Chemical Modification and Structure-activity Relationships of Pyripyropenes

3. Synthetic Conversion of Pyridine-pyrone Moiety

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Structure-activity relationships of the pyridine-pyrone moiety in pyripyropene A (**1**), a potent acyl-CoA : cholesterol acyltransferase (ACAT) inhibitor of fungal origin, were studied. Several kinds of aromatic or hetero ring substituents for the pyridine moiety were synthesized using unique degradation reaction, following by γ-acylation. All the six synthesized analogs decreased the inhibitory activity with 20 to 200 times larger IC_{50} values than that of **1**. Furthermore, the pyridine-pyrone substituent also dramatically decrease the inhibitory activity. Thus, the pyridine-pyrone moiety is important for eliciting potent ACAT inhibition.

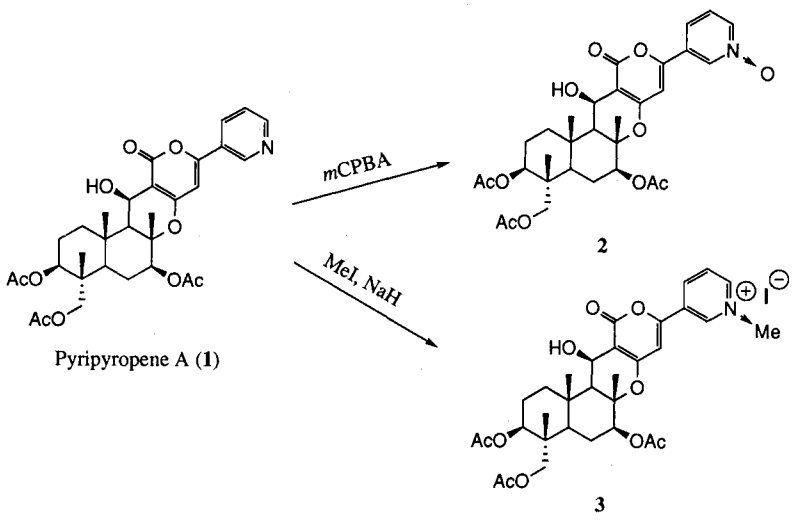
Pyripyropene A (**1**) is a metabolite isolated from a strain of *Aspergillus fumigatus* FO-1289 as potent and bioavailable acyl-CoA : cholesterol acyltransferase (ACAT) inhibitor[1]. The structure **1** consists of a poly-oxygenated sesquiterpene and a unique pyridine-pyrone moieties. In the preceding papers we have modified the four possible hydroxyl groups in the sesquiterpene moiety and showed structure-activity relationships[2~5]. However, it is still unclear whether or not the pyridine-pyrone moiety is important for potent ACAT inhibition. In this paper, we describe the synthesis and biological activity of pyridine-pyrone analogs of **1**.

Chemistry

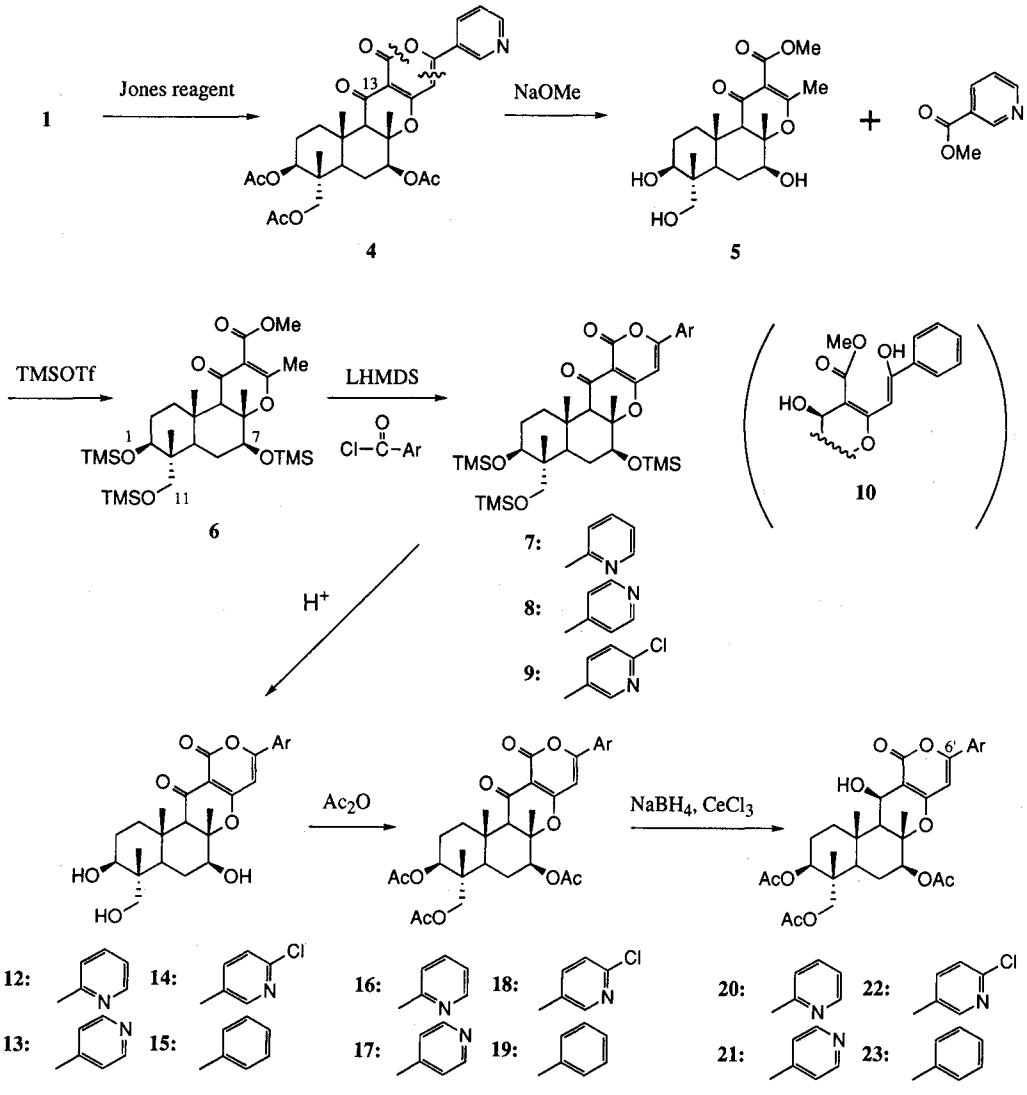
Synthesis of *N*-substituted derivatives is summarized in Scheme 1. We first synthesized *N*-oxide derivative (**2**) to expect that **2** shows similar inhibitory activity to **1** as reported for bioisostere[6]. Compound **2** was obtained by oxidation of **1** with 3-chloroperoxybenzoic acid (*m*CPBA). Methyl group was adopted as the smallest alkyl group of *N*-alkyl derivatives. Treatment of **1** with sodium hydride and methyl iodide gave *N*-methyl derivative (**3**).

