Structure−Activity Relationships of Phomactin Derivatives as Platelet Activating Factor Antagonists

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Phomactins, natural products isolated from the culture broth of marine fungus *Phoma* sp., were found to be active as PAF antagonists. This unique carbon skeleton led us to investigate the structure−activity relationship demonstrating that the lipophilicity at C-(7−8), acetoxy, (methoxycarbonyl)oxy, and 3-isoxazolyloxy substitution at C-20, and 2-β-OH configuration at C-2 are all required for the enhancement of inhibitor activity.

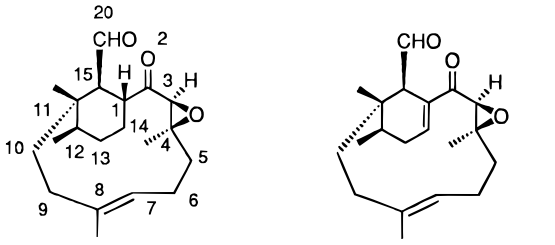
Platelet activating factor (PAF) is a naturally occurring ether phospholipid [1-*O*-alkyl-2(*R*)-acetylglyceryl-3-phosphorylcholine] that is a mediator of anaphylaxis released by a number of stimulated cells, such as basophils, neutrophils, platelets, and macrophages. PAF also causes platelet aggregation, chemotaxis, and degranulation of polymorphonuclear leukocytes, smooth muscle contraction, vascular permeability, and hypotension. Studies have further shown that PAF may be involved in many inflammatory, respiratory, and cardiovascular diseases.[1] Intensive efforts to find drugs which block the effects of PAF have resulted in the discovery of a number of specific PAF antagonists, some of which are being tested for their clinical effectiveness.[2]

During the screening of novel PAF antagonists from natural sources, we systematically screened lipophilic extracts of marine fungal isolates for inhibition of PAF-induced platelet aggregation and binding of PAF to its receptors and found that a marine fungus *Phoma* sp. produced novel PAF antagonists, phomactins.[3] They characteristically bear a unique bicyclo[9.3.1]pentadecane carbon skeleton. This led us to prepare derivatives to examine how each moiety contributes in binding the substrate to the PAF receptor.

As phomactin D (**1**) was identified as the most potent PAF antagonist in the phomactin series (inhibition of platelet aggregation IC_{50} 0.80 μM, inhibition of binding IC_{50} 0.12 μM),[3b] it was initiated as the lead compound and was dissected into three key fragments shown in Figure 1. Fragment A concerns the effect of addition at C-(7−8). Fragments B and C relate to the functional effect at C-20 and the configuration effect at C-2-O respectively. In this paper we describe the structure−activity relationship by virtue of modification in fragments A, B, and C.

Chemistry

On the basis of the structure of phomactin D (**1**), a series of analogues was prepared from a common intermediate **3,** prepared by DIBALH reduction of Sch 47918 (**2**) (phomactin C) (yield 56%).[3b] Efforts to afford **3** in higher yield by trying several reducing agents (namely K-, L-Selectride, LAH, $LiBHEt_3$, $NaBH_4$) were all unsuccessful and generated a 1,2-reduced product at C-2 only. Only DIBALH was chemoselective in affording **3** through 1,4-reduction and therefore was the reagent of choice (Scheme 1).



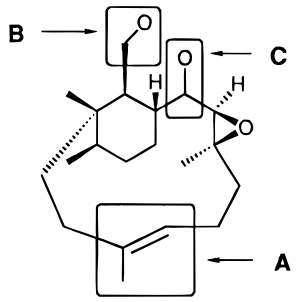


Figure 1. Structure of **1** shown in boxes with fragments of the molecule subjected to SAR investigation.

