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Laboratory note

Inhibitors of HMG-CoA reductase. Biological effects of A 6-[2-[2-(4-fluoro-3-methylphenyl)-4-substituted cyclohexe-1-en-1-yl]ethenyl]-4-hydroxy-3,4,5,6-tetrahydro-2h-pyran-2-one

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Summary – We report on a series of potent inhibitors of HMG-CoA reductase (HMGR) that were designed to examine the biological consequences that result from molecular changes in RG 12561. The introduction of functional groups on the cyclohexene nucleus of RG 12561 and the resulting inhibitor-enzyme interactions at remote binding sites of HMGR are discussed. Cellular membrane permeability may also contribute to potency. The HMGR inhibitory activity was measured utilizing solubilized enzyme. The compounds were evaluated as inhibitors of sterol biosynthesis *in vitro* using liver slices and *in vivo* after oral administration to the rat.

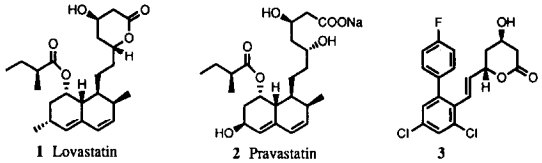
HMG-CoA reductase inhibitors / RG 12561 / cholesterol biosynthesis

Introduction

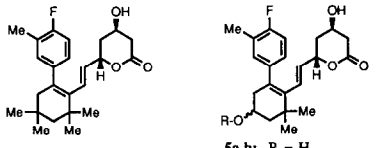
A causative link between the levels of plasma low density lipoprotein (LDL) cholesterol and the incidence of coronary heart disease (CHD) has been established. [1-3]. Reductions in plasma LDL have been secured through the agency of the HMG-CoA reductase (HMGR) inhibitors lovastatin (**1**) [4] and pravastatin (**2**) [5]. Decreased incidence of CHD has followed. Synthetic efforts which sought to replace the hexahydronaphthalene portion of **1** have produced a collection of potent HMGR inhibitors [6-14] of which Merck biphenyl **3** was the earliest prototype [15].

Biological differences between **1** [16, 17] and **2** [18, 19] have recently been reported. These compounds diverge chemically in the nature and stereochemistry of the C-6 substituent on the hexahydronaphthalene ring. Current hypotheses ascribe the pharmacological diversity of **2** and other synthetic inhibitors to their higher degree of hydrophilic character [20, 21].

We recently reported a series of synthetic inhibitors of HMGR that contained a phenylcyclohexene group as a surrogate for the hexahydronaphthalene nucleus of **1**. This effort culminated in the advancement of RG 12561 (**4**) to human clinical trials for the treatment of hypercholesterolemia [22, 23]. We envisaged that the chemical manipulation of the C-4 position of the cyclohexene nucleus of **4** could serve as a strategy to investigate interactions at remote binding sites of HMGR [24, 25]. Therefore, the goal was to synthesize and evaluate a C-4 hydroxy analog of RG 12561. In addition, biological-response questions regarding acyl derivatives of the hydroxy group could be addressed with compounds of this type. The preparation and biological assessment of compounds **5-9** is the subject of this report.



1 Lovastatin 2 Pravastatin 3

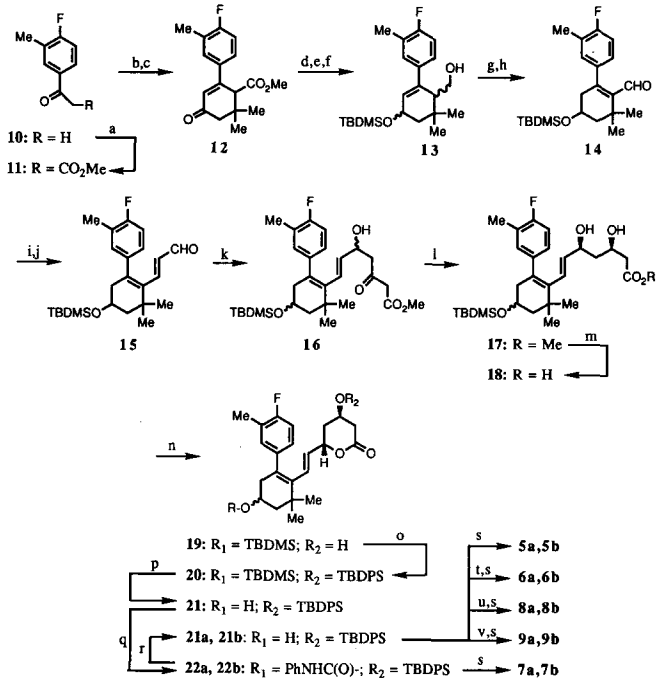


4 RG 12561

5a,b: R = H
6a,b: R = EtNHC(O)-
7a,b: R = PhNHC(O)-
8a,b: R = EtOC(O)-
9a,b: R = PhOC(O)-

Chemistry

The synthesis of compounds **5-9** is outlined in scheme 1. Sodium hydride induced carboxylation [26] of 4-fluoro-3-methylacetophenone (**10**) [27] provided β-keto ester **11** in 75% yield. Cyclohexenone **12** was formed in 64% yield by initial treatment of **11** with mesityl oxide and boron trifluoride etherate [28] at 0°C for 5 d followed by Triton-B in methanol at reflux [29]. Reduction of the ketone, protection of the alcohol as the t-butyldimethylsilyl (TBDMS) ether and LAH reduction of the ester converted **12** to **13** in 37% overall yield as a mixture of diastereomers. Sulfur trioxide pyridine complex oxidation of **13** followed by catalytic potassium t-butoxide isomerization to the α,β-unsaturated aldehyde was accomplished in nearly quantitative overall yield to give **14**. Acrolein **15** was prepared (62% yield) by treatment of **14** with the LDA derived anion of ethylidenecyclohexylamine



Scheme 1a. [a]Reagents: (a) NaH, $MeOCO_2Me$; (b) mesityl oxide, BF3·OEt, O°C; (c) Triton B, MeOH, heat; (d) $NaBH_4$; (e) TBDMSiCl, imidazole; (f) LAH,THF, 18h, 0°C; (g) SO_3·pyridine, DMSO; (h) KOt-Bu, THF; (i) LDA, $C_6H_{11}N{=}CHCH_3$; (j) SiO_2; (k) $CH_3COCH_2CO_2CH_3$, LDA; (l) Et_3B, $NaBH_4$, MeOH,-78°C; (m) NaOH, H_2O; (n) $ClCO_2Et$, Et_3N, CH_2Cl_2; (o) TBDPSiCl, imidazole; (p) HOAc, H_2O; (q) PhNCO, DMAP; (r) Cl_3SiH, Et_3N; (s) TBAF, HOAc; (t) EtNCO, DMAP; (u) $ClCO_2Et$, pyridine; (v) $ClCO_2Ph$, pyridine.