The synthetic conversion of pyridine moiety of **1** is summarized in Scheme 2. We have found the unique degradation reaction of pyridine as methyl nicotinate. The 13-hydroxyl group was oxidized with Jones reagent

Scheme 1. Synthesis of *N*-substituted derivatives.



Scheme 2. Synthetic conversion of pyridine moiety of pyripyropene A.

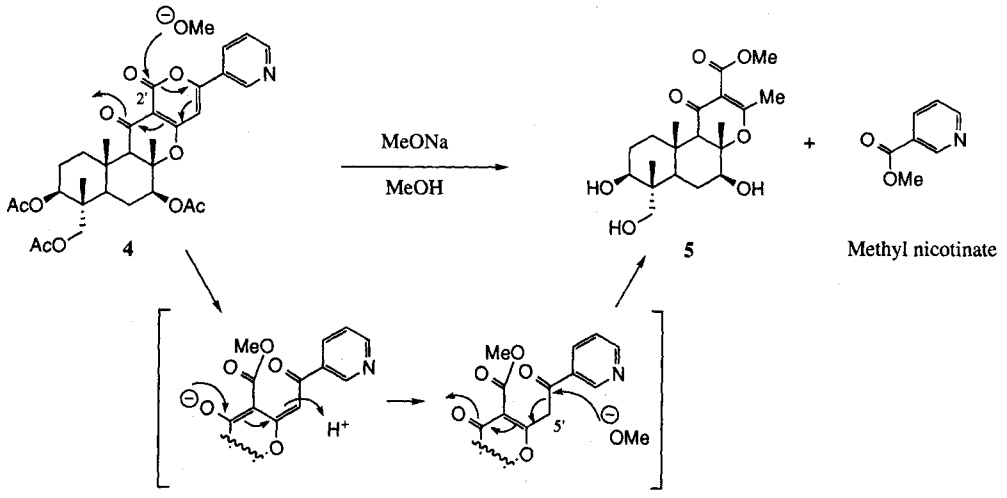


to give 13-oxopyripyropene A (**4**). Treatment of **4** with sodium methoxide in methanol formed compound **5**, whose three hydroxyl groups were protected as trimethylsilyl (TMS) ether by trimethylsilyl trifluoromethanesulfonate (TMSOTf) in the presence of 2,6-lutidine to obtain compound **6**. Possible mechanism of this degradation reaction is also shown in Scheme 3. The first methoxide anion may attack the C-2′ carbon of pyrone, and the intermediate α,β-unsaturated 1,5-diketone arises from proton transfer. Then, C–C bond cleavage may occur by retro-Claisen reaction[7] to afford **5** and methyl nicotinate. We used this unique reaction to investigate the biosynthesis of **1**[8].

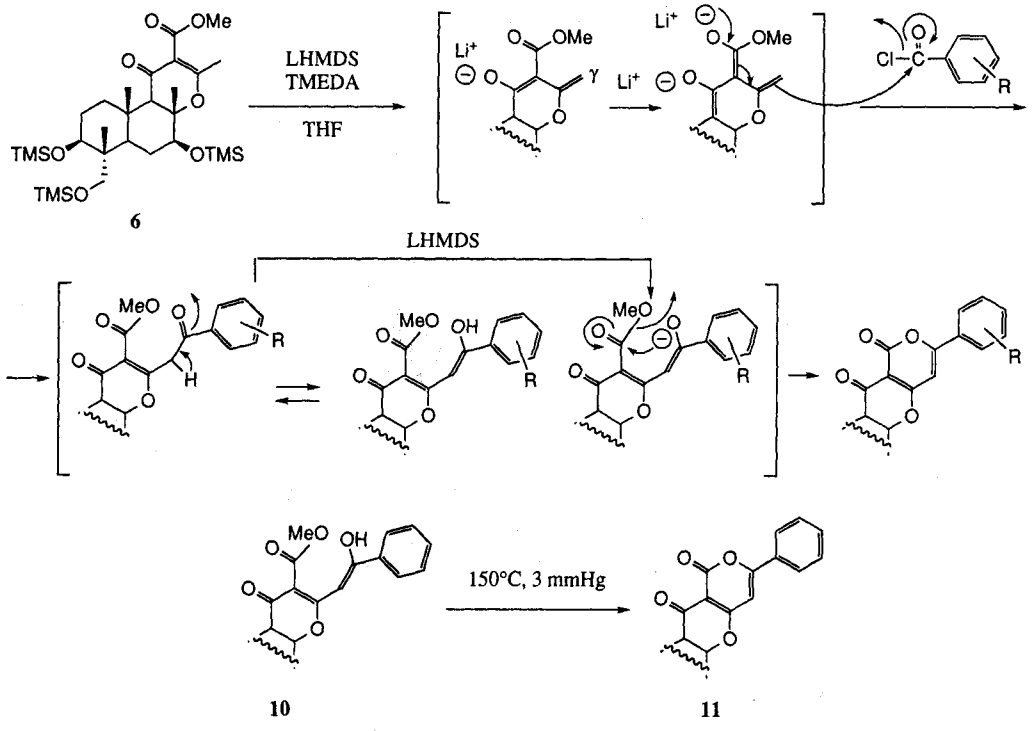
Compound **6** was added to the tetrahydrofuran solution of lithium bis(trimethylsilyl)amide (LHMDS) in the presence of *N,N,N′,N′*-tetramethylethylenediamine (TMEDA) to form dianion. Then the target aroyl halide was added to the reaction mixture to give 1,7,11-tri-*O*-TMS-6′-substituted pyripyropene A (**7**～**9**) (Scheme 2). Gamma-aldol reaction to form lactone reported by ADAMS *et al.*[9] and γ-alkylation of 3(2H)-furanone reported by SMITH *et al.*[10] were refered to this reaction. Mechanism of this acylation is shown in Scheme 4. The anion of γ-position may attack the aroyl halide to afford keto form intermediate, which is tautomerism of enol form. The presence of excess bases may re-construct 6-substituted-2-pyrone in a one-pot reaction. For example, treatment of benzoyl chloride with 2.5 equivalent of base formed enol derivative (**10**) in 80% yield, but using 5.0 equivalent of base with pyridine-2-carbonyl-chloride formed 2-pyrone form in 65% yield. Compound **10** was easily cyclized *in vacuo* at 150°C to give **11**.

