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Discovery of pyrrole-based hepatoselective ligands as potent inhibitors of HMG-CoA reductase

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Abstract—In an effort to identify hepatoselective inhibitors of HMG-CoA reductase, two series of pyrroles were synthesized and evaluated. Efforts were made to modify (3*R*,5*R*)-7-[3-(4-fluorophenyl)-1-isopropyl-4-phenyl-5-phenylcarbamoyl-1*H*-pyrrol-2-yl]-3,5-dihydroxy-heptanoic acid sodium salt **30** in order to reduce its lipophilicity and therefore increase hepatoselectivity. Two strategies that were explored were replacement of the lipophilic 3-phenyl substituent with either a polar function (pyridyl series) or with lower alkyl substituents (lower alkyl series) and attachment of additional polar moieties at the 2-position of the pyrrole ring. One compound was identified to be both highly hepatoselective and active in vivo. We report the discovery, synthesis, and optimization of substituted pyrrole-based hepatoselective ligands as potent inhibitors of HMG-CoA reductase for reducing low density lipoprotein cholesterol (LDL-c) in the treatment of hypercholesterolemia.

1. Introduction

Coronary heart disease (CHD) is the leading cause of death in most industrialized countries and affects 12–15 million people in the USA alone.[1] The major cause of CHD is hypercholesterolemia or elevated serum cholesterol levels, particularly non-high density lipoprotein cholesterol (non-HDL-c), which is most effectively lowered by the use of statin drugs such as; simvastatin, atorvastatin, and rosuvastatin.[2] Statins work by inhibiting 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, the rate limiting enzyme involved in the biosynthesis of cholesterol. When cholesterol biosynthesis is inhibited, the low density lipoprotein cholesterol (LDL-c) receptor is upregulated and LDL-c is rapidly cleared from the bloodstream.[3] In addition to lowering LDL-c, statins have been shown to lower very low density lipoprotein cholesterol (VLDL-c) and triglycerides, and sometimes raise HDL-c. Furthermore, results from multiple clinical studies have indicated that statins may be beneficial for restoring endothelial function, stimulating bone formation, decreasing vascular inflammation, and enhancing the stability of plaques associated with atherosclerosis.[4,5]

An adverse side effect occasionally associated with all statins is myalgia, mild muscle pain or weakness which generally increases with higher doses of the drug.[6,7] In rare cases, statins can cause rhabdomyolysis, a severe form of myopathy or muscle toxicity, which prompted cerivastatin's removal from the market.[8] In an effort to reduce the potential for myalgia, there has been increased emphasis on the design of more hepatoselective HMG-CoA reductase inhibitors. It is anticipated that such hepatoselectivity might reduce systemic exposure and therefore limit a compound's effect on muscle cells. One strategy for obtaining hepatoselectivity is to lower the lipophilicity of the drug. As an affirmation of this strategy, rosuvastatin ($\log D = -0.33$), the most potent and hydrophilic statin on the market, was shown in the clinic to be much more liver-selective than the more lipophilic statins, simvastatin and cerivastatin ($\log D > 1.5$).[9,10] The basis for greater hepatoselectivity of hydrophilic statins is presumably due to decreased passive permeability into non-hepatic cells, while active uptake into hepatocytes via the organic anion transport-

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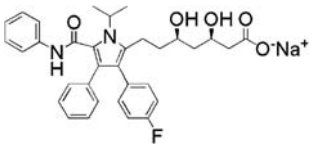


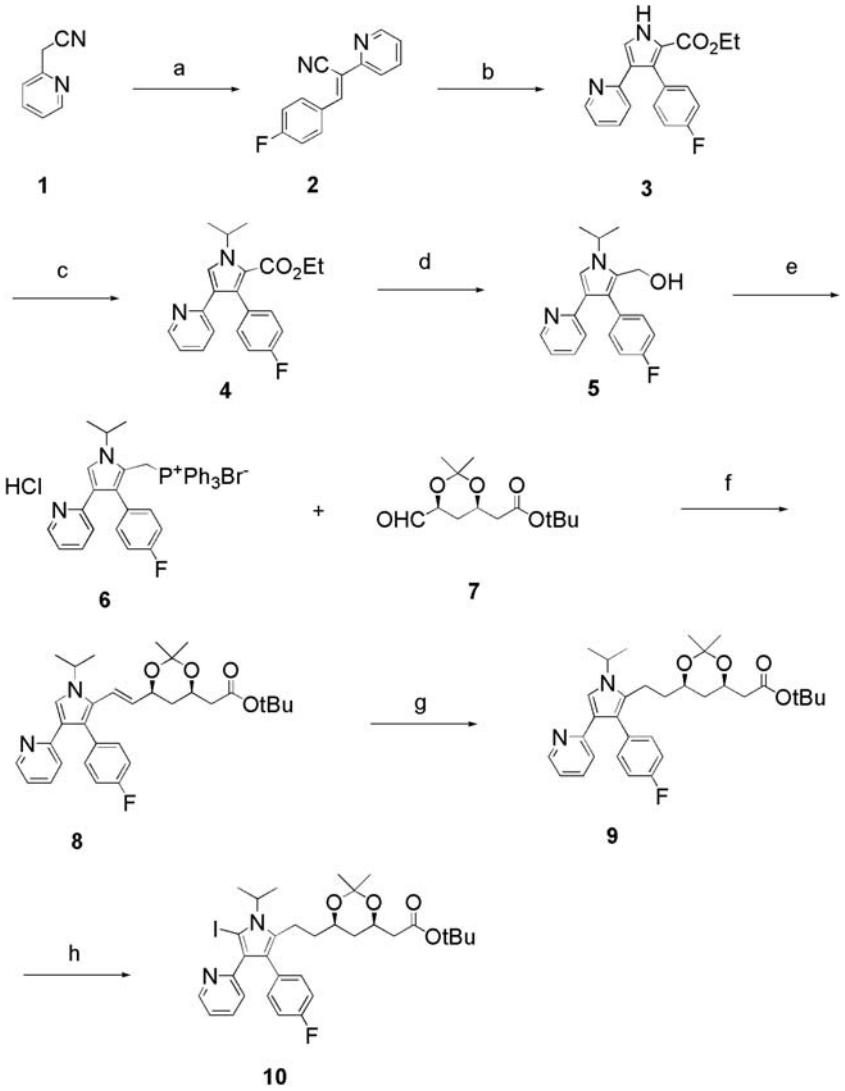
Figure 1. Compound **30**.

ing polypeptides is maintained. To obtain a more potent and hepatoselective statin, chemical modifications were implemented on atorvastatin because of its overall efficacy and safety profile compared to other statins currently on the market. Other coworkers in our group began making modifications on the core pyrole template by shifting the nitrogen atom over by one carbon unit while retaining the same functionality as in atorvastatin. This modification led to the preparation of **30**, which was found to be quite potent as an inhibitor of cholesterol in rat hepatocytes (IC_{50} = 0.43 nM), but did not exhibit the hepatoselectivity profile that we were searching for [11] (see Fig. 1).

To obtain a more hepatoselective statin, the lipophilicity of **30** was reduced by replacing the 4-phenyl substituent with either a polar pyridyl function or a lower alkyl moiety. Lipophilicity was further reduced by functionalizing the phenyl group in the amide position with polar substituents. Herein these changes in **30** will be the focus of our discussion. The synthesis and hepatoselectivity profile will also be described.

2. Chemistry

As outlined in Scheme 1, the preparation of the various pyridyl substituted pyrroles commenced with a Knoevenagel condensation of 2-pyridylacetonitrile **1** and 4-fluorobenzaldehyde to generate cyano-styrene **2**. Pyrrole formation was accomplished by treatment of **2** with ethyl isocyanoacetate in the presence of potassium *tert*-butoxide to afford pyrrole **3**.[12] Subsequently, N-alkylation of **3** using isopropyl iodide and potassium hydroxide provided *N*-isopropyl pyrrole **4**. Reduction

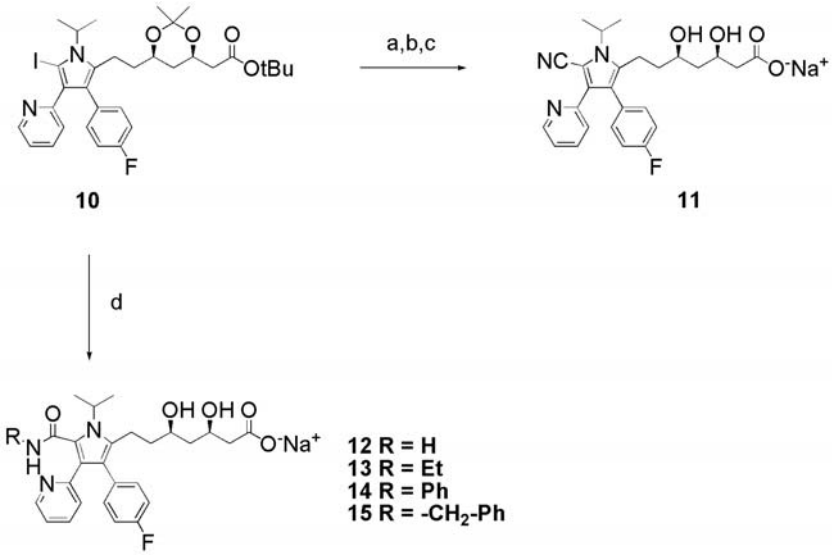


Scheme 1. Reagents and conditions: (a) 4-fluorobenzaldehyde, NaOEt, EtOH, 25 °C, 92%; (b) ethylisocyanoacetate, potassium *tert*-butoxide, THF, 0 °C, 88%; (c) KOH, *i*-propyl iodide, DMSO, 25 °C, 74%; (d) LAH, THF, −10 °C, 97%; (e) Ph_3PHBr, HCl, DCM, 25 °C, 99%; (f) NaHMDS, THF:DMSO, 78 °C, 66%; (g) Pd/C, MeOH, 25 °C, 53%; (h) NIS, DMF, 25 °C, 92%.