[30] and subsequent silica gel hydrolysis by HPLC of the hydroxy imine intermediate. The LDA derived dianion of methyl acetoacete [31] was added to **15** and produced **16** as a mixture of diastereomers. The erythro-diol **17** (only 3R, 5S stereochemistry shown) was obtained through the triethylborane/$NaBH_4$/MeOH [32] reduction technology (> 95% selectivity). Hydrolysis of **17** gave acid **18** in 58% overall yield. Lactonization of dihydroxy acid **18** with ethyl chloroformate and triethylamine furnished trans-lactone **19** (only 4β, 6α shown) in 72% yield. Protection of **19** as the t-butyldiphenylsilyl ether (TBDPS) provided **20**.

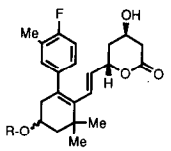
Removal of the TBDMS group of **20** in aqueous acetic acid provided alcohol **21** in 83% yield for the protection-deprotection sequence as an inseparable mixture of diastereomers. Exposing **21** to phenyl isocyanate and separation by HPLC gave racemic diastereomers **22a** (less polar) and **22b** (more polar) in a 3:2 ratio in 81% yield. The TBDPS moiety of **22a** and **22b** was cleaved with TBAF and provided **7a** and **7b**. Removal of the phenylcarbamate group of **22a** and **22b** with trichlorosilane and triethylamine [33] gave racemic diastereomers **21a** (66%) and **21b** (49%). Derivatization of both **21a** and **21b** with ethyl isocyanate, ethyl chloroformate and phenyl chloroformate and subsequent TBDPS removal provided **6a**, **8a** and **9a** and **6b**, **8b** and **9b**, respectively. Alcohols **5a** and **5b** were prepared from **21a** and **21b** by removal of the TBDPS protecting group in 71-6% yield.

An inconclusive 1H NMR analysis of **5a-9a** and **5b-9b**, and an inability to obtain X-ray quality single crystals of **5b**, **7a** and **7b** (Prof H Ammon, University Maryland, personal communication) as well as alcohol derivatives of **7b**, precluded the assignment of the relative C-4 stereochemistry on the cyclohexene ring.

Results and discussion

Compounds **5-9**, as racemic diastereomers, were evaluated for their ability to inhibit cell-free solubilized HMGR isolated from rat liver microsomes. For uniformity, all biological assays were conducted on the dihydroxy acids of **1**, **4**, **5a-9a** (the less tlc-polar series) and **5b-9b** (the more tlc-polar series). The results are shown in table I under *HMGR Inhibition*. In comparing each cyclohexene C-4 isomer (*ie* **5a** *vs* **5b**) the HMGR inhibition potency in the solubilized enzyme asssay was diminished (2-4-fold) for **5a-9a** *vs* **5b-9b**. The replacement of the C-4 gem-dimethyl group on the cyclohexene ring of **4** with a hydroxy group was deleterious to the enzyme inhibitory activity as seen by an approximate 10-fold potency reduction for **5a** and **5b** compared to **4**. This decline in activity is of somewhat greater magnitude than that of **1** *vs* **2** despite a similar molecular change. However,

Table I. Effects of substitution on phenylcyclohexene nucleus. Compounds **5a-9a, 5b-9b** are diastereomers. All compounds tested as their dihydroxy acid. See *Experimental procedures*; all compounds exhibited spectral data consistent with their structure. Analytical results are within ± 0.4% of the theoretical value.



Compound	*R*	*Formula*	*mp (°C)*	*HMGR Inhibition[a] IC50 (nM)*
1	Lovastatin	-	-	2.3
2	Pravastatin	-	-	8.9
± **4**	RG 12561	-	-	3.4
± **5a**[b]	H	$C_{22}H_{27}FO_4 \cdot 0.5\ H_2O$	53-7	46
± **5b**[c]	H	$C_{22}H_{27}FO_3 \cdot 0.5\ H_2O$	53-7	26
± **6a**	EtNHC(O)-	$C_{25}H_{32}FNO_5 \cdot 0.25\ H_2O$	60-3	3.7
± **6b**	EtNHC(O)-	$C_{25}H_{32}FNO_5 \cdot 0.25\ H_2O$	58-60	1.1
± **7a**	PhNHC(O)-	$C_{29}H_{32}FNO_5 \cdot 0.5\ H_2O$	78-82	1.2
± **7b**	PhNHC(O)-	$C_{29}H_{32}FNO_5 \cdot 0.5\ H_2O$	143-6	0.5
± **8a**	EtOC(O)-	$C_{25}H_{31}FO_6$	wax	10.3
± **8b**	EtOC(O)-	$C_{25}H_{31}FO_6$	wax	3.3
± **9a**	PhOC(O)-	$C_{29}H_{31}FO_6$	58-62	14.7
± **9b**	PhOC(O)-	$C_{29}H_{31}FO_6$	58-62	3.2

[a]See *Experimental* Section for *protocols*; [b]**a** = less polar diastereomer on tlc; [c]**b** = more polar diastereomer on tlc.

in vitro efficacy was regained by the attachment of acyl groups to the cyclohexene alcohol. Compounds **6a-9a** and **6b-9b** exhibited enhanced activity (3-40 and 10-50-fold) compared to **5a** and **5b** respectively. The carbamate group was slightly more effective than the carbonate in obtaining increased activity (compare **6a** and **7a** *vs* **8a** and **9a** and, also **6b** and **7b** *vs* **8b** and **9b**). No important differences were observed between ethyl and phenyl derivatives in either the carbamate or carbonate series. Compound **7b** provided the best enzyme inhibitory potency (IC_{50} = 0.5 nM) of this class of compounds compared to **1** (IC_{50} = 2.3 nM), **2** (IC_{50} = 8.9 nM) and **4** (IC_{50} = 3.4 nM).