TMS groups of **7**～**9** and **11** were deprotected to form **12**～**15**. 1,7,11-Hydroxyl groups of **12**～**15** were

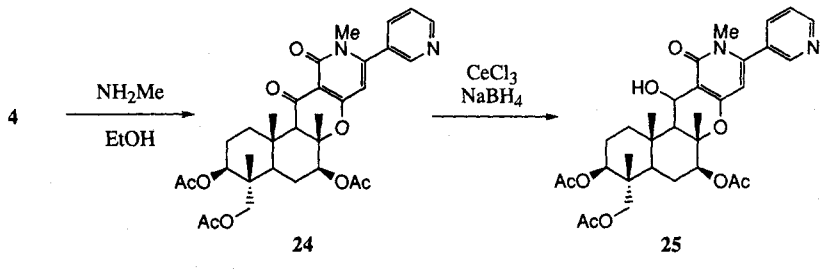
Scheme 3. Possible mechanism of degradation reaction.



Scheme 4. *C*-Acylation of γ-position of **6**.



Scheme 5. Synthesis of pyridone derivative.



acetylated (**16**~**19**), then reduction of the 13-ketone to hydroxyl group with sodium borohydride ($NaBH_4$) in the presence of cerium chloride ($CeCl_3$) afford 6'-substituted analogs of **1** (**20**~**23**) (Scheme 2).

Pyridone analog **24** was obtained by treatment of **4** with methylamine, and the position 13 ketone was reduced with sodium borohydride in the presence of cerium chloride to give **25** (Scheme 5).

Biological Activity

The *in vitro* ACAT inhibitory activity was assayed by our established method using rat liver microsomes[11]. The structure and ACAT inhibitory activity (IC_{50} value) of synthesized analogs are summarized in Table 1. IC_{50} values of all other intermediates, which have not shown in Table 1 were larger than 50 μM.

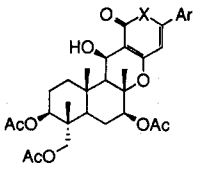
The *N*-substituted derivatives (**2** and **3**) showed about 20 and 50 times less inhibitory activity than **1**, respectively.

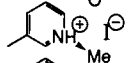
As for the position of nitrogen atom in the pyridine ring, **20**, **21** and **1** were prepared to compare the IC_{50} values with that of **1**. Interestingly, the two analogs having a nitrogen atom at the different position decrease the inhibitory activity. Moreover, even though the position of the nitrogen atom is the same as that of **1**, addition of chloride atom at the 6''-position (**22**) also significantly decreased the inhibitory activity. And **23** whose the pyridine ring of **1** was replaced by the benzene ring also decreased the activity with a similar IC_{50} value to **20**~**22**.

As for the pyrone moiety, the pyridone analog **25** showed the weakest inhibitory activity.

Taken together, all the prepared derivatives have dramatically decreased ACAT inhibitory activity. This results indicate that the pyridine-pyrone moiety of **1** is essential for eliciting ACAT inhibitory activity, suggesting that the moiety fits strictly to one of the critical sites of the ACAT molecule.

Table 1. *In vitro* ACAT inhibitory activity of synthesized analogs.



Compound	X	Ar	*In vitro* ACAT inhibitory activity (IC_{50}, μM)
1	O		0.089
2	O		2.0
3	O		4.7
20	O		9.1
21	O		17
22	O		11
23	O		10
25	NMe		27

Experimental

Reagents were obtained from commercial suppliers and were used without purification, unless otherwise noted. Column chromatography was carried out on silica gel (Kieselgel 60, 230~400 mesh, Merck, Art. 9385). For preparative TLC (PTLC), silica gel plate (Kieselgel 60 F-254, Merck, Art. 5715) was used. Mass spectra were obtained by using a JEOL model DX-300 mass spectrometer. IR spectra were taken with a Horiba model FT-210 spectrophotometer. ^1H NMR 270 MHz and ^{13}C NMR 76.5 MHz spectra were acquired on a JEOL EX-270 spectrometer. Chemical shifts are given in ppm with solvent peak ($CDCl_3$: 7.26 ppm, CD_3OD: 3.30 ppm) as the standard, and coupling constants (J) are given as Hz.

Compound 2

To a solution of **1** (20 mg, 0.034 mmol) in dried dichloromethane (CH_2Cl_2, 3 ml) was added 3-chloroperoxybenzoic acid (*m*CPBA, 12 mg, 0.070 mmol) and stirred at room temperature for 60 hours. Water was added to the reaction mixture, which was extracted with CH_2Cl_2. The organic layer was dried over anhydr Na_2SO_4 and evaporated. The residue was purified by PTLC (20 × 20 cm, CH_2Cl_2-MeOH (10:1)) to obtain **2** (20 mg, 100%). HR FAB-MS (*m/z*) Found: 600.2462 (M+H), Calcd for $C_{31}H_{38}O_{11}N$: 600.2445; IR (KBr) cm^{-1} 1730, 1190; ^1H NMR ($CDCl_3$) δ 0.88 (3H, s), 1.43 (3H, s), 1.68 (3H, s), 2.04 (3H, s), 2.08 (3H, s), 2.15 (3H, s), 3.69 (1H, d, J=11.9 Hz), 3.78 (1H, d, J=11.9 Hz), 4.78 (1H, dd, J=5.2, 11.1 Hz), 4.97 (1H, d, J=4.3 Hz), 4.98 (1H, m), 6.42 (1H, s), 7.36 (1H, m), 7.66 (1H, m), 8.27 (1H, d, J=6.6 Hz), 8.63 (1H, s).