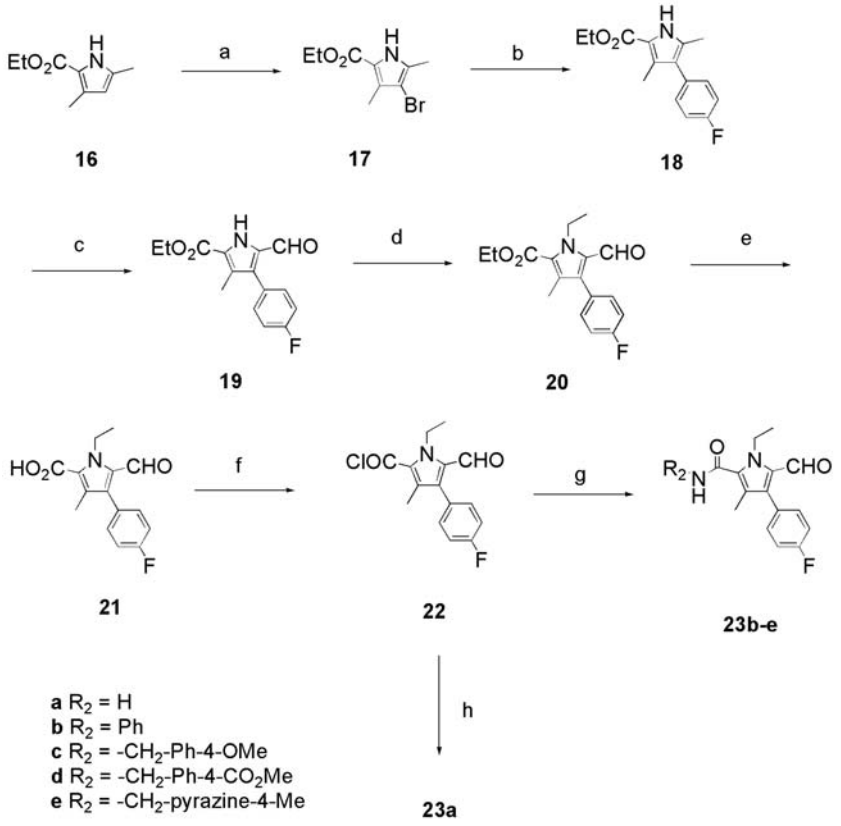
of the ethyl ester of pyrrole **4** to the corresponding alcohol **5** was accomplished using lithium aluminum hydride. Exposure of alcohol **5** to Ph_3PHBr in the presence of hydrochloric acid resulted in facile conversion to triphenyl phosphonium bromide **6**, which was then engaged in a Wittig olefination reaction with aldehyde **7** to generate olefin **8** as an inconsequential mixture of cis/trans stereoisomers.[13] The olefin of intermediate **8** was reduced by catalytic hydrogenation to afford **9**, which was then iodinated with *N*-iodosuccinimide to provide iodopyrrole **10** as a key intermediate for the preparation of analogs **11**–**15** as highlighted in Scheme 2. Treatment of iodopyrrole **10** with copper cyanide and potassium cyanide at elevated temperature in *N*,*N*-dimethylformamide followed by acetonide removal and subsequent base hydrolysis provided compound **11**. Separately, palladium-mediated carbonylative aminations of **10** with various amines (NH_3, $EtNH_2$, $PhNH_2$, and $PhCH_2NH_2$)[14] afforded, after deprotection and hydrolysis, amide analogs **12**–**15**.

Preparation of the lower alkyl pyrroles began with commercially available pyrrole ester **16** (Scheme 3), which was brominated with bromine under basic conditions to give the brominated pyrrole **17**.[15] Suzuki cross coupling of **17** with 4-fluoroboronic acid using tetrakis(triphenylphosphine)palladium(0) as catalyst provided the aryl pyrrole **18** in 74% yield.[16] Oxidation of the methyl substituent to the aldehyde was achieved under acidic conditions with ceric ammoniumnitrate as the oxidizing agent to provide the aldehyde pyrrole **19**.[16,17] The ethyl group was introduced by treatment of **19** with ethyl iodide and cesium carbonate to afford the N-alkylated pyrrole **20**. Saponification of the ester function with lithium hydroxide monohydrate at reflux, followed by acidification with hydrochloric acid, provided the acid aldehyde **21**.[17] Acid aldehyde **21** was treated with oxalyl chloride and a catalytic amount of *N*,*N*-dimethylformamide in dichloromethane to afford the acid chloride **22**, which was introduced into the next reaction without purification. Synthesis of the various substituted amide aldehydes **23a**–**e** was investigated using two different methods starting from their common acid chloride intermediate.

For example, the formamide aldehyde **23a** was prepared under modified Shotten–Baumann conditions from **22** and ammonia in aqueous ethyl acetate with sodium carbonate as base. Initial attempts to prepare amide aldehydes **23b**–**e** under these conditions failed to give the desired products, but instead, resulted in the formation of a significant amount of either an imine or decarboxylated side product. The remaining amides were eventually prepared in 54–73% yields when the amidations were conducted in dichloromethane using *N*,*N*-diisopropylethyl amine. Amide aldehydes **23a**–**e** were treated with the chiral ylide **24**[18] in refluxing toluene for 3 days to provide the corresponding Wittig products **25a**–**e** as inseparable mixtures of cis and trans isomers.[18] Reaction times were long and yields were relatively low presumably due to the steric nature surrounding the aldehyde function. Cleavage of the silyl ether function in **25a**–**e** to the alcohol amides **26a**–**e** was accomplished using either 48% hydrofluoric acid in acetonitrile or HF-pyridine in tetrahydrofuran.[19,20] HF-pyridine proved to be the reagent of choice when longer reaction times were required because it appeared to retard the formation of uncharacterized side products as indicated by thin layer chromatography. In either case, yields were greater when the starting materials were consumed in less than 2 h. Stereoselective reduction of the unsaturated ketone in **26a**–**e** with sodium borohydride, via chelation-control with diethylmethoxyborane, in a mixture of methanol and THF gave unsaturated diols **27a**–**e** in 82–88% yields.[20] Reduction of **27a**–**e** to the saturated diols **28a**–**e** was achieved under catalytic hydrogenation conditions over a period of 45 min to 2 h at low hydrogen pressure. High pressure conditions (50 psi) produced



Scheme 2. Reagents and conditions: (a) CuCN, KCN, DMF, 120 °C, 64%; (b) HCl, MeOH, 25 °C, 49%; (c) NaOH, MeOH, 25 °C, 91%; (d) i—RNH_2, CO (400 psi), $Pd(Ph_3P)_2Cl_2$, toluene, 100 °C; ii—HCl, MeOH, 25 °C; iii—NaOH, MeOH, 25 °C, 5–34% overall yield over three steps.



Scheme 3. Reagents and conditions: (a) Br_2, pyridine, DCM, 5 °C, 81%; (b) 4-fluorophenyl boronic acid, aq DMF, Na_2CO_3, $Pd(PPh_3)_4$, reflux, 71%; (c) $(NH_4)_2Ce(NO_3)_6$, acetic acid, aq THF, 25 °C, 65%; (d) EtI, Cs_2CO_3, acetonitrile, 25 °C, 87%; (e) i—LiOH–H_2O, aq THF, reflux, ii—aq HCl, 99%; (f) oxalyl chloride, DMF (cat), DCM, 25 °C, 100%; (g) R_2NH_2; $(i\text{-Pr})_2NEt$, DCM, 0–25 °C, 54–73%; (h) NH_3, Na_2CO_3, MeOH, aq ethyl acetate, −5 °C, 56%.

some side product identified as the monoalcohol of **28a**–**e** due to reductive elimination of the hydroxyl group at the 5-position of the side chain. However, shorter reaction times and lower hydrogen pressure (5 psi) minimized the formation of this unwanted side product. Finally, saponification of **28a**–**e** with sodium hydroxide in methanol at room temperature provided the target carboxylates **29a**–**e** in 62–95% yields. Similarly, **28d** was converted to the target dicarboxylate **29f** using excess base in a mixture of methanol and water at reflux (Scheme 4).

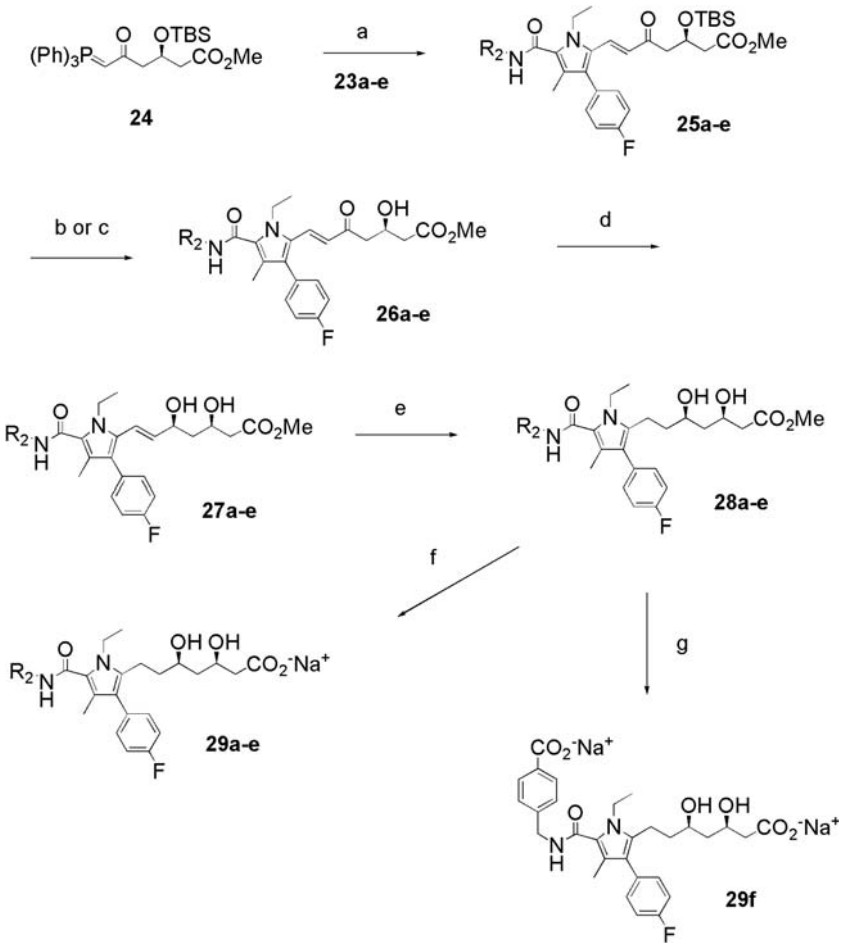
3. Biology

All new analogs were evaluated in a microsomal HMG-CoA reductase assay as well as in both hepatocyte and myocyte cellular assays.[21] The ratio of inhibition of cholesterol synthesis in heptaocytes versus myocytes was used to determine the hepatoselectivity of individual analogs. Compounds with sufficient in vitro potency and selectivity were then evaluated in an in vivo efficacy model for measuring inhibition of cholesterol synthesis. For the analogs **11**–**13** and **15** containing the pyridyl function, there was a correlation between lipophilicity and hepatoselectivity (Table 1). More polar compounds were less likely to inhibit cholesterol biosynthesis in muscle myocytes. This is likely a result of poor membrane permeability. In contrast, such compounds showed good hepatocyte activity suggesting that they may be actively transported. The lower alkyl analogs **29c**–**f** displayed the same lipophilic-hepatoselective trend as above. The only exception was found for compounds **29a** and **29b**. For **29a** loss in selectivity was due to decreased potency in liver hepatocytes. This loss in potency is not surprising since optimal binding to the receptor was not achieved due to the absence of the large phenyl group in the amide position. Similarly, diminished potency was also demonstrated for analogs **11**–**13**, which also lack the large lipophilic functionality in the 2-position of the pyrole ring.

Most of the compounds shown in Table 1 were tested in vivo using the mouse acute inhibition of cholesterol synthesis (MAICS) model. Two hepatoselective analogs **11** and **12** in the pyridyl series were found to maintain excellent in vivo activity. In the lower alkyl series, compound **29d** was the only analog to show modest in vivo activity (>60% inhibition) at the dose tested.

4. Conclusion

In summary, we have demonstrated that the hepatoselectivity of pyrrole-based HMG-CoA reductase inhibitors can in general be increased by decreasing their lipophilicity, consistent with literature precedent for other statins. Although most of the new compounds



Scheme 4. Reagents and conditions: (a) toluene, reflux, 53–67%; (b) HF–pyridine, THF, 25 °C, 35–68%; (c) 48% HF, acetonitrile, 5–25 °C, 70–74%; (d) diethylmethoxyborane, $NaBH_4$, 4:1 THF/MeOH, −60 °C, 82–88%; (e) 10% Pd/C, H_2 (5 psi), 1:1 THF/MeOH, 48–61%; (f) NaOH, aq MeOH, 25 °C, 62–95%; (g) excess NaOH, aq MeOH, reflux, 57%.

