8 Hz, 3 H), 1.34 (d, J = 7.0 Hz, 6 H), 1.92 (m, 2 H), 2.39 (q, J = 6.8 Hz, 2 H), 2.57 (d, J = 7.2 Hz, 2 H), 3.43 (m, 1 H), 3.63 (d, $J_{H,P}$ = 10.8 Hz, 3 H), 3.72 (s, 3 H), 4.31 (m, 1 H), 4.85 (m, 2 H), 7.12–7.28 (m, 5 H), 7.39–7.56 (m, 4 H); IR (CH_2Cl_2) 1734, 1636, 1510, 1221 cm^{-1}.

(*S*)-4-[[[5-Ethyl-4-(4-fluorophenyl)-2-(1-methylethyl)-6-phenyl-3-pyridinyl]methoxy]hydroxyphosphinyl]-3-hydroxybutanoic Acid, Disodium Salt (6p). A solution of compound **30p** (650 mg, 1.20 mmol) in dioxane (10 mL) was treated with 1 N NaOH (3.7 mL, 3.7 mmol), and the mixture was stirred at 55 °C for 3 h. The solvent was evaporated to give a white solid. The residue was slurried in warm H_2O and chromatographed on CHP-20P (25 mm × 100 mm) eluting in succession with H_2O (200 mL) and 50% MeOH in H_2O (400 mL). The desired fractions were pooled and evaporated, and the residue was taken up in H_2O and lyophilized to give **6p** (435 mg, 65%) as a white solid: TLC R_f 0.31 (8:1:1 CH_2Cl_2–HOAc–MeOH); ^1H NMR (CD_3OD, 270 MHz) δ 0.65 (t, J = 6.8 Hz, 3 H), 1.30 (d, J = 7.0 Hz, 6 H), 1.48 (dd, J = 7.6, 16.0 Hz, 2 H), 2.28 (q, J = 6.8 Hz, 2 H), 2.37 (m, 2 H), 3.66 (m, 1 H), 4.19 (m, 1 H), 4.64 (m, 2 H), 7.18–7.50 (m, 9 H); IR (KBr) 2935, 1581, 1510, 1404, 1222, 1020 cm^{-1}. Anal. ($C_{27}H_{29}FNNa_2O_6P \cdot H_2O$) C, H, F, N, P.

Biological Assays. Rat Hepatic HMG-CoA Reductase Inhibition. Rat hepatic HMG-CoA reductase activity is measured using a modification of the method described by Edwards.[21] Rat hepatic microsomes are used as a source of enzyme, and the enzyme activity is determined by measuring the conversion of the ^{14}C-HMG-CoA substrate to [^{14}C]mevalonic acid. Livers are removed from 2–4 cholestyramine-fed, decapitated, Sprague–Dawley rats, and homogenized in phosphate buffer A (potassium phosphate, 0.04 M, pH 7.2; KCl, 0.05 M; sucrose, 0.1 M; EDTA, 0.03 M, aprotinin, 500 KI units/mL). The homogenate is spun at 16000g for 15 min at 4 °C. The supernatant is removed and recentrifuged under the same conditions a second time. The second 16000g supernatant is spun at 100000g for 70 min at 4 °C. Pelleted microsomes are resuspended in a minimum volume of buffer A (3–5 mL per liver) and homogenized in a glass homogenizer. Dithiothreitol is added (10 mM), and the preparation is aliquoted, quick frozen in acetone/dry ice, and stored at −80 °C. The specific activity of a typical microsomal preparation is 0.68 nmol of mevalonic acid/mg of protein per minute. The reductase is assayed in 0.25 mL, which contains the following components at the indicated final concentrations: 0.04 M potassium phosphate, pH 7.2; 0.05 M KCl; 0.10 M sucrose; 0.03 M EDTA; 0.01 M dithiothreitol; 3.5 mM NaCl; 1% dimethyl sulfoxide; 50–200 μg of microsomal protein; 100 μM of ^{14}C-[D,L]-HMG-CoA (0.05 μCi, 30–60 mCi/mmol); 2.7 mM NADPH. Reaction mixtures are incubated at 37 °C. Under conditions described, enzyme activity increases linearly up to 300 μg of microsomal protein per reaction mixture and is linear with respect to incubation time up to 30 min. The standard incubation time chosen for drug studies is 20 min, which results in 12–15% conversion of HMG-CoA substrate to the mevalonic acid product. [D,L]HMG-CoA substrate is used as 100 μM, twice the concentration needed to saturate the enzyme under the conditions described. NADPH is used in excess at a level 2.7 times the concentration required to achieve maximum enzyme velocity. Standardized assays for the evaluation of inhibitors are conducted according to the following procedure. Microsomal enzyme is incubated in the presence of NADPH at 37 °C for 15 min. DMSO vehicle with or without test compound is added, and the mixture further incubated for 15 min at 37 °C. The enzyme assay is initiated by adding ^{14}C-HMG-CoA substrate. After 20 min of incubation at 37 °C, the reaction is stopped by the addition of 25 μL of 33% KOH. [^3H]Mevalonic acid (0.05 μCi) is added, and the reaction mixture allowed to stand at room temperature for 30 min. Fifty microliters of 5 N HCl is added to lactonize the mevalonic acid. Bromophenol blue is added as a pH indicator to monitor an adequate drop in pH. Lactonization is allowed to proceed for 30 minutes at room temperature. Reaction mixtures are layered onto 2 g of AG 1-X8 anion exchange resin (Biorad, formate form), poured in 0.7 cm (i.d.) glass columns, and eluted with 2.5 mL of H_2O. The first 0.5 mL is discarded, and the next 2.0 mL is collected and counted for both tritium and carbon-14 in 10.0 mL of Opti-fluor (Packard) scintillation fluid. Results are calculated as nanomoles mevalonic acid produced per 20 min and are corrected to 100% recovery of tritium. Drug effects are expressed as I_{50} values (concentration of drug producing 50% inhibition of enzyme activity) derived from composite dose response data from 2–5 experiments.