Fragment A. MCPBA oxidation of **1** and **3** gave the respective epoxides **4** and **5**, whereas oxidation of **1** and **3** with OsO_4 in the presence of *N*-methylmorpholine *N*-oxide provided **6** and **7**, respectively. In both reactions a single diastereomer was obtained, suggesting that oxidation occurred at the less hindered side of the double bond. In NOE experiments of **1** and **3**, irradiation of H-8Me resulted in enhancement of H-15 and H-20, whereas irradiation of H-7 resulted in enhancement of H-12. These results exemplified that the β-side of the C-(7−8) double bond is sterically less hindered than its α-side, thus the MCPBA and OsO_4 presumably attacks the double bond from the less hindered side. Hence the stereochemistry at C-7 and C-8 of **4**, **5**, **6**, and **7** was supposed as being 7*S* and 8*S*, respectively.

Fragment B. To clarify the substitution effects in the portion defined by fragment B, C-20-O derivatives were prepared. While **8** was prepared by condensation of **3** and acetic anhydride in pyridine, **9** and **10** were prepared by the condensation of **3** and *n*-propionyl chloride, phenyl chloroformate, respectively. Treatment of **10** with dimethylamine at −10 °C gave **12.** In a similar manner treatment of **10** with MeOH under basic conditions afforded methyl carbonate **11**.

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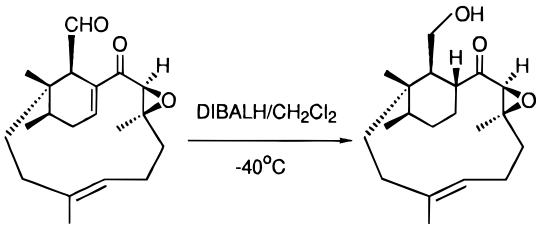
Scheme 1



2 **3**

Attempted condensation of **3** and 3-hydroxyisoxazole under Mitsunobu conditions was unyielding and resulted in recovery of the starting materials due to the strong hydrogen bond between C-20-OH and C-2-carbonyl. After unsuccessful attempts we found that the condensation of diol derivative **14** with 3-hydroxyisoxazole under the same condition gave isoxazole derivative **19**. Subsequent oxidation with PDC in the presence of 4A molecular sieves furnished the desired 2-keto derivative **13**.

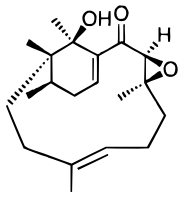
Fragment C. Reduction of all ketone derivatives **3**, **8**, **9**, and **12** with $NaBH_4$ proceeded efficiently with delivery of the hydride onto the α-face to give the corresponding 2-β-OH derivatives **14**, **15**, **16**, and **18**. The proton NMR revealed a coupling constant of 2.3−2.5 Hz between H-2 and H-3, confirming the β-orientation of the C-2 hydroxy. This selectivity is due to the steric hindrance at C-4Me and chelation between C-20-O and the boron atom.

Compound **17** was prepared by acylation of **14** with phenyl chloroformate followed by treatment with MeOH under basic conditions. Formation of **19** is explained in fragment B.

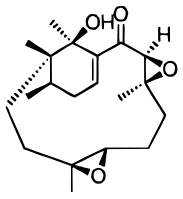
Results and Discussion

In this study a radioreceptor binding assay using rabbit platelets as the receptor source and [^{3}H]PAF as a ligand was employed to evaluate new compounds as potential PAF antagonists. L-652731 (Merck) was prepared as the reference compound.[4]

We have recently reported phomactin E (**20**) and F (**21**) as PAF antagonists.[5] Although **21** differs structurally to **20** only by the functionality present in fragment A, the former is less active than the latter (inhibition of binding phomactin E (**20**) IC_{50} 5.3 μM, phomactin F (**21**) IC_{50} 35.9 μM).