Table 1. Inhibition of cholesterol synthesis in rat liver microsomes, rat hepatocytes, and L6 myocytes in order of decreasing hepato selectivity and mouse acute inhibition of cholesterol synthesis

Compound	*C*log *D* (pH 7.4)	RM IC_{50}[a] (nM)	HEP IC_{50}[a] (nM)	L6 IC_{50}[a] (nM)	L6/HEP IC_{50}[a] (nM)	MAICS (%)
Ros	−0.33[8]	3.6	0.23	720	3130	−82
30	0.31	12	0.43	157	374	−75
Sim	>1.5[8]	18	1.3	229	175	−44
Cer	>1.5[8]	2.8	1.7	7	4	−92
Pyridyl	Analogs					
12	−1.83	5.3	4.9	18,609	3798	−73
13	−1.13	21	5.1	10,821	2122	nt
11	−0.75	4.8	(2.1)	1730	824	−72
15	0.42	3.7	0.15	28	187	nt
14	−1.04	(2.4)	0.17	28	165	nt
Lower	Alkyl	Analogs				
29f	−1.15	0.85	(0.11)	1760	16,000	−27
29e	−1.16	4.7	0.35	5225	14,929	−52
29c	0.80	1.6	0.24	184	770	nt
29a	−1.36	30	32	22,701	709	nt
29b	−0.57	2.8	(0.29)	102	349	−53
29d	0.86	1.6	(0.28)	26	93	−66

[a] Values in parentheses are given as an average of 2–3 experiments, Sim, simvastatin; Ros, rosuvastatin; Cer, cerivastatin; nt, not tested; ns, not selective; RM, HEP, and L6 are inhibition of cholesterol synthesis: of rat microsomal HMG CoA Reductase, in rat liver hepatocytes, and in rat muscle myocytes, respectively; L6/HEP, liver selective cholesterol inhibition; MAICS, mouse acute inhibition of cholesterol synthesis. All compounds were tested at a dose of 30 mg/kg in the MAICS assay with the exception of the following compounds: Ros, 10 mg/kg and **29e**, 100 mg/kg.

had in vivo activity inferior to the less hepatoselective lead **30**, we were successful in identifying one compound **12** with hepatoselectivity 10-fold higher than **30** that maintained robust in vivo activity.

5. Experimental

Melting points were determined on an Electro thermal Melting Point Apparatus and are uncorrected. Samples were characterized on a 400 MHz Nuclear Magnetic Resonance Spectrometer using either deuterated dimethylsulfoxide or deuterated chloroform. Mass spectra were determined on an LC Platform Mass Spectrometer using Chemical Ionization. A Rotoray Evaporator was used to remove solvents under reduced pressure. The precursor to intermediate **7** was purchased from Kaneka. Compound **24** was purchased from Bridge Organics. Standard sodium hydroxide solutions were purchased from Aldrich. All other reagents and starting materials were obtained from commercially available sources.

5.1. (*Z*)-3-(4-Fluorophenyl)-2-pyridin-2-yl-acrylonitrile (2)

To a solution of 4-fluorobenzaldehyde (52.5 g, 423 mmol) in EtOH (200 mL) at 25 °C were added pyridin-2-yl-acetonitrile **1** (50.0 g, 423 mmol) and NaOEt (151 g of 21% solution, 466 mmol). The reaction mixture was stirred at 25 °C for 0.5 h during which time a light brown precipitate developed. The solid was isolated by filtration and washed with EtOH (75 mL). The product was then dried under vacuum to afford the title compound (87 g, 92%), which was used without further purification: MS(APCI$^+$): *m*/*z* 225 (M+H); H NMR ($CDCl_3$) δ 8.62 (d, J = 4.0 Hz, 1H), 8.40 (s, 1H), 8.06–8.01 (m, 2H), 7.92–7.88 (m, 1H), 7.79 (d, J = 12.0 Hz, 1H), 7.41–7.33 (m, 3H).

5.2. 3-(4-Fluorophenyl)-1-isopropyl-4-pyridin-2-yl-1*H*-pyrrole-2-carboxylic acid ethyl ester (4)

A solution of **2** (25.0 g, 112 mmol) and ethyl isocyanoacetate (12.3 mL, 112 mmol) in THF (300 mL) was slowly added to a solution of KO*t*-Bu (223 mL of 1.0 M solution, 223 mmol) in THF (100 mL) at 0 °C. The resulting reaction mixture was stirred at 0 °C for 1.5 h after which time TLC indicated that the reaction was complete. The reaction mixture was transferred to a separatory funnel and ethyl acetate (500 mL) and water (200 mL) were added. The organic layer was separated, washed with brine, and dried over Na_2SO_4. Upon concentration of the organic layer, the crude product solidified to give 3-(4-fluorophenyl)-4-pyridin-2-yl-1*H*-pyrrole-2-carboxylic acid ethyl ester **3** (30.5 g, 88%) as a brown solid, which was utilized without further purification. To a solution of **3** (30.5 g, 98.3 mmol) in DMSO (100 mL) at 25 °C was added powdered KOH (24.8 g, 442 mmol) and the reaction mixture was stirred at 25 °C for 0.5 h. Subsequently, 2-iodopropane (26.5 mL, 265 mmol) was added dropwise to the suspension and the reaction mixture was stirred for an additional 0.5 h at 25 °C. Diethyl ether (300 mL) and water (100 mL) were then added and the organic layer was separated, dried (Na_2SO_4), and concentrated to give a crude oil, which was purified by silica gel chromatography (10–40% EtOAc/hexane) to give the title compound (23.2 g, 74%): MS(APCI$^+$): *m*/*z* 353 (M+H); H NMR (DMSO-d_6) δ 8.42 (d, J = 4.0 Hz, 1H), 7.79 (s, 1H), 7.47–7.43 (m, 1H), 7.20–7.04 (m, 5H), 6.65 (d, J = 12 Hz, 1H), 5.27 (sept, J = 7.9 Hz, 1H), 3.91 (q, J = 8.0 Hz, 2H), 1.46 (d, J = 8.0 Hz, 6H), 0.81 (t, J = 8.0 Hz, 3H).

5.3. [3-(4-Fluorophenyl)-1-isopropyl-4-pyridin-2-yl-1*H*-pyrrol-2-yl]-methanol (5)

To a solution of **4** (6.50 g, 18.4 mmol) in THF (120 mL) at −10 °C was slowly added lithium aluminum hydride (46.1 mL of 1.0 M in Et_2O, 46.1 mmol). The reaction mixture was stirred at −10 °C for 1 h after which time it was carefully quenched by slow addition of saturated NH_4Cl. Once the quench was complete, water was slowly added and the reaction mixture was extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and concentrated. The product was purified by silica gel chromatography (50–75% EtOAc/hexane) to afford the title compound (5.54 g, 97%) as a white solid: MS(APCI$^+$): *m*/*z* 311 (M+H); H NMR ($CDCl_3$) δ 8.47 (d, J = 7.6 Hz, 1H), 7.37 (br s, 1H), 7.30 (t, J = 14.0 Hz, 1H), 7.22–7.17 (m, 3H), 7.04–6.92 (m, 3H), 6.69 (d, J = 8.0 Hz, 1H), 4.59 (sept, J = 6.8 Hz, 1H), 4.49–4.48 (m, 2H), 1.51 (d, J = 6.8 Hz, 6H).

5.4. ((4*R*,6*S*-6-{(*E*,*Z*)-2-[3-(4-Fluorophenyl)-1-isopropyl-4-pyridin-2-yl-1*H*-pyrrol-2-yl]-vinyl}-2,2-dimethyl-[1,3]-dioxin-4-yl)-acetic acid *tert*-butyl ester (8)

To a solution of **5** (3.15 g, 10.1 mol) in CH_2Cl_2 (150 mL) were added triphenylphosphine hydrobromide (3.48 g, 10.2 mmol) and HCl (5.1 mL of 2.0 M solution in Et_2O, 10.1 mmol). The reaction mixture was stirred at 25 °C for 1 h after which time all starting material was consumed as determined by TLC. The organic layer was then washed with saturated $NaHCO_3$ and dried over Na_2SO_4. The organic layer was then concentrated under reduced pressure and dried under high vacuum for 12 h to afford [3-(4-fluorophenyl)-1-isopropyl-4-pyridin-2-yl-1*H*-pyrrol-2-ylmethyl]-triphenylphosphonium bromide **6** (6.36 g, 99%) as a yellow solid. To a solution of **6** (6.00 g, 8.93 mmol) in THF/DMSO (500 mL, 25:1) at −78 °C was added NaHMDS (9.12 mL of a 1.0 M solution in THF, 9.12 mmol). An orange color was noted as the base was added to the reaction mixture. The reaction mixture was stirred at −78 °C for 5 min after which time a solution of **7** (2.16 g, 8.35 mmol) in THF (20 mL) was added. The reaction mixture was stirred at −78 °C for 0.5 h and then allowed to warm to 25 °C over 1.5 h. The reaction was quenched by addition of saturated NH_4Cl. Ethyl acetate was then added and the organic layer was washed with water, dried (Na_2SO_4), and concentrated. The resulting oil was purified by silica gel chromatography (20–25% EtOAc/hexane) to provide the title compound (2.69 g, 66%) as a mixture of cis/trans isomers: MS(APCI$^+$): *m*/*z* 535 (M+H); H NMR [mixture cis/trans isomers] ($CDCl_3$) δ

8.51–8.49, 7.36–7.13, 7.01–6.94, 6.71–6.61, 6.36–6.26, 5.58–5.53, 5.37–5.35, 4.29–4.28, 4.10–4.07, 2.37–2.35, 2.26–2.29, 2.12–2.03, 1.49–1.34, 1.21–1.11.

5.5. ((4*R*,6*R*)-6-{2-[3-(4-Fluorophenyl)-1-isopropyl-4-pyridin-2-yl-1*H*-pyrrol-2-yl]-ethyl}-2,2-dimethyl-[1,3]dioxin-4-yl)-acetic acid *tert*-butyl ester (9)

To a solution of **8** (3.11 g, 5.82 mmol) in MeOH (100 mL) was added 10% Pd/C (300 mg). The reaction vessel was then evacuated and treated with hydrogen (50 psi) for 12 h at 25 °C. The reaction mixture was then filtered through a pad of Celite filter aid and the filtrate was concentrated. The resulting oil was purified by silica gel chromatography (30–50% EtOAc/hexane) to provide the title compound (1.65 g, 53%): MS(APCI$^+$): *m*/*z* 537.7 (M+H); H NMR ($CDCl_3$) δ 8.45 (d, J = 6.8 Hz, 1H), 7.33–7.14 (m, 5H), 7.01–6.88 (m, 3H), 6.65 (d, J = 8.0 Hz, 1H), 4.33 (sept, J = 6.8 Hz, 1H), 4.12–4.09 (m, 1H), 3.65–3.61 (m, 1H), 2.65–2.61 (m, 1H), 2.51 (dd, J = 15.2, 7.8 Hz, 1H), 2.49–2.46 (m, 1H), 2.32 (dd, J = 15.2, 4.0 Hz, 1H), 1.61–1.21 (m, 24H).

5.6. ((4*R*,6*R*)-6-{2-[3-(4-Fluorophenyl)-5-iodo-1-isopropyl-4-pyridin-2-yl-1*H*-pyrrol-2-yl]-ethyl}-2,2-dimethyl-[1,3]dioxin-4-yl)-acetic acid *tert*-butyl ester (10)

To a solution of **9** (0.80 g, 1.49 mmol) in DMF (8 mL) at 25 °C was added *N*-iodosuccinimide (0.309 g, 1.79 mmol). The reaction mixture was stirred at 25 °C for 1.5 h after which time DCM (50 mL) and saturated $NaHCO_3$ (50 mL) were then added and the organic layer was separated, washed with brine, and dried over Na_2SO_4. The organic layer was concentrated and the product was purified by silica gel chromatography (10% EtOAc/hexane) to give the title compound (0.912 g, 92%): MS(APCI$^+$): *m*/*z* 663 (M+H); H NMR ($CDCl_3$) δ 8.44 (d, J = 6.8 Hz, 1H), 7.55–7.52 (m, 1H), 7.07–7.05 (m, 1H), 7.01–6.96 (m, 5H), 4.59–4.55 (m, 1H), 4.17–4.14 (m, 1H), 3.83–3.79 (m, 1H), 3.21–3.18 (m, 1H), 2.35–2.11 (m, 2H), 1.69–1.24 (m, 24H).