Compound 3

To a solution of **1** (12 mg, 0.02 mmol) in dried THF (1 ml) were added sodium hydride (1.1 mg, 1 mg/ml in THF suspension, 0.048 mmol) and methyl iodide (100 μl, 1.6 mmol), and the mixture was stirred at 0°C for 2 hours and at room temperature for 3 hours. The reaction was stopped by adding water. The mixture was extracted with CH_2Cl_2, and the organic layer was dried over anhydr Na_2SO_4 and evaporated. The residue was purified by PTLC (10 × 10 cm, CH_2Cl_2-MeOH (10:1)) to

obtain **3** (4.4 mg, 29%). HR FAB-MS (*m/z*) Found: 598.2669 (M-I), Calcd for $C_{32}H_{40}O_{10}N$: 598.2652; IR (KBr) cm^{-1} 1730, 1250; ^1H NMR ($CDCl_3$) δ 0.89 (3H, s), 1.42 (3H, s), 1.65 (3H, s), 2.06 (3H, s), 2.15 (3H, s), 2.20 (3H, s), 3.74 (1H, d, J=12.0 Hz), 3.85 (1H, d, J=12.0 Hz), 4.72 (3H, s), 4.86 (1H, s), 4.86 (1H, m), 5.06 (1H, dd, J=5.0, 10.9 Hz), 7.24 (1H, s), 8.22 (1H, t, J=7.3 Hz), 8.84 (1H, d, J=5.9 Hz), 8.91 (1H, d, J=8.6 Hz), 9.84 (1H, s).

Compound **4**

To a solution of **1** (64 mg, 0.11 mmol) in 95% acetone in water (42 ml) was added Jones reagent (3 M-CrO_3-H_2SO_4 in water, 0.5 ml), and the mixture was stirred at room temperature for 3 hours. The reaction was quenched with 2-PrOH (0.5 ml), and the precipitate was filtered off and washed with acetone. The filtrate and washing were combined and dried up to give a green solid, which was diluted with EtOAc, and washed with water twice. The organic layer was dried over anhydr Na_2SO_4, and concentrated *in vacuo* to afford a pale yellow solid, which was purified by column chromatography (CH_2Cl_2-MeOH (50:1)) to obtain **4** (64 mg, 100%) as colorless powder. HR FAB-MS (*m/z*) 582.2350 (M+H) Calcd for $C_{31}H_{36}O_{10}N$: 582.2339; IR (KBr) cm^{-1} 1730, 1250; ^1H NMR ($CDCl_3$) δ 0.83 (3H, s), 1.19 (3H, s), 1.53 (3H, s), 1.99 (3H, s), 2.07 (3H, s), 2.13 (3H, s), 2.60 (1H, s), 2.74 (1H, d, J=13.5 Hz), 3.67 (1H, d, J=12.2 Hz), 3.73 (1H, d, J=12.2 Hz), 4.74 (1H, dd, J=5.3, 11.2 Hz), 5.20 (1H, dd, J=5.0, 11.2 Hz), 6.48 (1H, s), 7.41 (1H, dd, J=4.8, 8.1 Hz), 8.13 (1H, dt, J=2.0, 8.3 Hz), 8.70 (1H, s), 9.01 (1H, s).

Compound **5**

To a solution of **4** (547 mg, 0.94 mmol) in 70% MeOH in water (54.7 ml) was added sodium methoxide (291 mg, 5.4 mmol) and the mixture was stirred at room temperature for 18 hours. The reaction mixture was evaporated under the reduced pressure to remove MeOH and purified by ODS column chromatography (Senshu ODS-7515-12A, 50~100% MeOH in water) to afford **5** (289 mg, 80%) as colorless powder. HR FAB-MS (*m/z*) 383.2073 (M+H) Calcd for $C_{20}H_{31}O_7$: 383.2070; IR (KBr) cm^{-1} 1720, 1380; ^1H NMR ($CDCl_3$) δ 0.79 (3H, s), 1.08 (1H, m), 1.10 (3H, s), 1.26 (1H, d, J=13.5 Hz), 1.39 (3H, s), 1.45 (1H, m), 1.73 (2H, m), 1.84 (1H, m), 2.15 (3H, s), 2.35 (1H, s), 2.65 (1H, d, J=13.5 Hz), 3.34 (1H, d, J=10.6 Hz), 3.63 (1H, m), 3.64 (1H, d, J=10.6 Hz), 3.78 (3H, s), 3.97 (1H, dd, J=4.9, 11.4 Hz); ^{13}C NMR ($CDCl_3$) δ 11.75, 14.11, 16.07, 20.65, 26.51, 26.99, 37.11, 42.14, 45.30, 50.81, 52.01, 60.61, 68.32, 73.89, 76.73, 87.60, 111.42, 166.18, 173.75, 188.75.

Compound **6**

To a solution of **5** (67.2 mg, 0.18 mmol) in dry CH_2Cl_2 (2 ml) were added trimethylsilyl trifluoromethanesulfonate (TMSOTf, 153 μl, 0.85 mmol) and 2,6-lutidine (123 μl, 1.06 mmol) under argon, and the mixture was stirred at room temperature for 10 minutes. The reaction was stopped by adding water (5 ml), and the mixture was extracted with CH_2Cl_2 (5 ml) three times. The organic layer was dried over anhydr Na_2SO_4 and concentrated *in vacuo*. The colorless residue (120 mg) was purified by column chromatography (hexane - EtOAc (15:1~10:1)) to give **6** (95 mg, 90%) as colorless solid. HR FAB-MS (*m/z*) Found: 621.3095 (M+H), Calcd for $C_{29}H_{54}O_7Si_3$-Na: 621.3075; IR (KBr) cm^{-1} 1720, 1390; ^1H NMR ($CDCl_3$) δ 0.24 (9H, s), 0.28 (9H, s), 0.34 (9H, s), 0.75 (3H, s), 1.07 (3H, s), 1.33 (3H, s), 2.17 (3H, s), 2.36 (3H, s), 2.36 (1H, s), 2.58 (1H, d, J=13.5 Hz), 3.08 (1H, d, J=9.9 Hz), 3.33 (1H, d, J=9.9 Hz), 3.63 (1H, dd, J=4.8, 11.4 Hz), 3.78 (3H, s), 3.87 (1H, m).