The difference in inhibitory potency between series **5a-9a** *vs* **5b-9b** may indicate that **5b-9b** binds slightly more efficiently at remote binding sites. These variations between diastereomeric pairs cannot be attributed to conformational changes in the cyclohexene nucleus resulting from different C-4 appendages since within each series the compounds displayed similiar ^1H NMR spectra. The increased level of biological activity of **6a-9a** *vs* **5a** and **6b-9b** *vs* **5b** against the solubilized enzyme suggests that the carbamate and carbonate groups are likely involved in productive hydrogen bond acceptor or space filling interactions. No trend emerged for compounds **5-9** when a comparison of calculated log P and potency against solubilized enzyme was made [20, 21].

Therapeutically, inhibitors of HMGR should inhibit cholesterol biosynthesis in the liver since this is the primary organ of sterol synthesis. Therefore, compounds **5b-9b**, which displayed the better efficacy against solubilized enzyme, were evaluated *in vitro* for inhibition of cholesterol biosynthesis using rat liver slices. The results are summarized under **Liver Slices** in table II. Compounds **2** and **5b**, despite weaker HMGR inhibitory efficacies, displayed improved inhibition of cholesterol biosynthesis (IC_{50} = 78 and 79 nM, respectively) compared to **1** and **4** (IC_{50} = 116 and 300 nM, respectively). The cellular uptake of **2** and **5b** must be greater than **1** and **4** to account for this activity. Compounds **6b, 8b** and **9b** exhibited good inhibition of cholesterol biosynthesis in liver slices (IC_{50} = 28-83 nM) which correlated with their high HMGR inhibitory activity. Therefore, it is assumed **6b, 8b** and **9b** also have good membrane permeating abilities. However, **7b** was less effective at inhibiting cholesterol biosynthesis in liver slices (IC_{50} = 232 nM) than against solubilized enzyme.

Table II. Biological activities of more polar diastereomers.

Compound[a]	*Liver Slices*[b,c] *IC50 (nM)*	*Ex Vivo*[b,d] *% Inhibition at 1 mg/kg (N)*[e]
1	116	64 ± 4 (22)
2	78	20 ± 21 (8)
± **4**	300	69 ± 3 (30)
± **5b**	79	84 ± 4 (6)
± **6b**	83	92 ± 4 (6)
± **7b**	232	95 ± 1 (6)
± **8b**	28	87 ± 3 (6)
± **9b**	81	67 ± 10 (6)

[a]Compounds **5b-9b** are diastereomers. All compounds tested as their dihydroxy acid. [b]See *Experimental protocols.* [c]The IC50 value was determined from three concentrations. Each concentration was determined in triplicate; [d]A triplicate determination was used for each rat; [e]Number of rats used

Compounds **1, 2, 4**, and **5b-9b** were evaluated for inhibition of cholesterol biosynthesis in the rat at 1 mg/kg, po. The results are summarized in table II under *Ex Vivo % Inhibition.* Compound **7b**, with good inhibitory activity against the solubilized enzyme (IC_{50} = 0 5 nM), produced potent inhibition of cholesterol biosynthesis compared to **1, 2** and **4**. Moreover, compounds **5b, 6b** and **8b**, which exhibited similar HMGR inhibition compared to **1, 2** and **4**, were slightly more effective at inhibiting cholesterol biosynthesis in the rat. The introduction of either hydroxy or carbamate and carbonate derivatives to the C-4 position of the cyclohexene ring could play a role in oral absorption and hepatic uptake for this class of compounds. However, an *in vivo* metabolism of the carbamates and carbonates to alcohol **5b** cannot be excluded as an explanation for the similiar cholesterol biosynthesis inhibition of these compounds.

Experimental procedures

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were measured on a Varian EM-390, JEOL JNM FX-270 or Bruker AC-F 300 NMR spectrometer using tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ) from the Me_4Si resonance in the indicated solvent. Mass spectra were run on a Varian MATT 112. TLC analytical separations were conducted with E Merck silica gel 60 F-254 plates of 0.25 mm thickness and were visualized with UV or I_2. Normal-phase chromatographies were run by using gravity (Woelm Activity III/30 mm silica gel) or flash (EM Science Kieselgel 60, 70-230 mesh) conditions or on a Waters Prep LC/System 500 using Prep Pak-500/Silica cartridges and indicated solvents. The commercially available starting materials and anhydrous solvents were used as obtained.

*Methyl 4-flouro-3-methylbenzoylacetate (**11**)*

To a suspension of pentane-washed NaH (60%, 35.8 g, 0.894 mol) and dimethyl carbonate [26] (80.5 g, 90.1 mol) in 300 ml anhydrous refluxing ether was added 4-fluoro-3-methylacetophenone (**10**) [27] (68.0 g, 0.447 mol) dropwise during a period of 45 minutes. After the addition was complete, an additional 500 ml ether was added. The mixture was heated at reflex for 2 h, cooled to room temperature and poured onto ice water and acetic acid. The aqueous mixture was extracted thoroughly with ether. The combined organic layers were washed with water, $NaHCO_3$ and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* and distillation of the residue provided 70.4 gm (75%) of the oily product (bp 102-4°C, 0.4 torr) which slowly solidified upon standing. ^1H NMR ($CDCl_3$) δ 7.90-6.9 (m, 3H), 3.97 (s, 2H), 3.74 (s, 3H), 2.33 (s, 3H).