5.7. (3*R*,5*R*)-7-[5-cyano-3-(4-fluoro-phenyl)-1-isopropyl-4-pyridin-2-yl-1*H*-pyrrol-2-yl]-3,5-dihydroxy-heptanoic acid sodium salt (11)

To a solution of **10** (0.153 g, 1.71 mmol) and KCN (0.111 g, 1.71 mmol) in 10 mL of DMF was added CuCN (0.183 g, 2.05 mmol). The reaction mixture was heated to 120 °C for 2 h. After cooling to 25 °C, the reaction solvent was removed under reduced pressure and the resulting residue was purified by silica gel chromatography (20–50% EtOAc/hexane) to give (6-{2-[5-cyano-3-(4-fluorophenyl)-1-isopropyl-4-pyridin-2-yl-1*H*-pyrrol-2-yl]-ethyl}-2,2-dimethyl-[1,3]dioxan-4-yl)-acetic acid *tert*-butyl ester (0.512 g, 64%). To a solution of this compound in MeOH (10 mL) at 25 °C was added 1 N HCl (1.62 mL, 1.62 mmol). The reaction mixture was stirred at 25 °C for 2 h after which time the solvent was removed by evaporation and ethyl acetate (20 mL) was added. The organic layer was washed with saturated $NaHCO_3$, water, and brine prior to drying over Na_2SO_4. After concentration in vacuo, the product was purified by silica gel chromatography (50% EtOAc/hexane) to give (3*R*,5*R*)-7-[5-cyano-3-(4-fluorophenyl)-1-isopropyl-4-pyridin-2-yl-1*H*-pyrrol-2-yl]-3,5-dihydroxyheptanoic acid *tert*-butyl ester (0.082 g, 49%). To a solution of this compound in MeOH (10 mL) was added 1.03 N NaOH (0.159 mL, 0.163 mmol) and the reaction was stirred at 25 °C for 48 h. The reaction mixture was then concentrated and azeotroped with toluene (3× 25 mL). The product was dried under vacuum at 60 °C to give the title compound (0.069 g, 91%): MS(APCI$^+$): *m*/*z* 466 (M+H); H NMR (DMSO-d_6) δ 8.49 (d, J = 7.6 Hz, 1H), 7.58–7.49 (m, 2H), 7.19–7.15 (m, 1H), 7.12–7.07 (m, 3H), 6.84 (d, J = 9.2 Hz, 1H), 4.79 (br s, 1H), 4.67 (sept, J = 6.8 Hz, 1H), 4.24 (m, 1H), 3.67–3.65 (m, 1H), 3.27–3.21 (m, 1H), 2.65–2.61 (m, 1H), 2.47–2.42 (m, 1H), 1.93 (dd, J = 15.2, 4.0 Hz, 1H), 1.72 (dd, J = 15.2, 8.4 Hz, 1H), 1.58–1.16 (m, 10H).

5.8. (3*R*,5*R*)-7-[5-carbamoyl-3-(4-fluoro-phenyl)-1-isopropyl-4-pyridin-2-yl-1*H*-pyrrol-2-yl]-3,5-dihydroxy-heptanoic acid sodium salt (12)

A high pressure reactor was charged with **10** (0.465 g, 0.702 mmol), $Pd(PPh_3)_2Cl_2$ (0.064 g), and toluene (25 ml). The reactor was pressurized with ammonia (85 psi) and CO (400 psi) and then heated to 100 °C for 15 h. After cooling to 25 °C, the reaction solvent was removed under reduced pressure and the resulting residue was purified by silica gel chromatography to give (6-{2-[5-carbamoyl-3-(4-fluorophenyl)-1-isopropyl-4-pyridin-2-yl-1*H*-pyrrol-2-yl]-ethyl}-2,2-dimethyl-[1,3]dioxan-4-yl)-acetic acid *tert*-butyl ester (0.103 g, 25%). To a solution of this compound in MeOH (5 mL) was added 1 N HCl (0.533 mL, 0.533 mmol). The reaction mixture was stirred at 25 °C for 2 h after which time the solvent was removed by evaporation and ethyl acetate (20 mL) was added. The organic layer was washed with saturated $NaHCO_3$, water, and brine prior to drying over Na_2SO4. After concentration in vacuo, the product was purified by silica gel chromatography (50–100% EtOAc/hexane) to give (6-{2-[5-carbamoyl-3-(4-fluorophenyl)-1-isopropyl-4-pyridin-2-yl-1*H*-pyrrol-2-yl]-ethyl}-2,2-dimethyl-[1,3]dioxan-4-yl)-acetic acid *tert*-butyl ester (0.057 g, 59%). To a solution of this compound in MeOH (5 mL) was added 1.02 N NaOH (0.108 mL, 0.111 mmol) and the reaction mixture was stirred at 25 °C for 72 h. The reaction mixture was concentrated in vacuo and azeotroped with toluene (3× 25 mL). The product was dried under vacuum at 60 °C to give the title compound as a white solid (0.051 g, 96%): MS(APCI$^+$): *m*/*z* 484 (M+H); H NMR (DMSO-d_6) δ 8.40 (d, J = 7.6 Hz, 1H), 7.59–7.44 (m, 3H), 7.11–6.83 (m, 6 H), 4.74 (br s, 1H), 4.64 (sept, J = 6.8 Hz, 1H), 4.22 (m, 1H), 3.66–3.62 (m, 1H), 3.51–3.47 (m, 1 H), 2.66–2.62 (m, 1H), 2.49–2.43 (m, 1H), 1.93 (dd, J = 15.2, 4.0 Hz, 1H), 1.72 (dd, J = 14.8, 8.4 Hz, 1H), 1.53–1.14 (m, 10H).

5.9. 7-[5-Ethylcarbamoyl-3-(4-fluoro-phenyl)-1-isopropyl-4-pyridin-2-yl-1*H*-pyrrol-2-yl]-3,5-dihydroxy-heptanoic acid sodium salt (13)

Compound **13** was prepared according to the method described for **12** using ethyl amine in place of ammonia

to afford the title compound in 5% overall yield over three steps. MS(APCI$^+$): *m/z* 512 (M+H); H NMR (DMSO-d_6) δ 8.39–8.37 (m, 1H), 8.07–8.05 (m, 1H), 7.75–7.71 (m, 1H), 7.45–7.42 (m, 1H), 7.05–6.96 (m, 4H), 6.81–6.79 (m, 1H), 4.79–4.76 (m, 1H), 4.55–4.51 (m, 1H), 4.11 (m, 1H), 3.64–3.61 (m, 1H), 3.53–3.44 (m, 1H), 2.97 (q, J = 7.1 Hz, 2H), 2.63–2.59 (m, 1H), 1.96–1.91 (m, 1H), 1.73–1.69 (m, 1H), 1.49 (d, J = 6.8 Hz, 6H), 1.54–1.21 (m, 4H), 0.88 (t, J = 7.0 Hz, 5H).

5.10. (3*R*,5*R*)-7-[3-(4-fluoro-phenyl)-1-isopropyl-5-phenylcarbamoyl-4-pyridin-2-yl-1*H*-pyrrol-2-yl]-3,5-dihydroxy-heptanoic acid sodium salt (14)

Compound **14** was prepared according to the method described for **12** using aniline in place of ammonia to afford the title compound in 6% overall yield over three steps. MS(APCI$^+$): *m/z* 560 (M+H); H NMR (DMSO-d_6) δ 10.3 (s, 1H), 8.35 (d, J = 4.0 Hz, 1H), 7.68 (s, 1H), 7.44–7.39 (m, 3H), 7.24–7.14 (m, 2H), 7.06–6.93 (m, 5H), 6.79 (d, J = 8.0 Hz, 1H), 4.77 (br s, 1H), 4.64 (sept, J = 8.0 Hz, 1H), 4.22 (m, 1H), 3.66–3.60 (m, 1H), 3.58–3.53 (m, 1H), 2.70–2.61 (m, 1H), 2.41–2.47 (m, 1H), 1.94 (dd, J = 15.2, 4.0 Hz, 1H), 1.72 (dd, J = 15.2, 8.0 Hz 1H), 1.55–1.18 (m, 10H).

5.11. (3*R*,5*R*)-7-[5-Benzylcarbamoyl-3-(4-fluoro-phenyl)-1-isopropyl-4-pyridin-2-yl-1*H*-pyrrol-2-yl]-3,5-dihydroxy-heptanoic acid (15)

Compound **15** was prepared according to the method described for **12** using benzyl amine in place of ammonia to afford the title compound in 34% overall yield over three steps. MS(APCI$^+$): *m/z* 574 (M+H); H NMR (DMSO-d_6) δ 8.71 (t, J = 5.6 Hz, 1H), 8.24 (d, J = 4.0 Hz, 1H), 7.55–6.77 (m, 13H), 4.74 (s, 1H), 4.61–4.55 (m, 1H), 4.18 (d, J = 5.2 Hz, 2H), 3.75–3.59 (m, 1H), 3.57–3.43 (m, 1H), 2.77–2.58 (m, 1H), 2.55–2.38 (m, 1H), 1.95 (dd, J = 15.2, 4.0 Hz, 1H), 1.73 (dd, J = 14.8, 8.0 Hz, 1H) 1.58–1.03 (m, 4), 1.48 (d, J = 6.4 Hz, 6H).

5.12. 4-Bromo-3,5-dimethyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (17)

To a cold (5 °C) solution of 3,5-dimethyl-2-pyrrole-carboxylate **16** (23 g, 135 mmol) in 350 mL of dichloromethane was added pyridine (22 g, 284 mmol), followed by a solution of bromine (24 g, 148 mmol) in 100 mL of dichloromethane. The reaction mixture was stirred for 15 min and then poured into 1 L of ice cold 2.0 N aqueous sodium thiosulfate. The layers were separated and then the aqueous layer was extracted with dichloromethane (3× 250 mL). The combined organic layers were washed with ice cold 2.0 N hydrochloric acid (3× 500 mL), followed by 5% aqueous sodium bicarbonate (2× 500 mL) and brine (500 mL). The organic layer was dried (sodium sulfate), filtered, and then the filtrate was evaporated to afford an orange solid. Recrystallization from 750 mL of hexane provided 26.8 g (81%) as crystals; mp 138 °C (dec); MS(APCI$^+$) *m/z* 248 (M+2); ^{1}H NMR (d_6-DMSO) δ 11.70 (s, 1H), 4.16 (q, J = 7.07, 2H), 2.13 (s, 3H), 2.11 (s, 3H), 1.22 (t, J = 7.08 Hz, 3H).

