New Analogs of the Pyripyropene Family of ACAT Inhibitors via α-Pyrone Fragmentation and γ-Acylation/Cyclization

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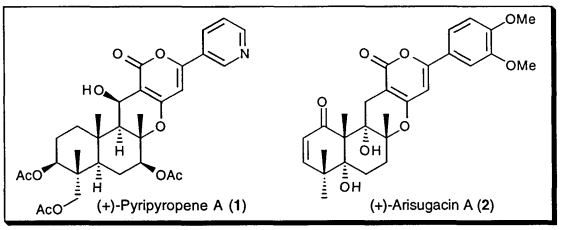
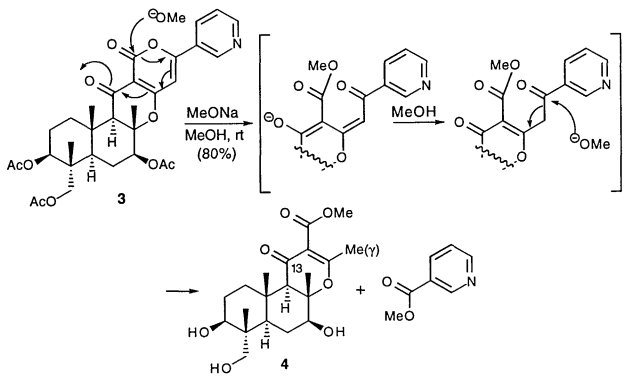
The pyridine moiety of pyripyropene A was replaced with other aromatic and heteroaromatic substituents via α-pyrone degradation followed by dienolate γ-acylation and in situ cyclization.

Inhibition of acyl-CoA:cholesterol acyltransferase (ACAT), the enzyme responsible for intracellular esterification of cholesterol, offers a promising new approach to the prevention and treatment of atherosclerosis and hypercholesterolemia.[1] Since 1993 we have reported the isolation, characterization, and initial biological evaluation of pyripyropenes A-L.[2] These novel polyketide-terpenoid metabolites rank as the most potent naturally occurring ACAT inhibitors in vitro,[3] with IC_{50} values as low as 58 nM,[2a] and also display oral bioavailability in hamsters.[2a] In addition to a total synthesis of (+)-pyripyropene A (**1**)[4a] and a biomimetic construction of (+)-pyripyropene E,[4b,c] we have prepared a number of analogs for analysis of structure-activity relationships.[5] Herein we describe the introduction of alternative aryl and heteroaryl substituents via degradative cleavage of the α-pyrone moiety followed by dienolate γ-acylation with aroyl chlorides and in situ cyclization. The acylation/cyclization protocol may also prove applicable to total synthesis of related structures such as (+)-arisugacin A (**2**),[6] an effective inhibitor of acetylcholinesterase.



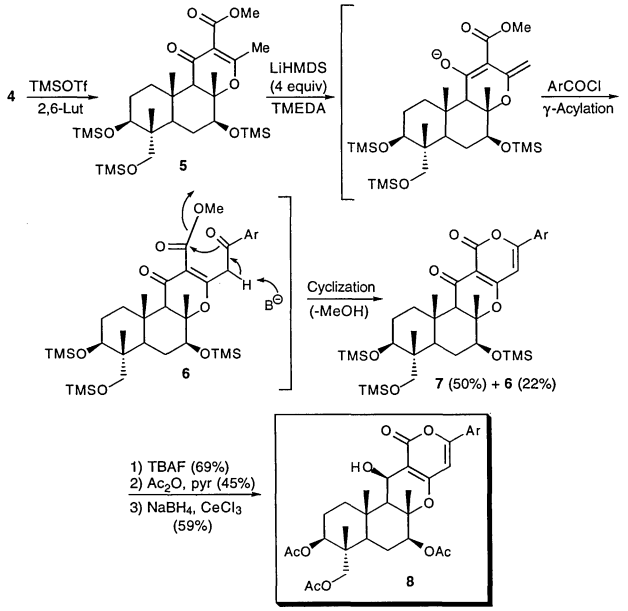
We recently discovered[7] that the α-pyrone moiety in the 13-oxo pyripyropene derivative **3**[5b,c] readily fragments upon exposure to MeONa in 70% aqueous MeOH, furnishing **4**[8] in 80% yield and the by-product methyl nicotinate (Scheme 1). Methanolysis of **1** under the same conditions gave only the corresponding tetraol[5a] (100% yield), indicating that the conjugated C(13) carbonyl group is required for cleavage.[9]

We envisioned reconstruction of the 6-substituted α-pyrone moiety via γ-acylation of **4** with an aroyl chloride and in situ cyclization, a tactic introduced by Parker and Resnick in their synthesis of racemic pyripyropene E.[4c] To this end, the hydroxyl groups of **4** were silylated to give **5**[10] [trimethylsilyl triflate (4.5 equiv), 2,6-lutidine (6 equiv), CH_2Cl_2, rt, 20 min, 90%]. We next devised an improved protocol for the requisite γ-acylation.[11] The dienolate of **5** was generated with LiHMDS (4 equiv) and TMEDA (1.2 equiv) in THF (0 °C, 0.5 h; rt, 1.5 h) and treated



Scheme I.