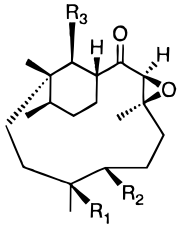
phomactin E (**20**)



phomactin F (**21**)

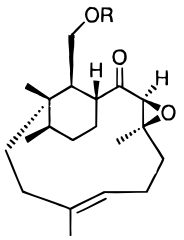
In order to determine whether the hydrophilic function in this fragment would have a negative effect on binding to the receptor, hydrophilic functions were introduced into phomactin D (**1**) and **3**. Inspection of the activities of compounds listed in Table 1 reveals that for a decrease in lipophilicity at C-7−8 there is a corresponding decrease in activity. These results suggest that a hydrophobicity in the vicinity of fragment A is essential to the binding.

Table 1. SAR of Fragment A



compd	$R_1 = R_2$	R_3	IC_{50} (μM)
4	−O−	CHO	17.0
5	−O−	CH_2OH	30.0
6	OH	CH_2OH	>280
7	OH	CH_2OH	>280
phomactin D (**1**)			0.12
3			1.3
L-652731			0.024

Table 2. SAR of Fragment B

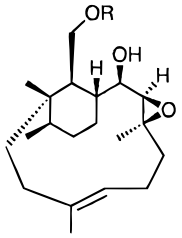


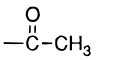
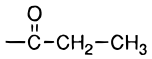
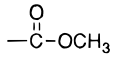
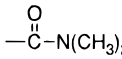
compd	R	IC_{50} (μM)
3	H	1.3
8	$-\overset{O}{\overset{\|}{C}}-CH_3$	0.41
9	$-\overset{O}{\overset{\|}{C}}-CH_2-CH_3$	1.0
10	$-\overset{O}{\overset{\|}{C}}-OPh$	0.55
11	$-\overset{O}{\overset{\|}{C}}-OCH_3$	0.026
12	$-\overset{O}{\overset{\|}{C}}-N(CH_3)_2$	0.18
13		0.05
L-652731		0.024

The derivatives acetoxy (**8**), *n*-propionyloxy (**9**), (dimethylcarbamoyl)oxy (**12**), (methoxycarbonyl)oxy (**11**), and isoxazolyloxy (**13**) (Table 2) were found to be more potent than the parent compound (**3**). Study of the SAR of PAF analogues shows that substitution at the C-2 position by acetoxy, [6] *n*-propionyloxy,[6] (dimethylcarbamoyl)oxy,[7] (methylcarbonyl)oxy,[8] and 3-isoxazolyloxy[9] have a strong affinity to the PAF receptor, while lyso-PAF (2-OH-PAF)[6] has no affinity. These results indicate that aforementioned functions in fragment B facilitate the binding.

The inhibitory activities of derivatives with the 2-β-OH configuration in fragment C were 1.0−8.1 times higher than those of the corresponding 2-keto deriva-

Table 3. SAR of Fragment C



compd.	R	IC_{50} (μM)
14	H	0.74
15		0.08
16		0.34
17		0.013
18		0.18
19		0.031
L-652731		0.024

tives. These results reveal that the 2-β-OH configuration is preferable in binding the substrate to the PAF receptor (Table 3).

In conclusion, the lipophilicity at C-(7−8), acetoxy, (methoxycarbonyl)oxy, and 3-isoxazolyloxy at C-20, and the 2-β-OH configuration at C-2 all enhance inhibitory activity over that of the lead compound **1**. The result obtained in this study will provide useful information for the interaction between the PAF receptor and its ligands. Further pharmacological characterizations of these compounds are in progress.

Experimental Section

4. To a solution of **1** (40.7 mg) in CH_2Cl_2 (5.0 mL) was added MCPBA (80−85%, 30 mg) at 0 °C. After 10 min the reaction mixture was diluted with saturated $NaHCO_3$ solution (30.0 mL) and CH_2Cl_2 (30.0 mL). The dichloromethane layer was concentrated, and the residue was subjected to silica gel column chromatography (hexane−EtOAc, 7:3) to give **4** (16.0 mg): colorless oil; HREIMS [*m*/*z* 334.2137; Δ −0.7 mmu $(M)^+$]; ^1H NMR (CD_3OD) δ 10.15 (1H, s), 3.54 (1H, s), 3.26 (1H, d, *J* = 12.6 Hz), 3.03 (1H, dd, *J* = 5.7, 3.2 Hz), 2.73 (1H, dt, *J* = 5.0, 12.6 Hz), 2.10−2.20 (3H, m), 1.55−1.94 (6H, m), 1.44−1.54 (2H, m), 1.39 (3H, s), 1.26−1.43 (2H, m), 1.25 (3H, s), 0.84 (3H, s), 0.82 (3H, d, *J* = 6.8 Hz).