Re-construction of 6-Substituted-2-pyrone (**7**~**11**)

Compound **7**

To a cooled (0°C) solution of lithium bis(trimethylsilyl)amide (LHMDS, 1 M solution in THF, 500 μl, 0.50 mmol) was added *N,N,N',N'*-tetramethylethylenediamine (TMEDA, 18 μl, 0.12 mmol), and after stirring for 10 minutes under argon, **6** (60 mg, 0.10 mmol) in THF solution (600 μl) was added. The mixture was stirred at 0°C for 30 minutes, then a cooling bath was removed and stirring was continued for 1.5 hours. To the resulting pale-yellow mixture was slowly added pyridine-2-carbonylchloride (52 mg, 0.30 mmol, prepared from piconilic acid by treatment with oxalyl chloride) at 0°C. The reaction mixture was stirred at that temperature for 3 hours, then quenched with AcOH (18 μl). The solution was extracted with EtOAc, and the organic layer was washed with satd NH_4Cl, water, and satd NaCl respectively, dried over anhydr Na_2SO_4 and evaporated. The residue was purified by column chromatography (CH_2Cl_2-MeOH (100:1~25:1)) to obtain **7** (46 mg, 65%) as colorless solid. HR FAB-MS (*m/z*) Found: 672.3201 (M+H), Calcd for $C_{34}H_{54}O_7NSi_3$: 672.3208; IR (KBr) cm^{-1} 1770, 1540; ^1H NMR ($CDCl_3$) δ 0.08 (9H, s), 0.12 (9H, s), 0.24 (9H, s), 0.60 (3H, s), 1.14 (3H, s), 1.43 (3H, s), 2.52 (1H, s), 2.67 (1H, d, J=13.5 Hz), 3.10 (1H, d, J=10.0 Hz), 3.36 (1H, d, J=10.0 Hz), 3.64 (1H, dd, J=4.8, 11.7 Hz), 3.99 (1H, m), 7.08 (1H, s), 7.40 (1H, m), 7.85 (1H, m), 8.09 (1H, d, J=7.9 Hz), 8.69 (1H, d, J=4.0 Hz); ^{13}C NMR ($CDCl_3$) δ −0.58, 0.61, 0.63, 12.65, 137.27, 14.88, 16.00, 28.84, 42.75, 43.11, 62.41, 65.37, 68.84, 77.20, 87.64, 98.47, 149.94, 172.92.

Compound **8**

In a similar manner to **7**, **6** (30 mg, 0.05 mmol, in 300 μl THF) was added to the mixture of LHMDS (1 M in THF, 200 μl, 0.20 mmol) and TMEDA (9 μl, 0.06 mmol), and after stirring for 0°C for 30 minutes and room temperature for 1.5 hours under argon, isonicotinoyl chloride hydrochloride (22 mg, 0.12 mmol) was added. The reaction mixture was stirred at 0°C for 1.5 hours and treatment in a similar manner to **7** give **8** (17 mg, 50%). HR FAB-MS (*m/z*) Found: 672.3201 (M+H),

Calcd for $C_{34}H_{54}O_7NSi_3$: 672.3208; IR (KBr) cm^{-1} 1770, 1540; ^1H NMR ($CDCl_3$) δ 0.09 (9H, s), 0.12 (9H, s), 0.23 (9H, s), 0.60 (3H, s), 1.14 (3H, s), 1.44 (3H, s), 2.51 (1H, s), 2.65 (1H, d, J=13.5 Hz), 3.09 (1H, d, J=9.9 Hz), 3.37 (1H, d, J=9.9 Hz), 3.63 (1H, dd, J=5.0, 11.6 Hz), 3.97 (1H, m), 6.47 (1H, s), 7.69 (2H, dd, J=1.7, 4.6 Hz), 8.79 (2H, dd, J=1.7, 4.6 Hz).

Compound **9**

In a similar manner to **7**, **6** (25 mg, 0.042 mmol, in 1 ml THF) was added to the mixture of LHMDS (1 M in THF, 250 μl, 0.25 mmol) and TMEDA (9 μl, 0.06 mmol), and after stirring for 0°C for 30 minutes and 1.5 hours under argon, *p*-chloronicotinoyl chloride (18 mg, 0.10 mmol, in 18 μl THF) was added. The reaction mixture was stirred at 0°C for 1 hour and room temperature for 3 hours, and treatment in a similar manner to **7** give **9** (15.4 mg, 52%). HR FAB-MS (*m/z*) Found: 706.2831 (M+H), Calcd for $C_{34}H_{53}O_7ClNSi_3$: 706.2818; IR (KBr) cm^{-1} 1770, 1540; ^1H NMR ($CDCl_3$) δ 0.09 (9H, s), 0.12 (9H, s), 0.23 (9H, s), 0.60 (3H, s), 1.14 (3H, s), 1.44 (3H, s), 2.50 (1H, s), 2.65 (1H, d, J=13.5 Hz), 3.09 (1H, d, J=9.9 Hz), 3.36 (1H, d, J=9.9 Hz), 3.63 (1H, dd, J=5.0, 11.6 Hz), 3.96 (1H, m), 6.37 (1H, s), 7.47 (1H, d, J=7.9 Hz), 8.11 (2H, dd, J=2.6, 8.6 Hz), 8.82 (2H, d, J=2.0 Hz).

Compound **10**

In a similar manner to **7**, **6** (30 mg, 0.05 mmol, in 300 μl THF) was added to the mixture of LHMDS (1 M in THF, 125 μl, 0.13 mmol) and TMEDA (9 μl, 0.06 mmol), and after stirring for 0°C for 2 hours under argon, benzoylchloride (30 μl, 0.26 mmol) was added. The reaction mixture was stirred at 0°C for 30 minutes and room temperature for 1.5 hours, and treatment in a similar manner to **7** to give **10** (28 mg, 80%). HR FAB-MS (*m/z*) Found: 703.3257 (M+H), Calcd for $C_{36}H_{59}O_8Si_3$: 703.3256; IR (KBr) cm^{-1} 1770, 1540; ^1H NMR ($CDCl_3$) δ 0.08 (9H, s), 0.10 (9H, s), 0.15 (9H, s), 0.55 (3H, s), 1.03 (3H, s), 1.31 (3H, s), 2.22 (1H, s), 2.47 (1H, d, J=13.3 Hz), 3.06 (1H, d, J=9.9 Hz), 3.33 (1H, d, J=9.9 Hz), 3.59 (1H, dd, J=5.0, 11.6 Hz), 3.85 (1H, m), 6.57 (1H, s), 7.51 (3H, m), 8.15 (2H, d, J=7.3 Hz).