5.13. 4-(4-Fluorophenyl)-3,5-dimethyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (18)

To a solution of **17** (27 g, 108 mmol) in 300 mL of *N*,*N*-dimethylformamide was added 4-fluoroboronic acid (22 g, 157 mmol), a solution of sodium carbonate (29 g, 278 mmol) in 75–80 mL of water, followed by tetrakis(triphenylphosphine)palladium(0) (4.2 g, 3.6 mmol). The reaction mixture was stirred at reflux for 19 h. The reaction mixture was diluted with 1 L of ethyl acetate and filtered through a bed of Celite filter aid. The filtrate was washed with 5% aqueous sodium carbonate (3× 1 L) and brine (3× 1 L). The organic layer was separated, dried (sodium sulfate), filtered, and then the filtrate was evaporated to give a gray solid. Recrystallization from 2.4 L of 66% aqueous acetonitrile treated with activated charcoal gave 20.2 g (71%) as light tan crystals; mp 174–175 °C; MS(APCI$^-$) *m/z* 260 (M−1); ^{1}H NMR (d_6-DMSO) δ 11.37 (s, 1H), 7.12–7.23 (m, 4H), 4.18 (q, J = 7.07, 2H), 2.13 (s 3H), 2.10 (s, 3H), 1.23 (t, J = 7.14, 3H).

5.14. 4-(4-Fluorophenyl)-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (19)

To a mixture of **18** (9.6 g, 37 mmol) in a mixture of 300 mL of tetrahydrofuran, 45 mL of acetic acid, and 100 mL of water was added ceric ammonium nitrate (80 g, 24 mmol) in portions for a period of a few minutes. The reaction mixture was stirred at 24 °C for 2 h and then poured into 1 kg of ice and water. The mixture was extracted with dichloromethane (4× 300 mL). The combined organic layers were washed with 5% aqueous sodium bicarbonate (4× 500 mL) and brine (500 mL). The organic layer was dried (sodium sulfate) and evaporated to provide an orange solid. Recrystallization from 40% aqueous acetonitrile afforded 6.6 g (65%) as crystals (yellow plates); mp 144–146 °C; MS(APCI$^+$) *m/z* 276 (M+1); ^{1}H NMR (d_6-DMSO) δ 12.60 (s, 1H), 9.49 (s, 1H), 7.33–7.41 (m, 2H), 7.17–7.26 (m, 2H), 4.26 (q, J = 7.09 Hz, 2H), 2.14 (s, 3H), 1.27 (t, J = 7.15 Hz, 3H).

5.15. 1-Ethyl-4-(4-fluorophenyl)-5-formyl-3-methyl-1*H*-pyrrole-2 carboxylic acid ethyl ester (20)

To a solution of **19** (6.6 g, 24 mmol) in 250 mL of acetonitrile was added cesium carbonate (12 g, 36 mmol), followed by 7.5 mL of ethyl iodide (15 g, 94 mmol). The reaction mixture was stirred at 24 °C for 21 h and then the mixture was filtered to remove insoluble material, which was washed several times with acetonitrile. The filtrate was evaporated to give a residue, which was dissolved in 250 mL of ethyl acetate. The organic layer was washed with brine (2× 250 mL), dried (sodium sulfate), filtered, and then the filtrate was evaporated to afford a residue. Purification by flash chromatography (silica gel, 10% ethyl acetate in hexane) gave 6.3 g (87%) of a viscous oil, which rapidly solidified into a solid; mp 69–71 °C; MS(APCI$^+$) *m/z* 304 (M+1); ^{1}H NMR

(d_6-DMSO) δ 9.37 (s, 1H), 7.32–7.39 (m, 2H), 7.21–7.28 (m, 2H), 4.62 (q, J = 6.90 Hz, 2H), 4.29 (q, J = 7.08 Hz, 2H), 2.06 (s, 3H), 1.22–1.32 (m, 6H).

5.16. 1-Ethyl-4-(4-fluorophenyl)-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid (21)

To a solution of **20** (7.0 g, 23 mmol) in 182 mL of 70% aqueous tetrahydrofuran was added lithium hydroxide monohydrate (5.3 g, 127 mmol). The reaction mixture was heated at reflux for 20 h and then added to 500 mL of water. The mixture was acidified with 1 N HCl to pH 2 to form a precipitate, which was triturated at 24 °C for 30 min. The mixture was filtered to collect a solid and then rinsed with 500 mL of water. The material was dried to provide 6.3 g (99%) of a yellow solid; mp 116–118 °C; MS(APCI$^+$) m/z 276 (M+1); ^{1}H NMR (d_6-DMSO) δ 12.38 (s, 1H), 9.36 (s, 1H), 7.32–7.38 (m, 2H), 7.20–7.28 (m, 2H), 4.66 (q, J = 6.94 Hz, 2H), 2.06 (s, 3H), 1.24 (t, J = 6.87 Hz, 3H).

5.17. 1-Ethyl-4-(4-fluorophenyl)-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid amide (23a)

To a mixture of **21** (3.2 g, 12 mmol) in 125 mL of dichloromethane were added 4 drops of *N*,*N*-dimethylformamide (cat), followed by 1.5 mL of oxalyl chloride (2.2 g, 17 mmol). The reaction mixture was stirred at 24 °C for 18 h and then evaporated to afford 3.4 g (100%) of a crude green oil as **22** 1-ethyl-4-(4-fluorophenyl)-5-formyl-3-methyl-1*H*-pyrrol-2-carbonyl chloride. To 55 mL of 80% aqueous ethyl acetate was added 6.4 mL of a 2 M solution of ammonia (0.22 g, 13 mmol) in methanol, followed by sodium carbonate (1.8 g, 17 mmol). The two-phase reaction mixture was cooled to −5 °C and then a solution of **22** (3.4 g, 12 mmol) in 11 mL of ethyl acetate was added. The reaction mixture was vigorously stirred at −5 °C for 3 h and then at room temperature for 3 days. The reaction mixture was diluted with 300 mL of ethyl acetate and then washed with 1 N hydrochloric acid (3× 50 mL), followed by saturated sodium bicarbonate (2× 50 mL) and brine (50 mL). The organic layer was dried (sodium sulfate), filtered, and then the filtrate was evaporated to provide a crude yellow-brown solid. Purification by flash chromatography (silica gel, 60% ethyl acetate in hexane) gave 1.78 g (56%) as a yellow solid; mp 181–182 °C; MS(APCI$^+$) m/z 275 (M+1); ^{1}H NMR (d_6-DMSO) δ 9.32 (s, 1H), 7.92 (d, J = 39.96 Hz, 2H), 7.40–7.32 (m, 2H), 7.31–7.22 (m, 2H), 4.42 (q, J = 7.16 Hz, 2H), 1.98 (s, 3H), 1.25 (t, J = 7.08 Hz, 3H).

5.18. 1-Ethyl-4-(4-fluorophenyl)-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid phenylamide (23b)

To a cold (0 °C) solution of aniline (2.2 g, 24 mmol) and *N*,*N*-diisopropylethylamine ('Hunig's base') in 125 mL of dichloromethane was added a solution of **22** (6.0 g, 20 mmol) in 125 mL of dichloromethane. The reaction mixture was stirred as the ice bath slowly melted for 22 h. The reaction mixture was diluted with 125 mL of dichloromethane and then the organic layer was washed with 2.0 N hydrochloric acid (3× 300 mL), followed by 5% aqueous sodium bicarbonate (300 mL) and brine (300 mL). The organic layer was dried (sodium sulfate), filtered, and then the filtrate was evaporated to afford a residue. Purification by flash chromatography (silica gel, 20–50% ethyl acetate in hexane) afforded 5.2 g (73%). A sample recrystallized from ethyl acetate/hexane gave crystals; mp 216–218 °C; MS(APCI$^+$) m/z 351 (M+1); ^{1}H NMR (d_6-DMSO) δ 10.63 (s, 1H); 9.35 (s, 1H), 7.72–7.64 (m, 2H), 7.44–7.22 (m, 6H), 7.12–7.05 (m, 1H), 4.40 (q, J = 7.11 Hz, 2H), 1.99 (s, 3H), 1.26 (t, J = 7.08 Hz, 3H).

5.19. 1-Ethyl-4-(4-fluorophenyl)-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid 4-methoxy-benzylamide (23c)

Compound **23c** was prepared by the procedure described for the preparation of compound **23b** using 4-methoxybenzylamine. Purification by flash chromatography (silica gel, 35% ethyl acetate in hexane) provided 3.7 g (60%) as an orange foamy solid; MS(APCI$^+$) m/z 395 (M+1); ^{1}H NMR (d_6-DMSO) δ 9.29 (s, 1H), 9.04 (t, J = 6.09 Hz, 1H), 7.37–7.29 (m, 2H), 7.28–7.18 (m, 4H), 6.89–6.80 (m, 2H), 4.39–4.29 (m, 4H), 3.68 (s, 3H), 1.91 (s, 3H), 1.19 (t, J = 7.09 Hz, 3H).

5.20. 4-({[1-Ethyl-4-(4-fluorophenyl)-5-formyl-3-methyl-1*H*-pyrrole-2-carbonyl]-amino}-methyl)-benzoic acid methyl ester (23d)

Compound **23d** was prepared by the procedure described for the preparation of compound **23b** using 4-(aminomethyl)benzoate hydrochloride. Purification by flash chromatography (silica gel, 35% ethyl acetate in hexane) gave 4.1 g (54%) as a tan solid; mp 143–145 °C; MS(APCI$^+$) m/z 423 (M+1); ^{1}H NMR (d_6-DMSO) δ 9.30 (s, 1H), 9.18 (t, J = 5.93 Hz, 1H), 7.93–7.87 (m, 2H), 7.47–7.41 (m, 2H), 7.37–7.31 (m, 2H), 7.28–7.21 (m, 2H), 4.51 (d, J = 6.00 Hz, 2H), 4.34 (q, J = 7.05 Hz, 2H), 3.79 (s, 3H), 1.94 (s, 3H), 1.19 (t, J = 7.04 Hz, 3H).

5.21. 1-Ethyl-4-(4-fluorophenyl)-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid (5-methyl-pyrazin-2-ylmethyl) amide (23e)

Compound **23e** was prepared by the procedure described for the preparation of compound **23b** using 2-(aminomethyl)-5-methylpyrazine. Purification by flash chromatography (silica gel, 60% ethyl acetate in hexane) gave 2.4 g (57%) as a tan solid; mp 91–93 °C; MS(APCI$^+$) m/z 381 (M+1); ^{1}H NMR (d_6-DMSO) δ 9.30 (s, 1H), 9.16 (t, J = 5.85 Hz, 1H), 8.48 (s, 1H), 8.44 (s, 1H), 7.39–7.20 (m, 4H), 4.55 (d, J = 5.94 Hz, 2H), 4.36 (q, J = 7.05 Hz, 2H), 2.42 (s, 3H), 1.96 (s, 3H), 1.20 (t, J = 7.04 Hz, 3H).

5.22. (*E*/*Z*)-(*R*)-3-(*Tert*-butyldimethylsilanyloxy)-7-[5-carbamoyl-1-ethyl-3-(4-fluorophenyl)-4-methyl-1*H*-pyrrol-2-yl]-5-oxo-hept-6-enoic acid methyl ester (25a)

To a solution of **23a** (1.6 g, 5.8 mmol) in 100 mL of toluene was added the wittig reagent **24** (4.6 g, 8.7 mmol). The reaction mixture was heated at reflux for 3 days

and then evaporated to give a crude red oil. Purification by flash chromatography (silica gel, 60–70% ethyl acetate in hexane) provided 1.62 g (53%) of a yellow foamy solid as a mixture of cis and trans isomers; mp 114–115 °C; MS(APCI$^+$) *m*/*z* 531 (M+1); ^{1}H NMR (d_6-DMSO) δ 7.75, 7.47, 7.42–7.27, 6.10, 4.58–4.59, 4.41, 3.66, 2.76, 2.54, 2.04, 1.35, 0.83, 0.06, 0.00.