5. **5** was prepared similarly to the procedure for **4** above. With **3** (50.0 mg) as starting material and using MCPBA (80−85%, 40.0 mg) and CH_2Cl_2 (5.0 mL), **5** (21.3 mg) was obtained: colorless oil; HREIMS [*m*/*z* 336.2314; Δ +1.3 mmu $(M)^+$]; ^1H NMR ($CDCl_3$) δ 4.46 (1H,s), 4.11 (1H, dd, *J* = 10.2, 3.2 Hz), 3.47 (1H, dd, *J* = 10.2, 10.8 Hz), 2.93−2.95 (1H, m), 2.86 (1H, s), 2.60 (1H, dt, *J* = 3.2, 10.8 Hz), 2.35−2.43 (1H, m), 2.16−2.17 (2H, m), 1.97−2.15 (1H, m), 1.91 (1H, brt, *J* = 14.1 Hz), 1.59−1.66 (4H, m), 1.47−1.58 (2H, m), 1.42 (3H, s), 1.30−1.40 (3H, m), 1.23 (3H, s), 0.81 (3H, d, *J* = 6.8 Hz), 0.65 (3H, s).

6. To a solution of **1** (57.8 mg) in CH_3CN-H_2O (3:1, 4mL) was added *N*-methylmorpholine *N*-oxide and OsO_4 (2% in H_2O, 500μL) at room temperature. After 1.5 h, the reaction mixture was diluted with saturated $NaHCO_3$ solution (20.0 mL) and extracted with EtOAc (30.0 mL) twice. The residue was subjected to silica gel column chromatography (hexane−EtOAc, 2:8) to give **6** (20.6 mg): colorless oil; HREIMS [*m*/*z* 352.2234; Δ −1.6 mmu $(M)^+$]; ^1H NMR ($CDCl_3$) δ 9.97 (1H, s), 5.30 (1H, s), 3.72 (1H, brd, *J* = 10.1 Hz), 3.68 (1H, s), 2.92 (1H, d, *J* = 3.4 Hz), 2.71 (1H, d, *J* = 2.1 Hz), 2.26−2.45 (3H, m), 1.32−2.04 (11H, m), 1.29 (3H, s), 1.20 (3H, s), 1.19 (3H, s), 0.90 (3H, d, *J* = 7.2 Hz).

7. **7** was prepared in a manner similar to the procedure for **6** above. With **3** (100.5 mg) as starting material and using OsO_4 (2%, 500 μL) and *N*-methylmorpholine *N*-oxide (49.1 mg), **7** (85.0 mg) was obtained: colorless oil; HREIMS [*m*/*z* 354.2385; Δ −2.1 mmu $(M)^+$]; ^1H NMR (CD_3OD) δ 3.88 (1H, s), 3.83 (1H, d, *J* = 10.4 Hz), 3.82 (1H, dd, *J* = 10.7, 2.0 Hz), 3.43 (1H, dd, *J* = 13.4, 10.7 Hz), 3.08 (1H, brd, *J* = 7.0 Hz), 2.56 (1H, brd, *J* = 10.8 Hz), 2.37−2.42 (1H, m), 2.34 (1H, dt, *J* = 2.0, 13.4 Hz), 1.86 (1H, dt, *J* = 2.4, 14.0 Hz), 1.54 (3H, s), 1.31−1.71 (7H, m), 1.25−1.29 (1H, m), 1.13 (3H, s), 0.78−1.09 (2H, m), 0.73 (3H, d, *J* = 6.7 Hz), 0.69 (3H, s).