*Methyl 2-(4-fluoro-3-methylphenyl)-4-oxo-6,6-dimethyl-cyclohex-2-enecarboxylate (**12**)*

A mixture of **11** (26.95 g, 0.128 mol), mesityl oxide (19.3 g, 0.192 mol) and boron trifluoride etherate (18.2 g, 0.128 mol) was kept under N_2 at 0-5°C for 5 d [28], poured into ice cold saturated $NaHCO_3$ (800 ml) and extracted with ether. The combined organic layers were washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was dissolved in 200 ml anhydrous methanol and Triton-B (25 ml of a 40% methanol solution). The solution was heated at reflux for 1 h, cooled to room temperature, acidified with 10% HCl and extracted with ether. The combined organic extracts were washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatile *in vacuo* provided a residue which was purified by HPLC using 11% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product rich fractions (R_f 0.52, 33% ethyl acetate in hexanes) provided 22.51 gm (65%) of the product which slowly solidified upon standing. Anal ($C_{17}H_{19}FO_3$) C,H. ^1H NMR ($CDCl_3$) δ 7.47-6.87 (m, 3H), 6.42 (s, 1H), 3.76-3.6 (m, 4H), 2.8 (d, 1H), 2.30 (m, 3H), 1.17 (s, 6H). MS, m/e 290 (M+).

*1-(4-Fluoro-3-methylphenyl)-3-(R,S)-(t-butyldimethylsilyl) oxy-5,5-dimethyl-6-(R,S)-hydroxymethylcyclohexene (**13**)*

To a solution of **12** (13.7 g, 47.2 mmol) in 100 ml methanol at 0-5°C was added portionwise $NaBH_4$ (2.68 g, 70.5 mmol). After the addition was complete the mixture was stirred 60 min, diluted with ether and quenched with dilute HCl. The organic layer was washed with water and brine and dried ($MgSO_4$). Removal of the solvents *in vacuo* provided an oily residue. To a solution of imidazole (9.75 g, 141.6 mmol) in 100 ml CH_2Cl_2 was added dropwise t-butyldimethylsilyl chloride (11.0 g, 70.8 mmol). The mixture was stirred 15 min and a solution of the above alcohol in 250 ml CH_2Cl_2 was added, stirred overnight and diluted with ether and water. The organic layer was washed with water and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* furnished the oily product. To a solution of this ester in 250 ml anhydrous THF at 0-5°C was added portionwise LAH (3.60 g, 94.4 mmol). The mixture was kept overnight at 0-5°C, quenched with water (3.6 ml), 15% NaOH (3.6 ml) and water (10.8 ml) and filtered. The filtrate was diluted with ether, washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided an oily residue which was purified by HPLC using 11% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions provided 6.55 gm (37% yield) of the oily product as a mixture of diastereomers. ^1H NMR ($CDCl_3$) δ

7.16-6.92 (m, 3H), 5.82 (d, 1H), 4.40-4.32 (m, 1H), 3.68-3.52 (m, 2H), 2.28 (d, 3H), 1.16 (s, 6H), 1.10 (s, 9H), 0.93-0.90 (2s, 6H). MS, m/e 378 (M+).

*2-(4-Fluoro-3-methylphenyl)-4-(t-butyldimethylsilyl)oxy-6,6-dimethylcyclohex-1-enecarboxaldehyde (**14**)*

To a solution of **13** (13.7 g, 36.2 mmol) and triethylamine (23.8 g) in 40 ml anhydrous DMSO was added dropwise a solution of sulfur trioxide-pyridine complex (17.6 g, 108.6 mmol) in 50 ml DMSO. After the addition was complete, the solution was stirred for 1 h and was diluted with ether and water. The organic layer was washed thoroughly with water and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided 13.57 gm of a brown oil which was dissolved in 100 ml anhydrous THF. Potassium t-butoxide (0.20 g, 1.80 mmol) was added. The solution was stirred for 2 h and diluted with ether and water. The organic layer was washed with water, saturated $NaHCO_3$ and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided 13.6 gm (99%) of the yellow, oily product, (R_f 0.66, 10% ethyl acetate in hexane) which was used without further purification. ^1H NMR ($CDCl_3$) δ 9.33 (s, 1H), 7.0-6.8 (m, 3H), 4.23-3.86 (m, 3H), 2.67-2.47 (m, 2H), 2.28 (m, 3H), 1.73-1.60 (m, 2H), 1.33 (s, 3H), 1.31 (s, 3H), 0.91 (s, 9H), 0.10 (s, 6H). MS, m/e 377 (M+1).

*3-(2-(4-Fluoro-3-methylphenyl)-4-(t-butyldimethylsilyl)oxy-6,6-dimethylcyclohex-1-en-1-yl)propenal (**15**)*

To a -78°C solution of LDA (68.4 mmol) in 75 ml anhydrous THF was added ethylidenecyclohexylamine [30] (8.58 g, 68.4 mol) in 100 ml anhydrous THF. The solution was warmed to 0-5°C for 30 min and cooled to –78°C. A solution of **14** (13.6 g, 36.0 mmol) in 100 ml anhydrous THF was added and the resulting mixture stirred for 2.5 h and quenched with H_2O and ether. The organic layer was washed with water and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was hydrolyzed and purified by HPLC using 3% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions provided 9.02 g (62%) of an orange oil. ^1H NMR ($CDCl_3$) δ 9.16 (d, 1H), 7.07-6.77 (m, 4H), 5.98 (dd, 1H), 4.23-3.86 (m, 3H), 2.60-2.40 (m, 2H), 2.26 (m, 3H), 1.81-1.53 (m, 2H), 1.33 (s, 3H), 1.26 (s, 3H), 0.90 (s, 9H), 0.08 (s, 6H). MS, m/e 402 (M+).