5.23. (*E*/*Z*)-(*R*)-3-(*Tert*-butyldimethylsilanyloxy)-7-[1-ethyl-3-(4-fluorophenyl)-5-(4-methoxybenzyl-carbamoyl)-4-methyl-1*H*-pyrrol-2-yl]-5-oxo-hept-5-enoic acid methyl ester (25c)

Compound **25c** was prepared from **23c** and **24** (2 equiv) by the procedure described for the preparation of **25a** to afford 3.95 g (67%) of a red tacky solid as a mixture of cis and trans isomers; MS(APCI$^+$) *m*/*z* 651 (M+1); ^{1}H NMR (d_6-DMSO) δ 8.89, 7.46, 7.42–7.27, 7.03–6.95, 6.10, 4.58–4.45, 4.35, 3.82, 3.66, 2.76, 2.54, 2.00, 1.33, 0.83, 0.06, 0.00.

5.24. (*E*/*Z*)-(*R*)-7-[5-Carbamoyl-1-ethyl-3-(4-fluoro-phenyl)-4-methyl-1*H*-pyrrol-2-yl]-3-hydroxy-5-oxo-hept-6-enoic acid methyl ester (26a)

To a solution of **25a** (2.1 g, 4.0 mmol) in 38 mL of anhydrous tetrahydrofuran was added 5.2 mL of a 70% solution of hydrofluoric acid in pyridine (5.6 g, 195 mmol). The reaction mixture was stirred in a plastic vessel at room temperature for 4 h and then the mixture was quenched with solid sodium bicarbonate to pH 8. The mixture was extracted with dichloromethane (100 mL) and then washed with brine (100 mL). The organic layer was dried (sodium sulfate), filtered, and then the filtrate was evaporated to afford a residue. Purification by flash chromatography (silica gel, 80–100% ethyl acetate in hexane, followed by 95% ethyl acetate in methanol) gave 1.08 g (65%) of a yellow solid as a mixture of cis and trans isomers; mp 150–152 °C; MS(APCI$^+$) *m*/*z* 417 (M+1); ^{1}H NMR (d_6-DMSO) δ 7.60, 7.32, 7.28–6.99, 5.97, 4.84, 4.26, 4.21–4.12, 3.52, 2.64–2.48, 2.42–2.22, 1.90, 1.22.

5.25. (*E*)-(*R*)-7-[1-Ethyl-3-(4-fluorophenyl)-4-methyl-5-phenylcarbamoyl-1*H*-pyrrol-2-yl]-3-hydroxy-5-oxo-hept-6-enoic acid methyl ester (26b)

To a solution of **23b** (2.5 g, 7.1 mmol) in 100 mL of toluene was added the Wittig reagent **24** (7.6 g, 14 mmol). The reaction mixture was heated at reflux for 2 days and then evaporated to give a crude orange oil. Purification by flash chromatography (silica gel, 20% ethyl acetate in hexane) provided 2.9 g (67%) of a mixture of cis and trans isomers as intermediate **25b** (*E*/*Z*)-(*R*)-3-(*tert*-butyldimethylsilanyloxy)-7-[1-ethyl-3-(4-fluorophenyl)-4-methyl-5-phenylcarbamoyl-1H-pyrrol-2-yl]-5-oxo-hept-6-enoic acid methyl ester. To a cold (5 °C) solution of **25b** (2.9 g, 4.8 mmol) in 50 mL of acetonitrile was added a solution of 1.1 mL of 48% aqueous hydrofluoric acid (1.3 g, 30 mmol) in 12 mL of acetonitrile. The reaction mixture was stirred as it warmed to room temperature for 1.5 h. The reaction mixture was added to 300 mL of ice cold 5% aqueous sodium bicarbonate and then extracted with ethyl acetate (4× 100 mL). The combined organic layers were washed with ice cold 5% sodium bicarbonate (3× 300 mL) and brine (300 mL). The organic layer was dried (sodium sulfate), filtered, and evaporated to afford a residue. Purification by flash chromatography (silica gel, 40–75% ethyl acetate in hexane) provided 1.75 g (74%) of an orange solid as the trans isomer; mp 139–141; MS(APCI$^+$) *m*/*z* 493 (M+1); ^{1}H NMR (d_6-DMSO) δ 10.33 (s, 1H), 7.71–7.62 (m, 2H), 7.41–7.18 (m, 7H), 7.09–6.99 (m, 1H), 6.02 (d, J = 16.18 Hz, 1H), 4.86 (d, J = 5.82 Hz, 1H), 4.27 (q, J = 7.23 Hz, 2H), 4.23–4.14 (m, 1H), 3.52 (s, 3H), 2.67–2.51 (m, 2H), 2.43–2.24 (m, 2H), 1.94 (s, 3H), 1.26 (t, J = 7.02 Hz, 3H).

5.26. (*E*/*Z*)-(*R*)-7-[1-Ethyl-3-(4-fluorophenyl)-5-(4-methoxybenzylcarbamoyl)-4-methyl-1*H*-pyrrol-2-yl]-3-hydroxy-5-oxo-hept-6-enoic acid methyl ester (26c)

Compound **26c** was prepared from **25c** by the procedure described for the preparation of **26a**. Purification by flash chromatography (silica gel, 60% ethyl acetate in hexane) afforded 3.07 g (68%) of a red tacky solid as a mixture of cis and trans isomers; MS(APCI$^+$) *m*/*z* 537 (M+1); ^{1}H NMR (d_6-DMSO) δ 8.73, 7.38–6.99, 6.92–6.79, 5.97, 4.84, 4.34, 4.27–4.12, 3.68, 3.52, 2.64–2.23, 1.86, 1.20.

5.27. 4-({[1-Ethyl-4-(4-fluorophenyl)-5-((*E*/*Z*)-(*R*)-5-hydroxy-6-methoxycarbonyl-3-oxo-hex-1-enyl)-3-methyl-1H-pyrrole-2-carbonyl]-amino}-methyl)-benzoic acid methyl ester (26d)

Compound **26d** was prepared from **23d** and **24** by the procedure described for the preparation of **26b**. Purification by flash chromatography (silica gel, 50–65% ethyl acetate in hexane) afforded 3.4 g (70%) of a red-orange foam as a mixture of cis and trans isomers; MS(APCI$^+$) *m*/*z* 565 (M+1); ^{1}H NMR (d_6-DMSO) δ 8.85, 7.94–7.85, 7.47–6.98, 5.97, 4.84, 4.49, 4.27–4.12, 3.79, 3.27, 2.64–2.22, 1.90, 1.20.

5.28. (*E*/*Z*)-(*R*)-7-{1-ethyl-3-(4-fluorophenyl)-4-methyl-5-[(5-methylpyrazin-2-ylmethyl)-carbamoyl]-1*H*-pyrrol-2-yl}-3-hydroxy-5-oxo-hept-6-enoic acid methylester (26e)

Compound **26e** was prepared from **23e** and **24** by the procedure described for the preparation of **26b** using a solution of hydrofluoric acid in pyridine. Purification by flash chromatography (silica gel, 80% ethyl acetate in hexane) afforded 1.06 g (35%) of a yellow tacky solid as a mixture of cis and trans isomers; MS(APCI$^+$) *m*/*z* 5.23 (M+1); ^{1}H NMR (d_6-DMSO) δ 8.83, 8.49–8.41, 7.36–7.15, 5.98, 4.84, 4.53, 4.27–4.12, 3.52, 2.63–2.23, 1.92, 1.21.

5.29. (*E*/*Z*)-(3*R*,5*S*)-7-[5-Carbamoyl-1-ethyl-3-(4-fluoro-phenyl)-4-methyl-1*H*-pyrrol-2-yl]-3,5-dihydroxy-hept-6-enoic acid methyl ester (27a)

To a cold (−60 °C) solution of **26a** (0.97 g, 2.3 mmol) in 37.5 mL of 80% tetrahydrofuran in methanol was added 2.3 mL of a 1 M solution of diethylmethoxyborane (0.23 g, 1 mmol) in tetrahydrofuran. The reaction mixture was stirred for 30 min and then powdered sodium borohydride (0.09 g, 2.3 mmol) was added. The reaction

mixture was stirred for 3 h at −60 °C and then warmed to 10 °C. Glacial acetic acid (3 mL) was added and then the mixture was stirred for 18 h as it warmed to room temperature. The mixture was diluted with 300 mL of ethyl acetate and then the organic layer was washed with saturated sodium bicarbonate (3× 50 mL) and brine (100 mL). The organic layer was dried (sodium sulfate), filtered, and then the filtrate was evaporated to give an oily residue. Purification by flash chromatography (silica gel, 100–95% ethyl acetate in methanol) provided 833 mg (86%) of a yellow solid as a mixture of cis and trans isomers; mp 82–84 °C; MS(APCI$^+$) *m*/*z* 401 (M+1); ^{1}H NMR (d_6-DMSO) δ 7.23, 7.16–7.11, 6.30, 5.38, 4.75, 4.66, 4.14, 4.08–4.00, 3.82–3.66, 3.52, 2.39–2.15, 1.94, 1.52–1.05.

5.30. (*E*/*Z*)-(3*R*,5*S*)-7-[1-Ethyl-3-(4-fluorophenyl)-4-methyl-5-phenylcarbamoyl-1*H*-pyrrol-2-yl]-3,5-dihydroxy-hept-6-enoic acid methyl ester (27b)

Compound **27b** was prepared by the procedure described for the preparation of **27a**. Purification by flash chromatography (silica gel, 60–75% ethyl acetate in hexane) provided 1.50 g (88%) of an off-white foam as a mixture of cis and trans isomers; MS(APCI$^+$) *m*/*z* 495 (M+1); ^{1}H NMR (d_6-DMSO) δ 10.00, 7.70–7.61, 7.31–7.10, 7.05–6.96, 6.35, 5.45, 4.79, 4.67, 4.18–4.03, 3.81–3.70, 3.52, 2.39–2.18, 1.98, 1.53–1.13.

5.31. (*E*/*Z*)-(3*R*,5*S*)-7-[1-Ethyl-3-(4-fluorophenyl)-5-(4-methoxybenzylcarbamoyl)-4-methyl-1*H*-pyrrol-2-yl]-3,5-dihydroxy-hept-6-enoic acid methyl ester (27c)

Compound **27c** was prepared by the procedure described for the preparation of **27a**. Purification by flash chromatography (silica gel, 70% ethyl acetate in hexane) afforded 2.43 g (86%) of a yellow foam as a mixture of cis and trans isomers; mp 49–51 °C; MS(APCI$^+$) *m*/*z* 539 (M+1); ^{1}H NMR (d_6-DMSO) δ 8.35, 7.25–7.09, 6.89–6.79, 6.30, 5.39, 4.75, 4.66, 4.32, 4.15–3.99, 3.80–3.69, 3.67, 3.26, 2.39–2.15, 1.90, 1.51–1.18, 1.13.

5.32. 4-({[5-((*E*/*Z*)-(3*S*,5*R*)-3,5-Dihydroxy-6-methoxycarbonyl-hex-1-enyl)-1-ethyl-4-(4-fluorophenyl)-3-methyl-1*H*-pyrrole-2-carbonyl]-amino}-methyl)-benzoic acid methyl ester (27d)

Compound **27d** was prepared by the procedure described for the preparation of **27a**. Purification by flash chromatography (silica gel, 60–75% ethyl acetate in hexane, gradient elution) gave 2.7 g (82%) of a light yellow foam as a mixture of cis and trans isomers; MS(APCI$^+$) *m*/*z* 549 (M+1)$^+$; ^{1}H NMR (d_6-DMSO) δ 8.47, 7.91–7.85, 7.46–7.38, 7.19–7.10, 6.31, 5.39, 4.76, 4.66, 4.47, 4.17–3.98, 3.79, 3.77–3.68, 3.52, 2.39–2.16, 1.94, 1.52–1.17, 1.13.