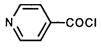
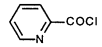
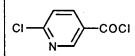
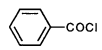
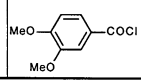
with isonicotinoyl chloride (2.5 equiv, 0 °C); LDA and ethyl nicotinate were employed previously.[4c] Intermediate **6** was immediately detected by TLC. Continued stirring at 0 °C for 1.5 h furnished the desired α-pyrone **7**[12] (50% yield) and uncyclized **6** (22%); longer reaction times did not improve the yield of **7**. Desilylation (TBAF, THF),[13] acetylation (Ac_2O, pyridine),[13] and stereocontrolled ketone reduction ($NaBH_4$, $CeCl_3$, MeOH)[13] afforded **8**, the 4-pyridyl analog of pyripyropene A.



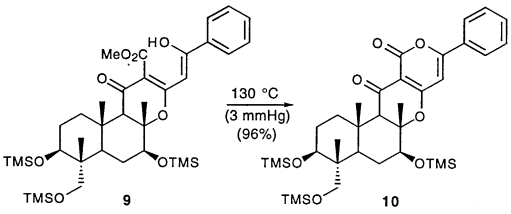
Scheme II.

As illustrated in Table 1, other aroyl chlorides[14] were utilized to prepare precursors of additional analogs. Pyridine-substituted analogs (entries 1-3) were routinely obtained in 50-

Table 1. γ-Acylation/cyclization reactions of pyripyropene analog **4**

Entry	Substrate (ArCOCl)	(equiv)	LiHMDS (equiv)	TMEDA (equiv)	Conditions	Yield (%) 7	6
1		2.5	4.0	1.2	0 °C, 1.5 h	50	22
2		3.0	5.0	1.2	0 °C, 3 h	65	
3		3.0	6.0	1.2	0 °C, 0.5 h; rt, 3 h	52	
4		3.0	2.5	1.2	0 °C, 0.5 h; rt, 1.5 h		80
5		2.0	5.0	1.2	0 °C, 0.5 h; rt, 1.5 h	26	

65% yields. Interestingly, reaction with benzoyl chloride (entry 4) gave the uncyclized γ-acyl enolic species **9** in 80% yield, but neither five equivalents of base nor longer reaction times led to more than 6% of the corresponding α-pyrone **10**. However, **9** did cyclize cleanly to **10** (96%) upon heating at 130 °C under reduced pressure (3 mmHg) (Scheme III).



Scheme III.

In summary, we have utilized our α-pyrone degradation and an improved γ-acylation/cyclization protocol to prepare five new analogs of the potent ACAT inhibitor pyripyropene A. All of the analogs proved to be ca. 100-fold less active than **1** suggesting that the pyridine moiety plays a significant role in binding to the enzyme.[15] We are currently investigating extensions of this methodology for the synthesis of arisugacin A.

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1 a) F. P. Bell, in "Pharmacological Control of Hyperlipidaemia," ed by R. Fears, J. R. Prous Science Publishers, Barcelona, Spain (1986), p 409. b) J. G. Heider, in "Pharmacological Control of Hyperlipidaemia," ed by R. Fears, J. R. Prous Science Publishers, Barcelona, Spain (1986), p 423. c) D. R. Sliskovic and A. D. White, *Trends Pharmacol. Sci.*, **12**, 194 (1991).

2 a) S. Ōmura, H. Tomoda, Y. K. Kim, and H. Nishida, *J. Antibiot.*, **46**, 1168 (1993). b) H. Tomoda, Y.-K. Kim, H. Nishida, R. Masuma, and S. Ōmura, *J. Antibiot.*, **47**, 148 (1994). c) Y.-K. Kim, H. Tomoda, H. Nishida, T. Sunazuka, R. Obata, and S. Ōmura, *J. Antibiot.*, **47**, 154 (1994). d) H. Tomoda, N. Tabata, D.-J. Yang, H. Takayanagi, H. Nishida, and S. Ōmura, *J. Antibiot.*, **48**, 495 (1995). e) H. Tomoda, H. Nishida, Y.-K. Kim, R. Obata, T. Sunazuka, S. Ōmura, J. Bordner, M. Guadliana, P. G. Dormer, and A. B. Smith, III, *J. Am. Chem. Soc.*, **116**, 12097 (1994).