8. To a solution of **3** (80.6 mg) in pyridine (2.0 mL) was added Ac_2O (0.5 mL) at room temperature. After 4 h the solvent was removed by evaporation, and the residue was subjected to silica gel column (hexane−EtOAc, 8:2) to give **8** (82.4 mg): colorless crystal; mp 108 °C; HREIMS [*m*/*z* 362.2479; Δ +2.2 mmu $(M)^+$]; ^1H NMR (CD_3OD) δ 5.28 (1H, brd, *J* = 9.0 Hz), 4.33 (1H, dd, *J* = 11.0, 3.3 Hz), 4.04 (1H, dd, *J* = 11.0, 10.9 Hz), 3.97 (1H, s), 3.34 (1H, dt, *J* = 3.3, 10.9 Hz), 2.52 (1H, dt, *J* = 2.4, 13.9 Hz), 2.29−2.38 (1H, m), 1.84−2.11 (7H, m), 1.93 (3H, s), 1.67 (3H, s), 1.27−1.62 (5H, m), 1.18 (3H, s), 0.89 (3H, d, *J* = 6.9 Hz), 0.70 (3H, s). Anal. ($C_{22}H_{34}O_4$) C, H.

9. To a solution of **3** (185.0 mg) in pyridine (3.0 mL) was added *n*-propionyl chloride (100 μL) at room temperature. After 15 min the reaction mixture was diluted with EtOAc (20.0 mL) and saturated $CuSO_4$ solution (20.0 mL). The organic layer was concentrated, and the residue was subjected to silica gel column chromatography (hexane−EtOAc, 8:2) to give **9** (139.9 mg): colorless crystal; mp 108 °C; HREIMS [*m*/*z* 376.2635; Δ +2.1 mmu $(M)^+$]; ^1H NMR (CD_3OD) δ 5.28 (1H, brd, *J* = 7.3 Hz), 4.35 (1H, dd, *J* = 10.9, 3.2 Hz), 4.03 (1H, t, *J* = 10.9 Hz), 3.98 (1H, s), 3.35 (1H, dt, *J* = 3.2, 10.9 Hz), 2.52 (1H, dt, *J* = 2.5, 13.7 Hz), 2.19−2.36 (3H, m), 1.85−2.11 (7H, m), 1.66 (3H, s), 1.27−1.62 (5H, m), 1.18 (3H, s), 1.07 (3H, t, *J* = 7.6 Hz), 0.90 (3H, d, *J* = 6.9 Hz), 0.71 (3H, s). Anal. ($C_{23}H_{36}O_4$) C, H.

10. To a solution of **3** (233.0 mg) and pyridine (2.0 mL) in CH_2Cl_2 (10.0 mL) was added phenyl chloroformate (220 μL) at room temperature. After 30 min the reaction mixture was diluted with EtOAc (20.0 mL) and washed with saturated $CuSO_4$ solution (20.0 mL). The organic layer was concentrated, and the residue was subjected to silica gel column (hexane−EtOAc, 9:1) to give **10** (266.0 mg): colorless crystal; mp 127 °C; HREIMS [*m*/*z* 440.2554; Δ −0.8 mmu $(M)^+$]; ^1H NMR (CD_3OD) δ 7.39 (2H, dd, *J* = 7.4, 7.6 Hz), 7.25 (1H, t, *J* = 7.4 Hz), 7.16 (2H, d, *J* = 7.6 Hz), 5.31 (1H, brd, *J* = 8.6 Hz), 4.53 (1H, dd, *J* = 10.8, 3.4 Hz), 4.29 (1H, dd, *J* = 10.8, 10.5 Hz), 3.94 (1H, s), 3.41 (1H, td, *J* = 10.5, 3.4 Hz), 2.40−2.54 (2H, m), 1.88−2.12 (7H, m), 1.71 (3H, s), 1.31−1.65 (5H, m), 1.19 (3H, s), 0.90 (3H, d, *J* = 6.9 Hz), 0.72 (3H, s). Anal. ($C_{27}H_{36}O_5$) C, H.