Compound **11**

Compound **10** (5 mg, 0.007 mmol) was heated at 150°C under the reduced pressure (3 mmHg) for 15 minutes. The residue was purified with PTLC (5×10 cm, hexane-EtOAc (5:1)) to give **11** (4.6 mg, 98%). HR FAB-MS (*m/z*) Found: 671.3257 (M+H), Calcd for $C_{35}H_{55}O_7Si_3$: 671.3256; IR (KBr) cm^{-1} 1770, 1540; ^1H NMR ($CDCl_3$) δ 0.08 (9H, s), 0.12 (9H, s), 0.23 (9H, s), 0.60 (3H, s), 1.15 (3H, s), 1.43 (3H, s), 2.29 (1H, s), 2.68 (1H, d, J=13.3 Hz), 3.10 (1H, d, J=9.9 Hz), 3.37 (1H, d, J=9.9 Hz), 3.64 (1H, dd, J=5.0, 11.6 Hz), 3.98 (1H, dd, J=5.1, 10.4 Hz), 6.34 (1H, s), 7.49 (3H, m), 7.85 (2H, dd, J=1.8, 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ −0.39, 0.61, 12.65, 14.97, 16.00, 26.97, 28.88, 37.11, 42.81, 43.25, 62.55, 63.95, 71.61, 77.20, 90.12, 96.48, 100.18, 126.43, 129.04, 130.46, 132.22, 157.52, 164.76, 172.69, 187.38.

Deprotection of Trimethylsilyl Groups (**12**~**15**)

Compound **12**

To a solution of **7** (40 mg, 0.06 mmol) in MeOH (2 ml) was added 2 N HCl (20 μl) and the mixture was stirred at room temperature for 1 hour. The reaction mixture was evaporated *in vacuo* and the residue was purified by reverse phase TLC (RP-18 F-254 S, Merck, Art. 15423, 10×10 cm×3 plates, 50% MeOH in water) to give **12** (21 mg, 76%) as colorless solid. HR FAB-MS (*m/z*) Found: 456.2040 (M+H), Calcd for $C_{25}H_{30}O_7N$: 456.2022; IR (KBr) cm^{-1} 1740, 1540; ^1H NMR ($CDCl_3$) δ 1.09 (3H, s), 1.19 (3H, s), 1.50 (3H, s), 2.51 (1H, s), 2.77 (1H, d, J=13.5, Hz), 3.40 (1H, d, J=10.6 Hz), 3.70 (1H, m), 3.72 (1H, d, J=9.9 Hz), 4.10 (1H, dd, J=4.8, 11.4 Hz), 7.19 (1H, s)), 7.42 (1H, ddd, J=1.0, 4.6, 7.6 Hz), 7.85 (1H, dd, J=1.7, 7.7 Hz), 8.07 (1H, d, J=8.0 Hz), 8.66 (1H, d, J=4.0 Hz).

Compound **13**

To a solution of **8** (10 mg, 0.02 mmol) in dried THF (1 ml) was added tetrabuthylammonium fluoride (TBAF, 1.0 M solution in THF, 10 μl, 0.01 mmol) and stirred at room temperature for 17 hours. The reaction mixture was evaporated *in vacuo* and the residue was purified by column chromatography (CH_2Cl_2-MeOH (10:1)) to give **13** (5 mg, 69%) as colorless solid. HR FAB-MS (*m/z*) Found: 456.1997 (M+H), Calcd for $C_{25}H_{30}O_7N$: 456.2022; IR (KBr) cm^{-1} 1750, 1540; ^1H NMR ($CDCl_3$) δ 0.70 (3H, s), 1.17 (3H, s), 1.47 (3H, s), 2.61 (1H, d, J=13.5 Hz), 2.77 (1H, s), 3.26 (1H, d, J=11.0 Hz), 3.55 (1H, d, J=11.0 Hz), 3.63 (1H, dd, J=5.0, 11.5 Hz), 4.06 (1H, m), 7.07 (1H, s), 7.89 (2H, dd, J=1.7, 4.6 Hz), 8.72 (2H, d, J=6.3 Hz).

Compound **14**

Compound **9** (10 mg, 0.014 mmol) was treated in a similar manner to **12** to give **14** (2.6 mg, 37%) as colorless solid. HR FAB-MS (*m/z*) Found: 490.2040 (M+H), Calcd for $C_{25}H_{29}O_7ClN$: 490.2022; IR (KBr) cm^{-1} 1700, 1620; ^1H NMR ($CDCl_3$) δ 0.87 (3H, s), 1.20 (3H, s), 1.49 (3H, s), 2.71 (1H, s), 2.78 (1H, d, J=13.2 Hz), 3.43 (1H, d, J=10.2 Hz), 3.73 (1H, d, J=11.5 Hz), 3.74 (1H, m), 4.12 (1H, m), 6.87 (1H, s), 7.44 (1H, d, J=8.6 Hz), 8.31 (1H, s), 8.92 (1H, s).

Compound **15**

Compound **11** (8.4 mg, 0.012 mmol) was treated in a similar manner to **13** to give **15** (4.4 mg, 77%) as colorless solid. HR FAB-MS (*m/z*) Found: 455.2086 (M+H), Calcd for $C_{26}H_{31}O_7$: 455.2070; IR (KBr) cm^{-1} 1740, 1540; ^1H NMR ($CDCl_3$) δ 0.84 (3H, s), 1.20 (3H, s), 1.51 (3H, s), 2.50 (1H, s), 2.79 (1H, d, J=13.3 Hz), 3.39 (1H, d, J=10.6 Hz), 3.70 (1H, d, J=10.6 Hz), 3.76 (1H, dd, J=5.0, 11.6 Hz), 4.12 (1H, dd, J=4.8, 11.4 Hz), 6.50

(1H, s), 7.50 (3H, m), 7.86 (2H, dd, J=1.4, 8.1 Hz).