3 For leading references to other ACAT inhibitors, see footnote 7 in reference 3a.

4 a) T. Nagamitsu, T. Sunazuka, R. Obata, H. Tomoda, H. Tanaka, Y. Harigaya, S. Ōmura, and A. B. Smith, III, *J. Org. Chem.*, **60**, 8126 (1995). b) A. B. Smith, III, T. Kinsho, T. Sunazuka, and S. Ōmura, *Tetrahedron Lett.*, **37**, 6461 (1996). c) For a related synthesis of recemic GERI-BP001 (identical to pyripyropene E), see: K. A. Parker and L. Resnick, *J. Org. Chem.*, **60**, 5726 (1995).

5 a) R. Obata, T. Sunazuka, Z. Li, H. Tomoda, and S. Ōmura, *J. Antibiot.*, **48**, 749 (1995). b) R. Obata, T. Sunazuka, H. Tomoda, Y. Harigaya, and S. Omura, *Bioorg. Med. Chem. Lett.*, **5**, 2683 (1995). c) R. Obata, T. Sunazuka, Z. Li, Z. Tian, Y. Harigaya, N. Tabata, H. Tomoda, and S. Ōmura, *J. Antibiot.*, **49**, 1133 (1996). d) R. Obata, T. Sunazuka, Y. Kato, H. Tomoda, Y. Harigaya, and S. Ōmura, *J. Antibiot.*, **49**, 1149 (1996).

6 S. Ōmura, F. Kuno, T. Sunazuka, K. Shiomi, R. Masuma, and Y. Iwai, *J. Antibiot.*, **48**, 745 (1995).

7 H. Tomoda, N. Tabata, Y. Nakata, H. Nishida, T. Kaneko, R. Obata, T. Sunazuka, and S. Ōmura, *J. Org. Chem.*, **61**, 882 (1996).

8 Preparation of **4**: A solution of **3** (547 mg, 0.94 mmol) in 70% aq. MeOH (55 mL) was treated with MeONa (291 mg, 5.7 equiv) and stirred at room temperature for 17 h. Concentration under reduced pressure and ODS reverse phase column chromatography (50% MeOH/H_2O eluant, employing ODS-7515-12 available from Senshu Scientific Co. Ltd.) gave **4** (289 mg, 80%) as a colorless powder: ^{1}H NMR ($CDCl_3$) δ 0.79 (3 H, s), 1.05 (1 H, m), 1.10 (3 H, s), 1.28 (1 H, m), 1.39 (3 H, s), 1.44 (1 H, m), 1.67 (2 H, m), 1.78 (1 H, m), 2.15 (3 H, s), 2.34 (1 H, s), 2.66 (1 H, m), 3.34 (1 H, d, J = 10.6 Hz), 3.63 (1 H, m), 3.64 (1 H, d, J = 10.6 Hz), 3.78 (3 H, s), 3.97 (1 H, dd, J = 4.8, 11.4 Hz); ^{13}C NMR ($CDCl_3$) δ 188.75, 173.75, 166.18, 111.43, 87.60, 76.73, 73.86, 68.32, 60.61, 52.01, 45.30, 42.14, 37.11, 26.99, 26.51, 20.65, 16.07, 14.11, 11.75; HRMS (FAB, *m*-NBA) *m/z* 383.2073 [(M+H)$^+$; calcd for $C_{20}H_{30}O_7$: 383.2069].

9 This fragmentation process appears to be unique, see: T. L. Ho, "Heterolytic Fragmentation of Organic Molecules," Wiley, New York (1993), p 73.

10 **5**: ^{1}H NMR ($CDCl_3$) δ 0.24 (9 H, s), 0.28 (9 H, s), 0.34 (9 H, s), 0.75 (3 H, s), 1.07 (3 H, s), 1.33 (3 H, s), 2.17 (3 H, s), 2.36 (3 H, s), 2.36 (1 H, s), 2.58 (1 H, d, J = 13.5 Hz), 3.08 (1 H, d, J = 9.9 Hz), 3.33 (1 H, d, J = 9.9 Hz), 3.63 (1 H, dd, J = 4.8, 11.4 Hz), 3.78 (3 H, s), 3.87 (1 H, m); HRMS (FAB, *m*-NBA/PEG 600/NaI) *m/z* 621.3095 [(M+Na)$^+$; calcd for $C_{29}H_{54}O_7Si_3Na$: 621.3075].