*Methyl 7-[2-(4-fluoro-3-methylphenyl)-4-(R,S)-(t-butyldimethylsilyl)oxy-6,6-dimethylcyclohex-1-en-1-yl]-5-(R,S)-hydroxy-3-oxohept-6-en-oate (**16**)*

To a –78°C solution of LDA (126 mmol) in 100 ml anhydrous THF was added dropwise methyl acetoacetate [31] (6.57 g, 56.0 mmol). The mixture was warmed to 0-5°C for 90 min and treated with the dropwise addition of propenal **15** (18.8 g, 46.7 mmol) in 100 ml anhydrous THF. The solution was stirred for 60 min and quenched with aqueous acetic acid and ether. The organic layer was washed with brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by HPLC using 20% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions provided 14.0 g (58%) of the product as an orange oil. (R_f 0.35, 50% ethyl acetate in hexane). ^1H NMR ($CDCl_3$) δ 6.97-6.77 (m, 3H), 6.0 (d, 1H), 5.27-4.87 (m, 1H), 4.40-3.93 (m, 4H), 3.72 (s, 3H), 3.37 (s, 2H), 2.53-2.20 (m, 5H), 1.18 (s, 3H), 1.14 (s, 3H), 0.90 (s, 9H), 0.10 (s, 6H)MS, m/e 519 (M+1).

*Erythro methyl-7-[2-(4-fluoro-3-methylphenyl)-4-(R,S)-(t-butyldimethylsilyl)oxy-6,6-dimethylcyclohex-1-en-1-yl]-3,5-dihydroxyhept-6-en-oate (**17**)*

A solution of **16** (7.80 g, 15.1 mmol) and triethylborane (22.6 ml of a 1M THF solution, 22.6 mmol) in 60 ml anhydrous THF was stirred for 5 min at room temperature, cooled to –78°C and $NaBH_4$ (0.654 g, 17.3 mmol) was added portionwise followed by the dropwise addition of methanol (10 ml). After 45 min aqueous H_2O_2 (30 ml of 30% H_2O_2 in 65 ml H_2O) was added dropwise [32]. After the addition was complete, the solution was diluted with ethyl acetate and H_2O. The organic layer was washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided 7.55 g (96% yield) of the oily product (R_f 0.35, 50% ethyl acetate in hexane) which was used without further purification. ^1H NMR ($CDCl_3$) δ 6.93-6.73 (m, 3H), 5.9 (d, 1H), 5.27-4.87 (m, 1H), 4.27-3.87 (m, 3H), 3.67 (s, 3H), 2.5-2.17 (m, 7H), 1.15 (s, 3H), 1.11 (s, 3H), 0.9 (s, 9H), 0.1 (s, 6H). MS, m/e 521 (M+1).

*Erythro 7-[2-(4-fluoro-3-methylphenyl)-4-(R,S)-(t-butyldimethylsilyl)oxy-6,6-dimethylcyclohex-1-en-1-yl]-3,5-dihydroxyhept-6-en-oic acid (**18**)*

A 0-5°C solution of ester **17** (7.55 g, 14.5 mmol) and NaOH (14.5 ml of a 1N aqueous solution) in 75 ml methanol was stirred for 60 min, warmed to room temperature, stirred for 60 min and approximately 75% of the vol was removed *in vacuo*. The residue was cooled to 0-5°C, acidified with 1N HCl to pH2 and extracted with ether. The organic layers were washed with brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided 6.90 gm (94% yield) of a solid which was used without further purification. ^1H NMR ($CDCl_3$) δ 2.22 (s, 3H), 1.16 (s, 3H), 1.11 (s, 3H), 0.9 (s, 9H), 0.1 (s, 6H). MS, m/e 488 (M-18).

*(Trans)(4β, 6α)-(E)-6-[2-[2-(4-fluoro-3-methylphenyl)-4-(R,S)-(t-butyldimethylsilyl)oxy-6,6-dimethylcyclohex-1-en-1-yl]ethenyl]4-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-one (**19**)*

To a solution of acid **18** (6.90 gm, 13.6 mmol) and triethylamine (1.65 g, 16.3 mmol) in 50 ml CH_2Cl_2 at -15°C was added dropwise ethyl chloroformate (1.52 g, 13.6 mmol) in 50 ml CH_2Cl_2. After the addition was complete the mixture was stirred for 30 min and diluted with ether and H_2O. The organic layer was washed with saturated $NaHCO_3$ and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* furnished a residue which was purified by HPLC using 33% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions provided 4.81 g (72% yield) of the solid product. ^1H NMR ($CDCl_3$) δ 6.94-6.76 (m, 3H), 6.00 (d, 2H), 5.16-5.04 (m, 1H), 4.96-4.84 (m, 1H), 4.12-3.96 (m, 2H), 2.68-2.24 (m, 4H), 2.22 (s, 3H), 1.8-1.1 (m, 4H), 1.18-1.06 (m, 6H), 0.88 (s, 9H), 0.08 (s, 6H). MS, m/e 489 (M+1).

*(Trans)-(4β, 6α)-(E)-6-[2-[2-(4-fluoro-3-methylphenyl)-4-(R,S)-(t-butyldimethylsilyl)oxy-6,6-dimethylcyclohex-1-en-1-yl]-ethenyl]4-(t-butyldiphenylsilyl)oxy-3,4,5,6-tetrahydro-2H-pyran-2-one (**20**)*

To a solution of imidazole (1.84 gm, 27.0 mmol) in 40 ml CH_2Cl_2 was added t-butylchlorodiphenylsilane (3.71 gm, 13.5 mmol). The mixture was stirred for 15 min and a solution of **19** (4.40 gm, 9.0 mmol) in 40 ml CH_2Cl_2 was added. The mixture was stirred overnight, diluted with ether, washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in*

vacuo provided 6.5 gm of the oily product (Rf 0.52, 50% ethyl acetate in hexane) which was taken forward without further purification. MS, m/e 727 (M+1).

*(Trans)-(4β, 6α)-(E)-6-[2-[2-(4-fluoro-3-methylphenyl)-4-(R,S)-hydroxy-6,6-dimethylcyclohex-1-en-1-yl]ethenyl]-4-(t-butyldiphenylsilyl)oxy-3,4,5,6-tetrahydro-2H-pyran-Z-one (**21**)*

A solution of **20** (6.5 gm) in 90 ml acetic acid, 30 ml anhydrous THF and 30 ml H_2O was stirred overnight and the vol reduced by 75% by concentration *in vacuo*. The residue was diluted with ether, washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided an oil which was purified on HPLC using 33% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions (R_f 0.41, 20% ethyl acetate in hexane) provided 4.66 gm of the solid product. ^1H NMR ($CDCl_3$) δ 7.76-6.76 (m, 13H), 6.01-5.88 (m, 1H), 5.16-4.96 (m, 2H), 4.28-3.92 (m, 2H), 2.16 (s, 3H), 1.22-1.08 (m, 6H), 1.0 (s, 9H). MS, m/e 612 (M+).