11. To a solution of **10** (46.1 mg) in MeOH (3.0 mL) was added one drop of 1 N NaOH at room temperature. After 45 min the mixture was concentrated, and the residue was subjected to silica gel column chromatography (hexane−EtOAc, 8:2) to give **11** (36.5 mg): colorless oil; HREIMS [*m*/*z* 378.2397; Δ −0.9 mmu $(M)^+$]; ^1H NMR (CD_3OD) δ 5.30 (1H, brd, *J* = 8.4 Hz), 4.36 (1H, dd, *J* = 10.7, 3.4 Hz), 4.10 (1H, dd, *J* = 10.7, 10.6 Hz), 3.90 (1H, s), 3.71 (3H, s), 3.33 (1H, dt, *J* = 3.4, 10.6 Hz), 2.52 (1H, dt, *J* = 2.3, 13.7 Hz), 1.85−2.41 (8H, m), 1.67 (3H, s), 1.23−1.62 (5H, m), 1.17 (3H, s), 0.89 (3H, d, *J* = 6.9 Hz), 0.70 (3H, s).

12. To a solution of **10** (122.1 mg) in CH_2Cl_2 (3.0 mL) was added dimethylamine (100 μL) at −10 °C. After 30 min the mixture was concentrated, and the residue was subjected to silica gel column chromatography (hexane−EtOAc, 8:2) to give **12** (107.4 mg): colorless crystal; mp 133 °C; HREIMS [*m*/*z*

391.2701; Δ −2.1 mmu $(M)^+$]; ^{1}H NMR (CD_3OD) δ 5.28 (1H, brd, J = 6.9 Hz), 4.38 (1H, dd, J = 10.7, 3.0 Hz), 3.98 (1H, s), 3.98 (1H, t, J = 10.7 Hz), 3.30 (1H, dt, J = 3.0, 10.7 Hz), 2.88 (3H, s), 2.80 (3H, s), 2.51 (1H, dt, J = 2.6, 13.7 Hz), 2.34−2.42 (1H, m), 1.86−2.12 (7H, m), 1.67 (3H, s), 1.50−1.62 (1H, m), 1.47−1.49 (1H, m), 1.28−1.42 (3H, m), 1.17 (3H, s), 0.90 (3H, d, J = 6.9 Hz), 0.70 (3H, s). Anal. ($C_{23}H_{37}NO_4$) C, H, N.

13. To a solution of **19** (20.0 mg) in CH_2Cl_2 (3.0 mL) was added 4A molecular sieves (500 mg) and PDC (48.2 mg) at room temperature. After 3 h the solution was filtered, and the filtrate was subjected to silica gel column (dichloromethane−acetone, 98:2) to give **13** (29.1 mg): colorless crystal; mp 143 °C; HREIMS [m/z 387.2426; Δ +1.7 mmu $(M)^+$]; UV; ^{1}H NMR (CD_3OD) δ 8.41 (1H, d, J = 1.8 Hz), 6.04 (1H, d, J = 1.8 Hz), 5.38 (1H, brd, J = 7.9 Hz), 4.64 (1H, dd, J = 10.1, 3.2 Hz), 4.14 (1H, t, J = 10.1 Hz), 4.06 (1H, s), 3.54 (1H, dt, J = 3.2, 10.1 Hz), 2.58 (1H, dt, J = 2.6, 13.8 Hz), 2.44−2.53 (1H, m), 1.93−2.17 (7H, m), 1.70 (3H, s), 1.26−1.68 (5H, m), 1.23 (3H, s), 0.94 (3H, d, J = 6.9 Hz), 0.77 (3H, s). Anal. ($C_{23}H_{33}NO_4$) C, H, N.