11 We previously observed regioselective γ-alkylation of related substrates: A. B. Smith, III and R. M. Scarborough, Jr., *Tetrahedron Lett.*, **44**, 4193 (1978).

12 **7**: ^{1}H NMR ($CDCl_3$) δ 0.09 (9 H, s), 0.12 (9 H, s), 0.23 (9 H, s), 0.60 (3 H, s), 1.14 (3 H, s), 1.44 (3 H, s), 2.51 (1 H, s), 2.65 (1 H, d, J = 13.5 Hz), 3.09 (1 H, d, J = 9.9 Hz), 3.37 (1 H, d, J = 9.9 Hz), 3.63 (1 H, dd, J = 5.0, 11.6 Hz), 3.97 (1 H, m), 6.47 (1 H, s), 7.69 (2 H, dd, J = 1.7, 4.6 Hz), 8.79 (2 H, dd, J = 1.7, 4.6 Hz); HRMS (FAB, *m*-NBA) *m/z* [672.3201(M+H)$^+$; calcd for $C_{34}H_{54}NO_7Si_3$: 672.3208].

13 A solution of **7** (10 mg, 0.02 mmol) in dry THF (1 mL) was treated with tetrabutylammoniumfluoride (TBAF, 1.0 M in THF, 10 μL) and stirred at room temperature for 17 h to give the corresponding triol (5 mg, 69%) as a colorless solid: IR (KBr) 3400, 1750, 1540 cm^{-1}; ^{1}H NMR (CD_3OD) δ 0.70 (3 H, s), 1.17 (3 H, s), 1.47 (3 H, s), 2.61 (1 H, d, J = 13.5 Hz), 2.77 (1 H, s), 3.26 (1 H, d, J = 11.0 Hz), 3.55 (1 H, d, J = 11.0 Hz), 3.63 (1 H, dd, J = 5.0, 11.5 Hz), 4.06 (1 H, m), 7.07 (1 H, s), 7.89 (2 H, dd, J = 1.7, 4.6 Hz), 8.72 (2 H, d, J = 6.3 Hz); HRMS (FAB, *m*-NBA) *m/z* 456.1997 [(M+H)$^+$; calcd for $C_{25}H_{30}O_7N$: 456.2022]. A solution of the triol (5 mg, 0.01 mmol) in dry pyridine (0.4 mL) was treated with acetic anhydride (0.2 mL) and DMAP (1 mg) and stirred at room temperature for 3 days to furnish the triacetate (2.7 mg, 45%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 0.87 (3 H, s), 1.24 (3 H, s), 1.56 (3 H, s), 2.04 (3 H, s), 2.12 (3 H, s), 2.18 (3 H, s), 2.63 (1 H, s), 2.78 (1 H, d, J = 13.9 Hz), 3.71 (1 H, d, J = 11.9 Hz), 3.77 (1 H, d, J = 11.9 Hz), 4.79 (1 H, dd, J = 5.3, 11.2 Hz), 5.25 (1 H, m), 6.57 (1 H, s), 7.69 (2 H, dd, J = 1.8, 4.5 Hz), 8.79 (2 H, d, J = 5.9 Hz); HRMS (FAB, *m*-NBA) *m/z* 584.2341 [(M+H)$^+$; calcd for $C_{31}H_{36}NO_{10}$: 584.2339]. A solution of the triacetate (2.7 mg, 0.005 mmol) in methanol (1 mL) was treated with $NaBH_4$ (2 mg) and $CeCl_3$•$7H_2O$ (2 mg) and stirred at 0 °C for 1 h to afford **8** (1.6 mg, 59%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.89 (3 H, s), 1.44 (3 H, s), 1.69 (3 H, s), 2.05 (3 H, s), 2.09 (3 H, s), 2.17 (3 H, s), 3.71 (1 H, d, J = 11.9 Hz), 3.79 (1 H, d, J = 11.9 Hz), 4.79 (1 H, dd, J = 5.1, 11.1 Hz), 5.00 (1 H, d, J = 3.6 Hz), 5.01 (1 H, m), 6.55 (1 H, s), 7.64 (2 H, d, J = 5.9 Hz), 8.74 (2 H, d, J = 5.6 Hz); HRMS (FAB, *m*-NBA) *m/z* 584.2488 [(M+H)$^+$; calcd for $C_{31}H_{38}NO_{10}$: 584.2496].

14 2-Pyridinecarbonyl chloride was prepared from picolinic acid by treatment with $(COCl)_2$. The other aroyl chlorides were commercially available.

15 The evaluations of biological activity will be described in detail elsewhere.