*(Trans)-(E)-6-[2-[2-(4-fluoro-3-methylphenyl)-4-(R,S)-phenylcarbamyloxy-6,6-dimethylcyclohex-1-en-1-yl]ethenyl]-4-(t-butyldiphenylsilyl)oxy-3,4,5,6-tetrahydro-2H-pyran-2-one (**22a, 22b**)*

A solution of **21** (6.25 gm, 10.2 mmol), phenylisocyanate (2.7 ml) and DMAP (0.25 gm) in 70 ml CH_2Cl_2 was stirred overnight, diluted with ether, washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by HPLC using 1.5% ethyl acetate in CH_2Cl_2 as the eluent. Concentration *in vacuo* of the product-rich fractions provided 3.50 gm of **22a** (less polar) (R_f 0.51, 2% ethyl acetate in chloroform) and 2.23 gm of **22b** (more polar) (R_f 0.42, 2% ethyl acetate in chloroform). **22a**: ^1H NMR ($CDCl_3$) δ 7.68-6.7 (m, 18H), 6.56 (bs, 1H), 6.06-5.90 (d, 1H), 5.20-5.0 (m, 3H), 4.24-3.88 (m, 1H), 2.16 (s, 3H), 1.24 (s, 3H), 1.12 (s, 3H), 1.02 (s, 9H). **22b** 7.64-6.76 (m, 18H), 6.56 (bs, 1H), 6.02 (d, 1H), 5.24-4.96 (m, 3H), 4.2-4.12 (m, 1H), 2.18 (s, 3H), 1.12 (s, 6H), 1.04 (s, 9H). MS, m/e 732 (M+1).

*(Trans)-(E)-6-[2-[2-(4-fluoro-3-methylphenyl)-4-(R,S)-hydroxy-6,6-dimethylcyclohex-1-en-1-yl]ethenyl]-4-(t-butyldiphenylsilyl)oxy-3,4,5,6-tetrahydro-2H-pyran-2-one (**21b**)*

A solution of **22b** (1.92 gm, 2.60 mmol), trichlorosilane (0.755 gm, 5.46 mmol) and triethylamine [33] (0.564 gm, 5.46 mmol) in 25 ml CH_2Cl_2 was stirred overnight at room temperature, diluted with ether, washed with H_2O, saturated NH_4Cl, water and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by HPLC using 33% ethyl acetate in hexanes. Concentrations *in vacuo* of the product-rich fractions (R_f 0.41, 20% ethyl acetate in hexane) provided 1.05 gm (66%) of solid **21b** and 0.36 gm (19%) of **22b**. ^1H NMR ($CDCl_3$) δ 7.68-6.72 (m, 13H), 6.03 (d, 1H), 5.16-4.92 (m, 2H), 4.24-4.0 (m, 2H), 2.18 (s, 3H), 1.20 (s, 3H), 1.15 (s, 3H), 1.05 (s, 9H). MS, m/e 612 (M+).

*(Trans)-(E)-6-[2-[2-(4-fluoro-3-methylphenyl)-4-(R,S)-hydroxy-6,6-dimethylcyclohex-1-en-1-yl]ethenyl]-4-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-one (**5b**)*

A solution of **21b** (0.160 gm, 0.261 mmol), TBAF (1.35 ml of a 1M THF solution, 1.35 mmol) and acetic acid (150 mL, 2.61 mmol) in 2 ml anhydrous THF was stirred at room temperature overnight, diluted with ethyl acetate, washed with water and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by flash chromatography using 66% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions (R_f 0.28, 80% ethyl acetate in hexane) furnished 70 mg (72%) of **5b**. mp 53-7°C. ^1H NMR ($CDCl_3$) δ 6.96-6.76 (m, 3H), 6.04 (d, 1H), 5.13 (dd, 1H), 5.0-4.88 (m, 1H), 4.2-4.0 (m, 2H), 2.72-2.12 (m, 4H), 2.20 (s, 3H), 1.16 (s, 6H). MS, m/e 374 (M+).

*(Trans)-(E)-6-[2-[2-(4-fluoro-3-methylphenyl)-4-(R,S)-ethylcarbarmyloxy-6,6-dimethylcyclohex-1-en-1-yl]ethenyl]4-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-one (**6b**).*

A solution of **21b** (0.146 gm, 0.238 mmol), ethyl isocyanate (38 mg, 0.524 mmol) and DMAP (6 mg, 0.048 mmol) in 2 ml CH_2Cl_2 was heated at reflux overnight, diluted with ether, washed with H_2O and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by MPLC using 25% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions provided the solid product. Removal of the t-butyldiphenylsilyl protecting group and purification was accomplished as above and provided **6b** (R_f 0.41, 80% ethyl acetate in hexane) as a solid, mp 58-60°C. ^1H NMR (C_6D_6) δ 6.9-6.55 (m, 3H), 5.95 (d, 1H), 5.45-5.25 (m, 1H), 5.08 (dd, 1H), 4.75-4.6 (m, 1H), 4.0-3.9 (m, 1H), 3.25-3.15 (m, 1H), 2.9-2.8 (m, 3H), 2.1 (s, 3H), 1.17 (s, 3H), 1.10 (s, 3H). MS, m/e 356 (M-89).