Inhibition of Cholesterol Synthesis in Freshly Isolated Rat Hepatocytes. Inhibitors of HMG-CoA reductase are evaluated for their ability to inhibit [^{14}C]acetate incorporation into cholesterol in freshly isolated rat hepatocyte suspensions using a modification of the methods originally described by Capuzzi.[22] Sprague–Dawley rats (180–220 g) are anesthetized with Nembutal (50 mg/kg). The abdomen is opened, and the first branch of the portal vein is tied closed. Two closing sutures are placed on the distal section of the portal vein, and the portal vein is canulated between the sutures and the first branching vein. The liver is perfused at a rate of 20 mL/min with prewarmed (37 °C) oxygenated buffer A ((HBSS, Hanks' Balanced Salt Solution) without calcium or magnesium containing 0.05% EDTA) after severing the vena cava to allow drainage of the effluent. The liver is additionally perfused with 200 mL of prewarmed oxygenated buffer B (HBSS containing 0.05% bacterial collagenase). Following perfusion with buffer B, the liver is excised and decapsulated in 50 mL of Waymouth's medium, allowing free cells to

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disperse into the medium. Hepatocytes are isolated either by low-speed centrifugation for 3 min at 50g at room temperature or by unit gravity sedimentation at 4 °C for 30–45 min. Pelleted hepatocytes are washed once in Waymouth's medium, counted, and assayed for viability by trypan blue exclusion. These hepatocyte enriched cell suspensions routinely show 70–90% viability. Hepatocytes are resuspended at 5×10^6 cells per 2.0 mL in incubation medium (IM) [0.02 M Tris-HCl (pH 7.4), 0.1 M KCl, 0.33 mM $MgCl_2$, 0.01 mM $MnCl_2$, 0.001 mM sodium succinate, 0.003 mM Coenzyme A, 0.33 mM sodium citrate, 0.67 mM nicotinamide, 0.23 mM NADP, 1.7 mM glucose-6-phosphate]. Test compounds are routinely dissolved in H_2O, DMSO, or DMSO–H_2O (1:3) and added to the IM. Final DMSO concentration in the IM is ≤1.0% and has no significant effect on cholesterol synthesis. Incubation is initiated by adding [^{14}C]acetate (58 mCi/mmol, 2 μCi/mL) and placing the cell suspensions (2.0 mL) in 35-mm tissue culture dishes at 37 °C for 2.0 h. Following incubation, cell suspensions are transferred to glass centrifuge tubes and spun at 50g for 3 min at room temperature. Cell pellets are resuspended and lysed in 1.0 mL of H_2O. Lipids are extracted essentially as described by Bligh and Dyer.[23] Following extraction, the lower organic phase is removed and dried under a stream of nitrogen and the residue resuspended in 100 μL $CHCl_3$–MeOH (2:1). The total sample is spotted on silica gel (LK6D) thin-layer plates and developed in CH_2Cl_2–acetone (60:1). Plates are scanned and counted using a BioScan automated scanning system. Radiolabel in the cholesterol peak (R_f 0.28) is determined and expressed as total counts per peak and as a percent of the label in the total lipid extract. Cholesterol peaks in control cultures routinely contain 5000–20000 dpm, and are approximately 30% of the label present in the total lipid extract. Drug effects (percent inhibition of cholesterol synthesis) are determined by comparing the percent of label in the cholesterol peak for control and drug treated cultures. Dose response curves are constructed from composite data from two or more studies and results are expressed as I_{50} values (concentration of drug which inhibits cholesterol synthesis 50%).