Acetylation of 1,7,11-Hydroxyl groups (**16**~**19**)

Compound **16**

To a solution of **12** (15 mg, 0.03 mmol) in dried pyridine (2 ml) were added acetic anhydride (0.5 ml, 5.3 mmol) and 4-dimethylaminopyridine (DMAP, 1 mg, 0.01 mmol), and the mixture was stirred at room temperature for 3 days. The reaction mixture was evaporated and purified by PTLC (20×20 cm, CH_2Cl_2-MeOH (25:1)) to give **16** (20 mg, 100%). HR FAB-MS (m/z) Found: 584.2349 (M+H), Calcd for $C_{31}H_{36}O_{10}N$: 582.2339; IR (KBr) cm^{-1} 1750, 1540; ^1H NMR ($CDCl_3$) δ 0.85 (3H, s), 1.21 (3H, s), 1.53 (3H, s), 2.02 (3H, s), 2.10 (3H, s), 2.14 (3H, s), 2.62 (1H, s), 2.78 (1H, d, J=13.9 Hz), 3.40 (1H, d, J=12.0 Hz), 3.72 (1H, d, J=12.0 Hz), 4.76 (1H, dd, J=5.3, 11.2 Hz), 5.23 (1H, dd, J=4.8, 10.8 Hz), 7.14 (1H, s), 7.41 (1H, dd, J=4.6, 7.6 Hz), 7.84 (1H, t, J=7.6 Hz), 8.06 (1H, d, J=7.9 Hz), 8.65 (1H, d, J=4.3 Hz); ^{13}C NMR ($CDCl_3$) δ 13.73, 15.98, 16.53, 21.24, 21.51, 21.58, 23.22, 25.16, 37.20, 37.59, 40.85, 45.02, 62.61, 65.21, 73.84, 77.68, 87.44, 99.08, 101.56, 124.22, 126.67, 137.79, 148.10, 150.46, 157.52, 163.45, 170.24, 170.91, 171.43, 173.19, 186.86.

Compound **17**

Compound **13** (5 mg, 0.03 mmol) was treated in a similar manner to **16** to give **17** (2.7 mg, 45%). HR FAB-MS (m/z) Found: 584.2341 (M+H), Calcd for $C_{31}H_{36}O_{10}N$: 582.2339; IR (KBr) cm^{-1} 1740, 1250; ^1H NMR ($CDCl_3$) δ 0.87 (3H, s), 1.24 (3H, s), 1.56 (3H, s), 2.04 (3H, s), 2.12 (3H, s), 2.18 (3H, s), 2.63 (1H, s), 2.78 (1H, d, J=13.9 Hz), 3.71 (1H, d, J=11.9 Hz), 3.77 (1H, d, J=11.9 Hz), 4.79 (1H, dd, J=5.3, 11.2 Hz), 5.25 (1H, m), 6.57 (1H, s), 7.69 (2H, dd, J=1.8, 4.5 Hz), 8.79 (2H, d, J=5.9 Hz).

Compound **18**

Compound **14** (4 mg, 0.008 mmol) was treated in a similar manner to **16** to give **18** (4.5 mg, 89%). HR FAB-MS (m/z) Found: 617.2341 (M+H), Calcd for $C_{31}H_{35}O_{10}NCl$: 617.2339; IR (KBr) cm^{-1} 1740, 1240; ^1H NMR ($CDCl_3$) δ 0.87 (3H, s), 1.23 (3H, s), 1.56 (3H, s), 2.04 (3H, s), 2.12 (3H, s), 2.17 (3H, s), 2.55 (1H, s), 2.78 (1H, d, J=13.5 Hz), 3.71 (1H, d, J=11.9 Hz), 3.77 (1H, d, J=11.6 Hz), 4.78 (1H, dd, J=5.1, 11.1 Hz), 5.23 (1H, m), 6.48 (1H, s), 7.48 (1H, d, J=8.6 Hz), 8.12 (1H, dd, J=2.5, 8.4 Hz), 8.83 (1H, d, J=2.3 Hz).

Compound **19**

Compound **15** (6 mg, 0.013 mmol) was treated in a similar manner to **16** to give **19** (6.8 mg, 89%). HR FAB-MS (m/z) Found: 581.2387 (M+H), Calcd for $C_{32}H_{37}O_{10}$: 581.2384; IR (KBr) cm^{-1} 1740, 1540; ^1H NMR ($CDCl_3$) δ 0.86 (3H, s), 1.23 (3H, s), 1.55 (3H, s), 2.80 (1H, d, J=13.5 Hz), 2.62 (1H, s), 2.80 (1H, d, J=13.9 Hz), 3.70 (1H, d, J=12.2 Hz), 3.76 (1H, d, J=12.2 Hz), 4.78 (1H, dd, J=5.1, 11.2 Hz), 5.22 (1H, m), 7.49 (3H, m), 7.85 (2H, d, J=7.9 Hz).

Reduction of 13-Ketone (**20**~**23**)

Compound **20**

To a solution of **16** (15 mg, 0.026 mmol) in MeOH (0.6 ml) were added cerium (III) chloride heptahydrate ($CeCl_3 \cdot 7H_2O$, 12 mg, 0.032 mmol) and sodium borohydride ($NaBH_4$, 2.4 mg, 0.063 mmol), and the mixture was stirred at room temperature for 30 minutes. The reaction mixture was evaporated and purified by PTLC (20×20 cm, CH_2Cl_2-MeOH (25:1)) to give **20** (7.7 mg, 35%). HR FAB-MS (m/z) Found: 584.2500 (M+H), Calcd for $C_{31}H_{38}O_{10}N$: 584.2496; IR (KBr) cm^{-1} 1740, 1240; ^1H NMR ($CDCl_3$) δ 0.88 (3H, s), 1.43 (3H, s), 1.66 (3H, s), 2.03 (3H, s), 2.08 (3H, s), 2.14 (3H, s), 2.84 (1H, s), 3.68 (1H, d, J=11.9 Hz), 3.72 (1H, d, J=11.9 Hz), 4.78 (1H, dd, J=5.3, 11.2 Hz), 5.00 (1H, d, 4.3 Hz), 5.01 (1H, m), 7.10 (1H, s), 7.35 (1H, ddd, J=1.3, 4.7, 7.6 Hz), 7.81 (1H, dt, J=1.9, 7.8 Hz), 7.98 (1H, d, J=8.3 Hz), 8.63 (1H, dt, J=1.0, 4.0 Hz).