5.33. (*E*/*Z*)-(3*R*,5*S*)-7-{1-Ethyl-3-(4-fluorophenyl)-4-methyl-5-[(5-methyl-pyrazin-2-ylmethyl)-carbamoyl]-1*H*-pyrrol-2-yl}-3,5-dihydroxy-hept-6-enoic acid methyl ester (27e)

Compound **27e** was prepared by the procedure described for the preparation of **27a**. Purification by flash chromatography (silica gel, 95% ethyl acetate in methanol) gave 755 mg (87%) of a yellow solid as a mixture of cis and trans isomers; mp 47–50 °C; MS(APCI$^+$) *m*/*z* 525 (M+1); ^{1}H NMR (d_6-DMSO) δ 8.48–8.40, 7.20–7.08, 6.31, 5.40, 4.76, 4.66, 4.50, 4.15–4.00, 3.81–3.67, 3.52, 2.42, 2.39–2.16, 1.96, 1.53–1.19, 1.14.

5.34. (3*R*,5*R*)-7-[5-Carbamoyl-1-ethyl-3-(4-fluorophenyl)-4-methyl-1*H*-pyrrol-2-yl}-3,5-dihydroxy-heptanoic acid methyl ester (28a)

To a solution of **27a** (0.74 g, 1.8 mmol) in 35 mL of 50% tetrahydrofuran in methanol was added 10% Pd/C (0.10 g, 0.09 mmol). The reaction mixture was stirred at room temperature under hydrogen (5 psi) atmosphere between 45 min and 2 h. Purification by flash chromatography (silica gel, 95% ethyl acetate in methanol) provided 423 mg (57%) as an off-white solid; mp 98–100 °C; MS(APCI$^+$) *m*/*z* 421 (M+1); ^{1}H NMR (d_6-DMSO) δ 7.18–7.12(m, 4H), 7.08 (s, 2H), 4.70 (d, J = 5.7 Hz, 1H), 4.52 (d, J = 5.11 Hz, 1H), 4.11 (q, J = 7.17 Hz, 2H), 3.93–3.82 (m, 1H), 3.54–3.43 (m, 4H), 2.66–2.13 (m, 4H), 1.96 (s, 3H), 1.53–1.26 (m, 4H), 1.14 (t, J = 7.03 Hz, 3H).

5.35. (3*R*,5*R*)-7-[1-Ethyl-3-(4-fluorophenyl)-4-methyl-5-phenylcarbamoyl-1*H*-pyrrol-2-yl]-3,5-dihydroxy-heptanoic acid methyl ester (28b)

Compound **28b** was prepared by the procedure described for the preparation of **28a**. Purification by flash chromatography (silica gel, 50–75% ethyl acetate in hexane, gradient elution) provided 580 mg (58%) as a white foam; MS(APCI$^+$) *m*/*z* 497 (M+1); ^{1}H NMR (d_6-DMSO) δ 9.88 (s, 1H), 7.66–7.62 (m, 2H). 7.31–7.12 (m, 6H), 7.04–6.95 (m, 1H), 4.70 (d, J = 5.21, 1H), 4.54 (d, J = 5.03 Hz, 1H), 4.10 (q, J = 7.12 Hz, 2H), 3.94–3.83 (m, 1H), 3.55–3.44 (m, 4H), 2.68–2.54 (m, 1H), 2.49–2.15 (m, 3H), 2.00 (s, 3H), 1.57–1.28 (m, 4H), 1.19 (t, J = 6.94 Hz, 3H).

5.36. (3*R*,5*R*)-7-(1-Ethyl-3-(4-fluorophenyl)-5-(4-methoxybenzylcarbamoyl)-4-methyl-1*H*-pyrrol-2-yl]-3,5-dihydroxy-heptanoic acid methyl ester (28c)

Compound **28c** was prepared by the procedure described for the preparation of **28a**. Purification by flash chromatography (silica gel, 80% ethyl acetate in hexane) afforded 673 mg (67%) as a white foam; MS(APCI$^+$) *m*/*z* 541 (M+1); ^{1}H NMR (d_6-DMSO) δ 8.19 (t, J = 6.02 Hz, 1H), 7.24–7.09 (m, 6H), 6.87-6.79 (m, 2H), 4.69 (d, J = 5.14 Hz, 1H), 4.52 (d, J = 5.13 Hz, 1H), 4.31 (d, J = 6.03 Hz, 2H), 4.06 (q, J = 7.16 Hz, 2H), 3.93–3.82 (m, 1H), 3.67 (s, 3H), 3.54–3.43 (m, 4H), 2.64–2.14 (m, 4H), 1.93 (s, 3H), 1.54–1.27 (m, 4H), 1.11 (t, J = 7.01 Hz, 3H).

5.37. 4-({[5-((3*R*,5*R*)-3,5-Dihydroxy-6-methoxycarbonyl-hexyl)-1-ethyl-4-(4-fluorophenyl)-3-methyl-1*H*-pyrrole-2-carbonyl]-amino}-methyl)-benzoic acid methyl ester (28d)

Compound **28d** was prepared by the procedure described for the preparation of **28a**. Purification by flash

chromatography (silica gel, 75–80% ethyl acetate in hexane, gradient elution) gave 930 mg (48%) as a white foam; MS(APCI$^+$) *m/z* 569 (M+1); ^{1}H NMR (d_6-DMSO) δ 8.32 (t, J = 6.01 Hz, 1H), 7.93–7.84 (m, 2H), 7.47–7.38 (m, 2H), 7.20–7.10 (m, 4H), 4.69 (d, J = 5.20 Hz, 1H), 4.52 (d, J = 5.11 Hz, 1H), 4.46 (d, J = 6.11 Hz, 2H), 4.07 (q, J = 7.21 Hz, 2H), 3.93–3.83 (m, 1H), 3.79 (s, 3H), 3.53–3.44 (m, 4H), 2.66–2.13 (m, 4H), 1.97 (s, 3H), 1.55–1.26 (m, 4H), 1.10 (t, J = 6.94 Hz, 3H).

5.38. (3*R*,5*R*)-7-{1-Ethyl-3-(4-fluorophenyl)-4-methyl-5-[(5-methyl-pyrazin-2-ylmethyl)-carbamoyl]-1*H*-pyrrol-2-yl}-3,5-dihydroxy-heptanoic acid methyl ester (28e)

Compound **28e** was prepared by the procedure described for the preparation of **28a**. Purification by flash chromatography (silica gel, 95% ethyl acetate in methanol) afforded 261 mg (61%) of an off-white tacky solid; MS(APCI$^+$) *m/z* 527 (M+1); ^{1}H NMR (d_6-DMSO) δ 8.44 (s, 1H), 8.41 (s, 1H), 8.30 (t, J = 5.90 Hz, 1H), 7.19–7.11 (m, 4H), 4.70 (d, J = 5.20 Hz, 1H), 4.52 (d, J = 5.08 Hz, 1H), 4.49 (d, J = 5.83 Hz, 2H), 4.07 (q, J = 7.25 Hz, 2H), 3.93–3.82 (m, 1H), 3.54–3.43 (m, 4H), 2.64–3.13 (m, 7H), 1.99 (s, 3H), 1.55–1.27 (m, 4H), 1.11 (t, J = 6.97 Hz, 3H).

5.39. (3*R*,5*R*)-7-[5-Carbamoyl-1-ethyl-3-(4-fluorophenyl)-4-methyl-1*H*-pyrrol-2-yl]-3,5-dihydroxy-heptanoic acid sodium salt (29a)

To a solution of **28a** (0.36 g, 0.85 mmol) in 10 mL of methanol was added 0.91 mL of a 1.028 N aqueous solution of sodium hydroxide (0.04 g, 0.94 mmol). The reaction mixture was stirred at room temperature for 3 h and then evaporated in vacuo to give a semi-solid residue. Purification by triturating in 50 mL of diethyl ether gave 326 mg (89%) as a white solid; mp 150 °C (dec); MS(APCI$^-$) *m/z* 405 (M−Na); ^{1}H NMR (d_6-DMSO) δ 7.53 (s, 1H), 7.19–7.11 (m, 4H), 7.08 (s, 2H), 4.70 (s, 1H), 4.10 (q, J = 6.96 Hz, 2H), 3.69–3.59 (m, 1H), 3.54–3.43 (m, 1H), 2.63–2.30 (m, 2H), 2.01–1.65 (m, 5H), 1.51-0.99 (m, 7H).

5.40. (3*R*,5*R*)-7-[1-Ethyl-3-(4-fluorophenyl)-4-methyl-5-phenylcarbamoyl-1*H*-pyrrol-2-yl]-3,5-dihydroxy-heptanoic acid sodium salt (29b)

To a solution of **28b** (0.53 g, 1.1 mmol) in 18 mL of 66% aqueous absolute ethanol was added 1.04 mL of a 1.028 N aqueous solution of sodium hydroxide (0.04 g, 1.1 mmol). The reaction mixture was stirred at room temperature for 2 h and then evaporated in vacuo to give a semi-solid residue. The residue was suspended in acetone and evaporated again three times. Purification by triturating from 75 mL of diethyl ether gave 470 mg (87%) as a white solid; mp 198–200 °C; MS(APCI$^-$) *m/z* 481 (M−Na); ^{1}H NMR (d_6-DMSO) δ 9.92 (s, 1H), 7.72–7.33 (m, 3H), 7.31–7.08 (m, 6H), 7.05–6.92 (m, 1H), 4.72 (s, 1H), 4.18–3.99 (m, 2H), 3.71–3.57 (m, 1H), 3.56–3.43 (m, 1H), 2.71–2.23 (m, 2H), 2.09–1.64 (m, 5H), 1.54–0.95 (m, 7H).

5.41. (3*R*,5*R*)-7-[1-Ethyl-3-(4-fluorophenyl)-5-(4-methoxybenzylcarbamoyl)-4-methyl-1*H*-pyrrol-2-yl]-3,5-dihydroxy-heptanoic acid sodium salt (29c)

Compound **29c** was prepared by the procedure described for the preparation of **29a** to provide 544 mg (95%) as a white solid; MS(APCI$^-$) *m/z* 527 (M−Na); ^{1}H NMR (d_6-DMSO) δ 8.22 (t, J = 6.02 Hz, 1H), 7.57 (s, 1H), 7.24–7.10 (m, 6H), 6.86–6.78 (m, 2H), 4.70 (s, 1H), 4.31 (d, J = 6.10 Hz, 2H), 4.06 (q, J = 7.08 Hz, 2H), 3.70–3.58 (m, 4H), 3.53–3.43 (m, 1H), 2.64–2.31 (m, 2H), 1.96–1.65 (m, 5H), 1.50–1.06 (m, 7H).

5.42. (3*R*,5*R*)-7-[1-Ethyl-3-(4-fluorophenyl)-5-(4-methoxycarbonyl-benzylcarbamoyl)-4-methyl-1*H*-pyrrol-2-yl]-3,5-dihydroxy-heptanoic acid sodium salt (29d)

Compound **29d** was prepared by the procedure described for the preparation of **29b** to provide 190 mg (62%) as a white solid; MS(APCI$^-$) *m/z* 553 (M−Na); ^{1}H NMR (d_6-DMSO) δ 8.35 (t, J = 5.95 Hz, 1H), 7.91–7.83 (m, 2H), 7.59 (br s, 1H), 7.45–7.38 (m, 2H), 7.20–7.10 (m, 4H), 4.70 (br s, 1H), 4.45 (d, J = 5.99 Hz, 2H), 4.06 (q, J = 7.00 Hz, 2H), 3.80 (s, 3H), 3.69–3.58 (m, 1H), 3.53–3.42 (m, 1H), 2.64–2.32 (m, 2H), 2.00–1.65 (m, 5H), 1.51–1.06 (m, 7H).