Inhibition of Cholesterol Synthesis in Human Skin Fibroblasts. Human skin fibroblasts (passage 7-27) are grown in minimal essential medium (MEM, Gibco) containing 10% fetal calf serum. For each experiment, stock cultures are trypsinized to disperse the cell monolayer, counted, and plated in 35-mm tissue culture wells (5×10^5 cells/2.0 mL). Cultures are incubated for 18 h at 37 °C in 5% CO_2/95% humidified room air. Cholesterol biosynthetic enzymes are induced by removing the serum containing medium, washing the cell monolayers with MEM, adding 1.0 mL of MEM containing 1.0% fatty acid free bovine serum albumin, and incubating the cultures an additional 24 h. Test compounds are dissolved in H_2O, DMSO, or DMSO–EM (1:3) (final DMSO concentration in cell cultures ≤1.0%) and added to the cultures, and the cultures are preincubated for 30 min at 37 °C in 5% CO_2/95% humidified room air. Following preincubation with drugs, sodium [1-^{14}C]acetate (2.0 μCi/mL, 58 mCi/mmol) is added, and the cultures are reincubated for 4 h. After incubation, the culture medium is removed and the cell monolayer is scraped into 1.0 mL of H_2O. Lipids in the lysed cell suspension are extracted as described for hepatocyte suspensions. The organic phase is dried under nitrogen, and the residue is resuspended and analyzed as described for hepatocytes. Cholesterol peaks in control cultures routinely contain 8000–12000 dpm and are approximately 15% of the label present in the total lipid extract.

Inhibition of cholesterol synthesis is determined as described for hepatocytes. Results are expressed as I_{50} values and are derived from composite dose response curves from two or more experiments.

In Vivo Cholesterol Biosynthesis Inhibition in Rats. The methods used for intravenous (iv) and oral (po) drug testing were adapted from a procedure originally described by Sandoz.[24] Male Sprague–Dawley rats (200–300 g) were adapted to a reverse light cycle for 7–10 days and fed Purina rat chow (no. 5001) ad libitum. In order to measure cholesterol synthesis, sodium [1-^{14}C]acetate (1–3 mCi/mmol) (25 μCi/100 g of body weight) was injected intraperitoneally (ip) 2 h before the mid-dark point in the diurnal cycle. Two hours after the mid-dark point animals were anesthetized ip with ketamine/xylazine and bled into EDTA-treated centrifuge tubes from the abdominal aorta. Plasma was obtained by centrifugation at 1100g for 10 min. One-milliliter plasma samples were aliquoted and either processed directly or frozen at −20 °C. For iv testing, the salt forms of test compounds were routinely dissolved in saline and injected iv into the tail vein 5 min before [^{14}C]acetate injection. For po testing, drugs were dissolved in saline and given by gavage 30 min before [^{14}C]acetate injection. Cholesterol synthesis was measured by determining the level of ^{14}C-labeled nonsaponifiable lipid present in 1 mL of plasma; the method used is a modification of the method described by Dugan.[25] One milliliter physiological saline was added to 1 mL of plasma, followed by the addition of 5.0 mL of 10% KOH in absolute ethanol. Samples were mixed and saponified at 75 °C for 1 h. After cooling, approximately 0.02 μCi (44,000 dpm) [1,2-^{3}H]cholesterol (40–60 Ci/mmol) was added to each sample. Samples were extracted once with 5 mL of petroleum ether, and the organic phase was backwashed with 5 mL of saline. This extraction procedure resulted in 50–90% recovery of the added [^{3}H]cholesterol internal standard. The extracts were dried in glass vials, and the residue resuspended in 0.5 mL of $CHCl_3$–MeOH (2:1). Samples were counted for both ^{3}H and ^{14}C in 10 mL of Optifluor scintillation fluid. The [^{3}H]cholesterol internal standard recovery value from each sample was used to correct each sample to 100% recovery of [^{14}C]cholesterol. In early experiments, sample extract residues were redissolved in 100 mL of $CHCl_3$–MeOH (2:1) and chromatographed on silica gel (Whatman LK6D) thin-layer plates using either hexanes–Et_2O–HOAc (75:25:1) or CH_2Cl_2–acetone (60:1). Using either chromatographic system, greater than 90% of the ^{14}C-label cochromatographed with authentic cholesterol. Thus, to simplify the method, the TLC step was omitted in subsequent experiments and results were calculated as ^{14}C-labeled nonsaponifiable plasma lipid values, of which, greater than 90% of the ^{14}C-label is authentic cholesterol. The percent inhibition of cholesterol synthesis was derived by comparing ^{14}C-labeled nonsaponifiable plasma lipid values per milliliter of plasma from control and drug-treated animal groups (4–5 rats/group). Percent inhibition is plotted relative to the log drug dose and a linear best fit regression line is determined for each experiment. Mean ED_{50} values (level of drug required to suppress cholesterol synthesis in vivo by 50%) were calculated from two or more experiments.

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