Compound **21**

Compound **17** (4 mg, 0.006 mmol) was treated in a similar manner to **20** to give **21** (0.6 mg, 21%). HR FAB-MS (m/z) Found: 584.2488 (M+H), Calcd for $C_{31}H_{38}O_{10}N$: 584.2496; IR (KBr) cm^{-1} 1740, 1240; ^1H NMR ($CDCl_3$) δ 0.89 (3H, s), 1.44 (3H, s), 1.69 (3H, s), 2.05 (3H, s), 2.09 (3H, s), 2.17 (3H, s), 2.87 (1H, s), 3.71 (1H, d, J=11.9 Hz), 3.79 (1H, d, J=11.9 Hz), 4.79 (1H, dd, J=5.1, 11.1 Hz), 5.00 (1H, d, J=3.6 Hz), 5.01 (1H, m), 6.55 (1H, s), 7.64 (2H, d, J=5.9 Hz), 8.74 (2H, d, J=5.6 Hz).

Compound **22**

Compound **18** (4 mg, 0.006 mmol) was treated in a similar manner to **20** to give **22** (0.5 mg, 12%). HR FAB-MS (m/z) Found: 618.2836 (M+H), Calcd for $C_{31}H_{37}O_{10}NCl$: 618.2812; IR (KBr) cm^{-1} 1740, 1250; ^1H NMR ($CDCl_3$) δ 0.89 (3H, s), 1.25 (3H, s), 1.44 (3H, s), 2.05 (3H, s), 2.09 (3H, s), 2.16 (3H, s), 3.69 (1H, d, J=11.3 Hz), 3.79 (1H, d, J=11.3 Hz), 4.77 (1H, m), 4.99 (2H, m), 6.44 (1H, s), 7.43 (1H, d, J=8.3 Hz), 8.05 (1H, dd, J=2.4, 8.3 Hz), 8.78 (1H, d, J=2.4 Hz).

Compound **23**

Compound **19** (6 mg, 0.010 mmol) was treated in a similar manner to **20** to give **23** (6.1 mg, 100%). HR FAB-MS (m/z) Found: 605.2407 (M+Na), Calcd for $C_{32}H_{38}O_{10}Na$: 605.2363; IR (KBr) cm^{-1} 1740; ^1H NMR ($CDCl_3$) δ 0.89 (3H, s), 1.44 (3H, s), 1.69 (3H, s), 2.04 (3H, s), 2.09 (3H, s), 2.16 (3H, s), 2.49 (1H, s), 3.71 (1H, d, J=9.9 Hz), 3.79 (1H, d, J=9.9 Hz), 4.79 (1H, dd, J=5.5, 11.1 Hz), 4.99 (1H, d, J=4.3 Hz), 5.00 (1H, m), 6.39 (1H, s), 7.45 (3H, m), 7.79 (2H, m); ^{13}C NMR ($CDCl_3$) δ 13.23, 16.25, 17.45, 20.79, 21.13, 21.21, 22.73, 25.20, 36.19, 37.85, 40.33, 45.37, 54.77, 60.31, 64.87,

73.62, 77.20, 82.89, 98.22, 102.14, 125.61, 128.90, 130.98, 131.03, 159.96, 162.55, 164.46, 170.06, 170.49, 170.94.

Compound **24**

To a solution of **2** (13 mg, 0.02 mmol) in EtOH (2 ml) was added 25% methylamine in water (150 μl, 1.2 mmol) and the mixture was stirred at room temperature for 18 hours. The reaction mixture was dried up and extracted with CH_2Cl_2, and the organic layer was washed with water, dried over anhydr Na_2SO_4 and evaporated. The residue was purified by PTLC (15×20 cm, CH_2Cl_2-MeOH (20:1)) to obtain **24** (7 mg, 52%). HR FAB-MS (*m/z*) Found: 595.2634 (M+H), Calcd for $C_{32}H_{39}O_9N_2$: 595.2656; IR (KBr) cm^{-1} 1740, 1240; ^1H NMR ($CDCl_3$) δ 0.86 (3H, s), 1.23 (3H, s), 1.51 (3H, s), 2.02 (3H, s), 2.10 (6H, s), 2.58 (1H, s), 2.82 (1H, d, J=13.9 Hz), 3.29 (3H, s), 3.72 (2H, m), 4.77 (1H, dd, J=5.8, 10.7 Hz), 5.18 (1H, dd, J=4.1, 10.4 Hz), 5.79 (1H, s), 7.46 (1H, dd, J=4.6, 7.3 Hz), 7.69 (1H, d, J=7.9 Hz), 8.63 (1H, s), 8.75 (1H, d, J=4.3 Hz); ^{13}C NMR ($CDCl_3$) δ 13.30, 15.44, 16.05, 20.78, 21.13, 22.86, 24.67, 36.84, 37.00, 40.43, 44.67, 62.30, 64.87, 73.59, 77.20, 85.28, 96.41, 101.24, 123.58, 135.65, 148.05, 150.87, 151.63, 167.58, 169.92, 170.53, 187.33.

Compound **25**

Compound **24** (7 mg, 0.012 mmol) was treated in a similar manner to **20** to give **25** (50 mg, 70%). HR FAB-MS (*m/z*) Found: 597.2836 (M+H), Calcd for $C_{32}H_{41}O_9N_2$: 597.2812; IR (KBr) cm^{-1} 1740, 1640; ^1H NMR ($CDCl_3$) δ 0.89 (3H, s), 1.45 (3H, s), 1.64 (3H, s), 2.05 (3H, s), 2.08 (3H, s), 2.11 (3H, s), 3.33 (3H, s), 3.59 (1H, s), 3.70 (1H, d, J=11.9 Hz), 3.79 (1H, d, J=11.9 Hz), 4.80 (1H, dd, J=5.5, 11.1 Hz), 4.98 (1H, dd, J=4.9, 10.9 Hz), 5.07 (1H, dd, J=2.0, 4.3 Hz), 5.85 (1H, s), 7.42 (1H, ddd, J=0.8, 4.8, 7.9 Hz), 7.66 (1H, dt, J=2.0, 8.3 Hz), 8.62 (1H, d, J=1.7 Hz), 8.72 (1H, dd, J=1.5, 4.8 Hz).

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