14. To a solution of **3** (50.0 mg) in dry EtOH (5.0 mL) was added $NaBH_4$ (20.0 mg) at room temperature. After 3 h the solvent was removed by evaporation, and the residue was subjected to silica gel to give **14** (45.5 mg): colorless crystal; mp 112−113 °C; HREIMS [m/z 322.2488; Δ −2.0 mmu $(M)^+$]; ^{1}H NMR (CD_3OD) δ 5.13 (1H, brd, J = 9.0 Hz), 4.70 (1H, dd, J = 4.7, 2.5 Hz), 3.76 (1H, dd, J = 11.5, 4.1 Hz), 3.67 (1H, dd, J = 11.5, 3.7 Hz), 2.96 (1H, d, J = 2.5 Hz), 2.41 (1H, dt, J = 2.6, 13.5 Hz), 2.28 (1H, ddd, J = 11.7, 4.1, 3.7 Hz), 1.78−2.12 (9H, m), 1.57 (3H, s), 1.54 (3H, s), 1.40−1.45 (1H, m), 0.87−1.27 (3H, m), 0.84 (3H, d, J = 6.9 Hz), 0.70 (3H, s). Anal. ($C_{20}H_{34}O_3$) C, H.

15. **15** was prepared similarly to the procedure for **14** above. With **8** (62.4 mg) as starting material and using $NaBH_4$ (30.0 mg) and EtOH (5.0 mL), **15** (59.4 mg) was obtained: colorless crystal; mp 117 °C; HREIMS [m/z 364.2599; Δ −1.4 mmu $(M)^+$]; ^{1}H NMR (CD_3OD) δ 5.16 (1H, brd, J = 9.2 Hz), 4.46 (1H, dd, J = 4.5, 2.5 Hz), 4.29 (1H, dd, J = 12.1, 3.8 Hz), 4.10 (1H, dd, J = 12.1, 4.2 Hz), 2.88 (1H, d, J = 2.5 Hz), 2.57 (1H, ddd, J = 11.4, 4.2, 3.8 Hz), 2.43 (1H, dt, J = 2.6, 13.6 Hz), 1.73−2.08 (9H, m), 2.05 (3H, s), 1.55 (3H, s), 1.55 (3H, s), 1.43−1.48 (1H, m), 1.03−1.31 (3H, m), 0.86 (3H, d, J = 6.9 Hz), 0.68 (3H, s). Anal. ($C_{22}H_{36}O_4$) C, H.

16. **16** was prepared similarly to the procedure for **14**. With **9** (90.8 mg) as starting material and using $NaBH_4$ (30.0 mg) and EtOH (4.0 mL), **16** (85.8 mg) was obtained: colorless crystal; mp 118 °C; HREIMS [m/z 378.2773; Δ +0.3 mmu $(M)^+$]; ^{1}H NMR (CD_3OD) δ 5.16 (1H, brd, J = 8.3 Hz), 4.46 (1H, dd, J = 4.4, 2.4 Hz), 4.31 (1H, dd, J = 12.1, 3.6 Hz), 4.09 (1H, dd, J = 12.1, 4.5 Hz), 2.90 (1H, d, J = 2.4 Hz), 2.59 (1H, ddd, J = 11.4, 3.6, 4.5 Hz), 2.40−2.57 (1H, m), 2.36 (2H, q, J = 7.6 Hz), 1.74−2.06 (9H, m), 1.55 (3H, s), 1.54 (3H, s), 1.43−1.48 (1H, m), 1.03−1.32 (3H, m), 1.14 (3H, t, J = 7.6 Hz), 0.86 (3H, d, J = 6.9 Hz), 0.69 (3H, s). Anal. ($C_{23}H_{38}O_4$) C, H.