5.43. (3*R*,5*R*)-7-{1-Ethyl-3-(4-fluorophenyl)-4-methyl-5-[(5-methyl-pyrazin-2-ylmethyl)-carbamoyl]-1*H*-pyrrol-2-yl}-3,5-dihydroxy-heptanoic acid sodium salt (29e)

Compound **29e** was prepared by the procedure described for the preparation of **29a** to provide 189 mg (90%) as an off-white solid; MS(APCI$^-$) *m/z* 511 (M−Na); ^{1}H NMR (d_6-DMSO) δ 8.44 (s, 1H), 8.41 (s, 1H), 8.34 (t, J = 5.87 Hz, 1H), 7.60 (s, 1H), 7.19–7.11 (m, 4H), 4.71 (s, 1H), 4.48 (d, J = 5.58 Hz, 2H), 4.06 (q, J = 7.05 Hz, 2H), 3.68–3.57 (m, 1H), 3.53–3.43 (m, 1H), 2.66–2.30 (m, 5H), 1.98 (s, 3H), 1.94–1.65 (m, 2H), 1.52–1.06 (m, 7H).

5.44. (3*R*,5*R*)-7-[5-(4-carboxy-benzylcarbamoyl)-ethyl-3-(4-fluorophenyl)-4-methyl-1*H*-pyrrol-2-yl]-3,5-dihydroxy-heptanoic acid disodium salt (29f)

To a solution of **28d** (0.60 g, 1.1 mmol) in a mixture of 10 mL of methanol and 2 mL of water was added 5.2 mL of a 1.028 M aqueous solution of sodium hydroxide (0.21 g, 5.3 mmol). The reaction mixture was stirred at reflux for 2 h and then evaporated to give a semi-solid residue, which was suspended in 100 mL of 20% methanol in dichloromethane. The mixture was filtered, the filtrate evaporated, and the final residue was triturated in 75 mL of diethyl ether for 18 h. The solid was filtered and washed several times with fresh diethyl ether to provide, after drying, 350 mg (57%); mp >280 °C; MS(APCI$^-$) *m/z* 538 (M−Na+1); ^{1}H NMR (d_6-DMSO) δ 8.26 (t, J = 6.04 Hz, 1H), 7.79–7.71 (m, 2H), 7.44 (s, 1H), 7.22–7.10 (m, 6H), 4.69 (s, 1H), 4.38 (d, J = 5.71 Hz, 2H), 4.07 (q, J = 6.94 Hz, 2H), 3.70–3.59 (m, 1H), 3.54–3.43 (m, 1H), 2.65–2.32 (m, 2H), 2.02–1.66 (m, 5H), 1.52–1.07 (m, 7H).

[3-14C]-HMGCoA (57.0 mCi/mmol) was purchased from Amersham Biosciences, UK. HMGCoA, mevalonolactone, Beta-NADPH (Beta-Nicotinamide Adenine Dinucleotide Phosphate, Reduced form) were purchased from Sigma Chemical Co. AG 1-8X resin was purchased from Bio-Rad Laboratory.

5.45. Isolation of rat liver microsomes

Male Charles River Sprague–Dawley rats were fed with 2.5% cholestyramine in rat chow diets for 7 days before sacrificing. Livers were minced and homogenized in a sucrose homogenizing solution in an ice bath 10 times. Homogenates were diluted into a final volume of 200 mL and centrifuged for 15 min. with a Sorvall Centrifuge at 5 °C, 10,000 rpm (12,000*g*). The upper fat layer was removed and the supernatant decanted into fresh tubes. This step was repeated one more time before transferring the supernatant into ultracentrifuge tubes and centrifuged at 36,000 rpm (105,000*g*) for an hour at 5 °C. The resulting supernatant was discarded and the pellet was added to a total of 15 mL of 0.1 M KH_2PO_4. Pellets were homogenized gently by hand about 10 times. Samples were pooled and diluted into a total of 60 mL buffer. The protein concentration of the homogenate was determined by the Lowry Method using a BCA (Bicinchoninic acid) kit from Pierce Chemical Company. Aliquots (1 mL) of microsomes were kept frozen in liquid nitrogen.

5.46. HMG-CoA reductase assay (in vitro) procedure

DMSO (1 μL) or 1 μL of DMSO containing a test compound at a concentration of between 0.1 and 1 nM was placed into each well of a Corning 96-well plate. A volume of 34 μL buffer [100 mM NaH_2PO_4, 10 mM imidazole, and 10 mM EDTA, (ethylenediamine-tetraacetic acid)] containing 35 μg/mL rat liver microsomes was added into each well. After incubation for 30 min on ice, 15 μL of ^{14}C-HMGCoA (0.024 μCi) with 15 mM NADPH, 25 mM DTT, (dithiothreitol) was added and incubated at 37 °C for an additional 45 min. The reaction was terminated by the addition of 10 μL of 2 M HCl, followed by the addition of 5 μL of 0.10 M mevalonolactone. Plates were incubated at room temperature for 60 min to allow lactonization of mevalonate to mevalonolactone. The incubated samples were applied to columns containing 300 μL of AGI-X8 anion exchange resin in a Corning filter plate. The eluates were collected into Corning 96-well capture plates. Scintillation cocktail (Ultima-Flo-M) was added into each well and plates counted on a Trilux Microbeta Counter. The IC_{50} values were calculated with GraphPad software (Prism).

5.47. Procedure for sterol biosynthesis (cell assay) in rat hepatocytes, cell culture, compound treatment, cell labeling, cholesterol extraction, and data analysis

Frozen rat hepatocyctes purchased from Xeno Tech were seeded on a 6-well collagen 1 coated plates at a density of 105 cells/per well. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco, No. 11054-020) containing 10% fetal bovine serum (FBS) and 10 mM Hepes (*N*-2-hydroxyethyl-piperazie-N^1-2-ethane sulfonic acid) (Gibco No. 15630-080) for 24 h. The cells were pre-incubated with compounds for 4 h and then labeled by incubating in medium containing 1 μCi/mL of ^{14}C acetic acid for an additional 4 h. After labeling, the cells were washed twice with 5 nM MOPS (3-[*N*-morpholino]propane sulfonic acid) solution containing 150 mM NaCl and 1 nM EDTA, and collected in the lysis buffer containing 10% KOH and 80% ethanol by volume. In order to separate labeled cholesterol from labeled non-cholesterol lipids, the cells lysate were subjected to saponification at 60 °C for 2 h. The lysates were then combined with 0.5 volume of H_2O and 2 volumes of hexane, followed by 30 min of vigorous shaking. After the separation of the two phases, the upper-phase solution was collected and combined with 5 volumes of scintillation cocktail. The amount of ^{14}C cholesterol was quantified by liquid scintillation counting. The IC_{50} values were calculated with GraphPad software (Prism 3.03).

5.48. Procedure for sterol biosynthesis (cell assay) in L6 rat myoblasts, cell culture, compound treatment, cell labeling, cholesterol extraction, and data analysis

L6 rat myoblasts purchased from ATCC (CRL-1458) were grown in T-150 vented culture flasks and seeded on 12-well culture plates at a density of 60,000 cells per well. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat inactivated fetal bovine serum (FBS) for 72 h until reaching confluence. The cells were pre-incubated in media with compound and 0.20% DMSO (dimethylsulfoxide) for 3 h and then labeled by incubating in medium containing compound, 0.20% DMSO, and 1 μCi/mL of ^{14}C acetic acid for an additional 3 h. After labeling, the cells were washed once with 1× PBS and then lysed overnight at 4 °C in buffer containing 10% KOH and 78% ethanol by volume. Lipid ester bonds were hydrolyzed by saponification of the lysates at 60 °C for 2 h. Sterols (including cholesterol) were extracted from saponified lysates by combining with 3 volumes of hexane and mixing by pipette 6 times. The upper organic phase solution was collected and combined with an equal volume of 1 N KOH in 50% methanol and mixed by pipette 6 times. The upper organic phase was collected in a scintilant-coated plate and hexanes were removed by evaporation at room temperature for 3 h. The amount of ^{14}C cholesterol was quantified by scintillation counting in a Triflux 1450 plate reader (Wallac). The IC_{50} values were calculated from % inhibitions relative to negative controls versus compound concentration on Microsoft excel 2000 data analysis wizard using a sigmoid inhibition curve model with formula: $y = B_{max}(1 - (X^n/K^n + X^n)) + Y_2$, where K is the IC_{50} for the inhibition curve, X is inhibitor concentration, Y is the response being inhibited, and $B_{max} + Y_2$ is the limiting response as X approaches zero.

5.49. Procedure for cholesterol synthesis inhibition effects of the test compounds in mice

5.49.1. In vivo study procedure. Male C57/BL6 mice 8 weeks old obtained from Charles River Laboratories

were randomly placed into nine groups with 10 mice per group and acclimated for a minimum of 7 days to a reversed 12 h light (6:00 a.m. to 6:00 p.m.), 12 h dark (6:00 p.m. to 6:00 a.m.) since natural peak cholesterol synthesis is mid-diurnal during the dark period in mice. On the test day, a single dose of the test compounds or vehicle (control group) was administered by oral gavage 2 h prior to the middle of the dark period. A single intraperitoneal injection of 25 μCi ^{14}C sodium acetate (Amersham Biosciences UK Limited) was administered 30 min after drug administration. At 4 h post ^{14}C sodium acetate administration, mice were euthanized by CO_2 asphyxiation and exsanguinated by cardiac puncture for plasma collection. Individual animal plasma samples were acquired by placing 0.8 mL of whole blood into centrifuge tubes containing 20 μL EDTA and centrifuged. Plasma was then transferred into new tubes and stored at −20 °C until assayed for ^{14}C labeled cholesterol.

5.49.2. Drug preparation. Test compounds were prepared in vehicle (1.5% carboxymethylcellulose, 0.15% Tween 20 in water) by polytron and vortex mixing and orally administered using a dose volume of 0.1 mL/10 g body weight.

5.49.3. Assay for plasma ^{14}C labeled cholesterol. Frozen plasma samples (0.4 mL) were allowed to thaw, and an equal volume of physiological saline was added to each sample along with 0.025 μCi ^{3}H-cholesterol (Perkin-Elmer Life Sciences Inc., Boston, Mass). Freshly prepared 10% KOH solution was then added at 2.5 mL per sample. Samples were then vortexed and saponified at 75 °C for 1 h. After the samples cooled, saponified ^{14}C labeled synthesized cholesterol was extracted by adding 2.5 mL petroleum ether (Mallinckrodt No. 4976 Petroleum Ether) per sample, shaken for 10 min, followed by centrifuging for 10 min at 0 °C. The organic phase (1.4 mL) was transferred to new tubes and 5 mL of the scintillation cocktail (Beckman–Coulter, Ready Gel, liquid scintillation cocktail) was added. Each sample was then vortexed. Samples were allowed to sit overnight to minimize chemoluminescence before being placed in a scintillation counter and counted until statistical same counts for deteriorations per minute (DPMS) for ^{3}H and ^{14}C were achieved. The percent recovery of the ^{3}H-cholesterol in each sample was used to mathematically correct the ^{14}C counts for any differences in recovery.

5.49.4. Data analysis. Effects of the test samples on percent inhibition of cholesterol synthesis measured in plasma were determined by comparing the amount of ^{14}C sodium acetate incorporated into ^{14}C labeled cholesterol in the plasma of compound treated mice versus the amount in vehicle treated mice.

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References and notes

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