*(Trans)-(E)-6-[2-[2-(4-fluoro-3-methylphenyl)-4-(R,S)-phenylcarbarmyloxy-6,6-dimethylcyclohex-1-en-1-yl]ethenyl]4-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-one (**7b**).*

A solution of **22b** (0.272 gm, 0.372 mmol), TBAF (1.9 ml of a 1M THF solution, 1.86 mmol) and acetic acid (0.223 gm, 3.72 mmol) and 3 ml anhydrous THF was stirred at room temperature overnight, then diluted with ether and water. The organic layer was washed with water and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* provided a residue which was purified by flash chromatography using 50% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions (R_f 0.41, 67% ethyl acetate in hexane) gave 0.123 gm (67%) of **7b** as a solid. mp 143-6°C. ^1H NMR ($CDCl_3$) δ 7.48-6.76 (m, 8H), 6.54 (s, 1H), 6.04 (d, 1H), 5.24-5.04 (m, 2H), 5.0-4.84 (m, 1H), 4.2-4.04 (m, 1H), 2.92-2.26 (m, 4H), 2.24 (s, 3H), 2.0-1.4 (m, 4H), 1.24 (s, 3H), 1.20 (s, 3H). MS, m/e 494 (M+1).

*(Trans)-(E)-6-[2-[2-(4-fluoro-3-methylphenyl)-4-(R,S)-ethoxycarbonyloxy-6,6-dimethylcyclohex-1-en-1-yl]ethenyl]-4-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-one (**8b**).*

A solution of **21b** (0.245 gm, 0.40 mmol) and ethyl chloroformate (90 mg, 0.80 mmol) in 3 ml anhydrous pyridine was heated at 60°C for 5 h, cooled to room temperature and diluted with ether and water. The organic layer was washed with water and brine and dried ($MgSO_4$). Removal of the volatiles *in vacuo* furnished an oil which was purified by flash chromatography using 17% ethyl acetate in hexanes as the eluent. Concentration *in vacuo* of the product-rich fractions provided 0.167 gm (61%) of solid. Removal of the silyl ether and purification as described above gave **8b** (R_f 0.44, 67% ethyl acetate in hexane) as a wax. ^1H NMR ($CDCl_3$) δ 6.95-6.8 (m, 3H), 6.1 (d, 1H), 5.22 (dd, 1H), 5.15-4.9 (m, 2H), 4.2 (q, 2H), 4.15 (m, 1H), 2.85-2.33 (m, 4H), 2.25 (s, 3H), 2.05-1.4 (m, 4H), 1.35 (t, 3H), 1.21 (s, 3H), 1.19 (s, 3H). MS, m/e 447 (M+l).

*(Trans)-(E)-6-[2-[2-(4-fluoro-3-methylphenyl)-4-(R,S)-phenoxycarbonyloxy-6,6-dimethycyclohex-1-en-1-yl]ethenyl]-4-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-one (**9b**).*

To a solution of **21b** (0.231 gm, 0.377 mmol) in 3 ml anhydrous pyridine and 3 ml CH_2Cl_2 was added dropwise phenyl chloroformate (0.121 gm, 0.754 mmol). The mixture was stirred at 25°C for 2 h and diluted with ether and water. The organic layer was washed with water and brine and dried ($MgSO_4$). Removal of the volatiles and purification of the residue using flash chromatography using 17% ethyl acetate in hexanes as the eluent provided 0.245 gm (89%) of the solid product. Removal of the silyl ether and purification of the residue as descrided provided **9b** (R_f 0.43, 67% ethyl acetate in hexane) as a solid. mp 58-62°C. ^1H NMR ($CDCl_3$) δ 7.4-6.75 (m, 8H), 6.03 (d, 1H), 5.2-5.0 (m, 2H), 5.0-4.8 (m, lH), 4.2-4.1 (m, 1H), 2.24 (s, 3H), 1.22 (s, 3H), 1.21 (s, 3H). MS, m/e 494 (M+1).

Biological methods

To obtain sodium salts the lactones **1, 4, 5a-7b** and **5b-7b** were dissolved in a small amount of ethanol, then incubated with 1.2 mole equivalents NaOH for 10 min at 37°C. For lactones **8a, 8b, 9a** and **9b** 1.15 mole equivalents NaOH and 3 h at 25°C was used for the hydrolysis. This procedure results in complete conversion of lactones to their Na-salts as confirmed by thin layer chromatography (silica gel plates and ethyl acetate:hexane (50:50) as eluent).

Animals

Male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 110-130 g were kept on a reverse light cycle (lights 3 pm–3 am) for 14 d prior to use. Throughout the period of adaptation, the rats had free access to a low cholesterol diet (# 5012, Purina Mills, Inc, Richmond, IN) unless otherwise indicated.

Inhibition of partially purified rat hepatic HMG-CoA reductase

Rats were fed *ad lib* a rodent meal chow (Purina # 5001) containing 2% cholestyramine for 5 d prior to sacrifice at the mid-dark period. Liver microsomes were prepared and HMGR was solubilized from the microsomes by a freeze-thaw cycle in high ionic strength buffer (50 mM potassium phosphate, pH 7.0, 100 mM sucrose, 50 mM KCl, 30 mM EDTA and 2 mM dithiothreitol). The enzyme preparation was stored at -80°C in small aliquots and was used up to 6 months after preparation without loss of activity. Prior to use, the enzyme was activated at 37°C for 30 min. The reaction mixture contained, in 240 µl: 0.14 M potassium phosphate buffer (pH 7.0); 0.18 M KCl; 3.5 mM EDTA; 10 mM dithiothreitol; 0.1 mg/ml BSA; (^{14}C)HMG-CoA (20 µM final concentration; 3.9 Ci/mol; CFA-577, Amersham Company, Arlington Heights, IL), and 70 µg of solubilized enzyme protein with or without inhibitors (in 10 µl DMSO or glass distilled water). The reaction was initiated with 0.2 mM NADPH. After an incubation period of 10 min, the reaction product, (^{14}C)mevalonolactone, was quantitated by the method of Alberts.

In vitro inhibition of cholesterol biosynthesis in rat livers

The assay was similar to that described by Tsujita *et al* [18]. Rats were sacrificed, the livers were removed and transferred to chilled oxygenated Krebs-Ringer-bicarbonate buffer (pH 7.4). The livers were then chopped into 0.8 mm^3 cubes using a McIlwain tissue slicer (Brinkmann Instruments, Westbury, NY), and were suspended in the same buffer. Aliquots of the suspension containing about 100 mg tissue were pipetted, in triplicate, into culture tubes which contained (^{14}C)sodium octanoate (300 µM, 6.67 Ci/mol) and inhibitor. The assay vol was 1 ml. After saponification, an aliquot of the mixture was assayed for protein concentration using a commercially available assay kit (BioRad Laboratories, Richmond, CA).