17. To a solution of **4** (130.0 mg) and pyridine (200 μL) in CH_2Cl_2 (10.0 mL) was added phenyl chloroformate (60.7 μL) at room temperature. After 30 min the reaction mixture was diluted with EtOAc (20.0 mL) and washed with $CuSO_4$ solution (20.0 mL). The organic layer was concentrated to give an oil (137.1 mg). To a solution of this oil (52.0 mg) in MeOH (3.0 mL) was added 1 drop of NaOH at room temperature. After 3 h the mixture was concentrated, and the residue was subjected to silica gel column chromatography (hexane−EtOAc, 8:2) to give **17** (45.8 mg): colorless oil; HREIMS [m/z 380.2589; Δ +2.7 mmu $(M)^+$]; ^{1}H NMR (CD_3OD) δ 5.13 (1H, brd, J = 9.2 Hz), 4.45−4.81 (1H, m), 4.32 (1H, dd, J = 11.6, 3.9 Hz), 4.16 (1H, dd, J = 11.6, 4.1 Hz), 3.72 (3H, s), 2.82 (1H, d, J = 2.5 Hz), 2.53 (1H, ddd, J = 11.4, 4.1, 3.9 Hz), 2.40 (1H, dt, J = 2.7, 13.5 Hz), 1.71−2.04 (9H, m), 1.51 (3H, s), 1.51 (3H, s), 1.40−1.45 (1H, m), 1.01−1.29 (3H, m), 0.83 (3H, d, J = 6.7 Hz), 0.65 (3H, s).

18. **18** was prepared similarly to the procedure for **14**. With **12** (44.0 mg) as starting material and using $NaBH_4$ (20.0 mg) and EtOH (4.0 mL), **18** (35.6 mg) was obtained: colorless crystal; mp 126−127 °C; HREIMS [m/z 393.2887; Δ +0.8 mmu $(M)^+$]; ^{1}H NMR (CD_3OD) δ 5.15 (1H, brd, J = 9.3 Hz), 4.52−4.96 (1H, m), 4.34 (1H, dd, J = 11.6, 3.3 Hz), 4.05 (1H, dd, J = 11.6, 4.7 Hz), 2.95 (3H, s), 2.92 (4H, s), 2.60 (1H, ddd, J = 11.0, 4.7, 3.3 Hz), 2.44 (1H, dt, J = 2.4, 13.8 Hz), 1.76−2.07 (9H, m), 1.54 (6H, s), 1.43−1.48 (1H, m), 1.04−1.32 (3H, m), 0.86 (3H, d, J = 6.9 Hz), 0.70 (3H, s). Anal. ($C_{23}H_{39}O_4$) C, H, N.

19. To a solution of **14** (60.0 mg), 3-hydroxyisoxazole (79.5 mg), and triphenylphosphine (244.2 mg) was added DEAE (146 μL) at room temperature. After 2.0 h solvent was removed by evaporation, and the residue was subjected to silica gel column (hexane−EtOAc, 7:3) to give **19** (50.0 mg): colorless crystal; mp 178 °C; HRFABMS [m/z 390.1654 Δ +0.9 mmu $(M + H)^+$]; ^{1}H NMR (CD_3OD) δ 8.41 (1H, d, J = 1.8 Hz), 6.16 (1H, d, J = 1.8 Hz), 5.21 (1H, brd, J = 9.3 Hz), 4.56 (1H, dd, J = 4.6, 2.5 Hz), 4.43 (1H, dd, J = 11.0, 3.6 Hz), 4.29 (1H, dd, J = 11.0, 4.3 Hz), 2.99 (1H, d, J = 2.5 Hz), 2.75 (1H, ddd, J = 11.7, 4.3, 3.6 Hz), 2.48 (1H, dt, J = 2.6, 13.5 Hz), 1.84−2.22 (9H, m), 1.59 (3H, s), 1.58 (3H, s), 1.48−1.53 (1H, m), 1.09−1.40 (3H, m), 0.90 (3H, d, J = 6.9 Hz), 0.74 (3H, s). Anal. ($C_{23}H_{35}NO_4$) C, H, N.

Supporting Information Available: The data of ^{13}C NMR, IR, and mass spectra (4 pages). Ordering information is given on any current masthead page.

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