Ex vivo inhibition of cholesterol biosynthesis in rat liver

Inhibitors were given orally (9-11 am) as a solution in glass distilled water (salts of hydroxyacids) to 18 h fasted rats. One hour after the rats were sacrificed, the livers were removed and cholesterol biosynthesis in the liver was determined similar to that described for *in vitro* rat liver assay.

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References

1 Stamler J, Wentworth D, Neaton JD (1986) *J Am Med Assoc*, 256, 2823–2828
2 Leaf A (1989) *N Engl J Med* 321, 680–684
3 Grundy SM (1990) *J Am Med Assoc* 264, 3053–3059
4 See *The Merck Index* (1989) Vol 11, 5460
5 Review: McTavish D, Sorkin EM (1991) *Drugs* 42, 65–89
6 Kathawala FG (1991) *Med Res Rev* 11, 121–146
7 Barton J, Neuenschwander K, Regan J (1990) *Current Card Patents* 2, 597–616
8 Roth BD, Sliskovic DR, Trivedi BK (1989) *Ann Rep Med Chem* 24, 147–156
9 Lee TJ (1987) *Trends Pharmacol Sci* 8, 442–446
10 Parker RA, Clark RW, Sit S-Y, Lanier TL, Grosso RA, Wright JJK (1990) *J Lipid Res* 31, 1271–1282

11 Beck G, Kessler K, Baader E, Bartmann W, Bergmann A, Granzer E, Tendralla H, Kerekjarto B, Krause R, Paulus E, Schubert W, Wess G (1990) *J Med Chem* 33, 52–60

12 Prugh JD, Alberts AW, Deana AA, Gilfillian JL, Huff JW, Smith RL, Wiggans JM (1990) *J Med Chem* 33, 758–765

13 Sliskovic DR, Roth BD, Wilson MW, Hoefle ML, Newton RS (1990) *J Med Chem* 33, 31–38.

14 Roth BD, Blankley CJ, Chucholowski AW, Ferguson E, Hoefle ML, Ortwine DF, Newton RS, Sekerke CS, Sliskovic DR, Stratton CD, Wilson MW (1991) *J Med Chem* 34, 357–366

15 Stokker GE, Alberts AW, Anderson PS, Cragoe Jr EJ, Deana AA, Gilfillan JF, Hirshfield J, Holtz WJ, Hoffman WF, Huff JW, Lee TJ, Novello FC, Prugh JD, Rooney CS, Smith RL, Willard AK (1986) *J Med Chem* 29, 170–181

16 Germershausen JI, Hunt VM, Bostedor RG, Bailey PJ, Karkas JD, Alberts AW (1989) *Biochem Biophys Res Commun* 158, 667–675

17 Gerson RJ, MacDonald JS, Alberts AW, Chen J, Yudkovitz JB, Greenspan MD, Rubin LF, Bokelman DL (1990) *Exp Eye Res* 50, 65–78

18 Tsujita Y, Kuroda M, Shimada Y, Kazuhiko T, Arai M, Kaneko I, Tanaka M, Masuda H, Tarumi C, Watanake Y, Fuji S (1986) *Biochem Biophys Acta* 877, 50–60

19 Mosley ST, Kalinkowski SS, Schafer BL, Tanaka RD (1989) *J Lipid Res* 30, 1411–1420

20 Roth BD, Bocan TMA, Blankley CJ, Chucholowski AW, Creger PL, Creswell MW, Ferguson E, Newton RS, O'Brien P, Picard JA, Roark WH, Sekerke CS, Sliskovic DR, Wilson MW (1991) *J Med Chem* 34, 463–466

21 Prabhakar YS, Saxena AK, Dass MJ (1989) *Drug Des Del* 4, 97–108

22 Neuenschwander K, Regan J, Scotese A, Kosmider B, Bruno J, Amin D, Gustafson S, Weinacht J, Perrone M (1990) *Abst Am Chem Soc 200th National Meeting* Washington, DC, August 26-31, MEDI 125

23 Amin D, Gustafson S, Weinacht J, Cornell S, Neuenschwander K, Kosmider B, Scotese A, Regan J, Perrone M (1992) *Pharmacology* (in press)

24 For C-5 simvastatin analogs see: Duggan ME, Alberts AW, Bostedor R, Chao Y, Germershausen JI, Gilfillan JL, Halczenko W, Hartman GD, Hunt V, Imagire JS, Schwartz MS, Smith RL, Stubbs RJ (1991) *J Med Chem* 34, 2489–2495

25 For C-6 lovastatin analogs see: Inamine ES, Lee T-J, Houck DR, Hensens OD, Halczenko W, Smith RL (1987) *European Patent Appl* 0251625, June 22

26 Fuqua SA, Parkhurst RM, Silverstein RM (1964) *Tetrahedron Lett* 20, 1625–1632

27 Buu-Hoi NP, Jacquignon P (1952) *J Chem Soc* 4173–4175.

28 Rubinstein H (1962) *J Org Chem* 27, 3886–3887

29 Walker GN (1955) *J Amer Chem Soc* 77, 3664–3667

30 Wittig G, Reiff H (1968) *Angew Chem Int Edit Engl* 7, 7–14

31 Huckin SN, Weiler L (1974) *J Am Chem Soc* 96, 1082–1087

32 Narasaka K, Pai H (1984) *Tetrahedron Lett* 40, 2233–2238

33 Pirkle WH, Hauske JR (1977) *J Org Chem* 42, 2781–2785.

34 Alberts AW, Chen J, Kuron G, Hunt V, Huff J, Hoffman C, Rothrock J, Lopez M, Joshua H, Harris E, Patchett A, Monaghn R, Currie S, Stapley E, Albers-Schonberg G, Hensen O, Hirchfield J, Hoogsteen K, Liesch J, Springer J (1980) *Proc Natl Acad Sci USA* 77, 3957–3961