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Chemical Study on Hapalosin, a Cyclic Depsipeptide Possessing Multidrug Resistance Reversing Activities: Synthesis, Structure and Biological Activity[1]

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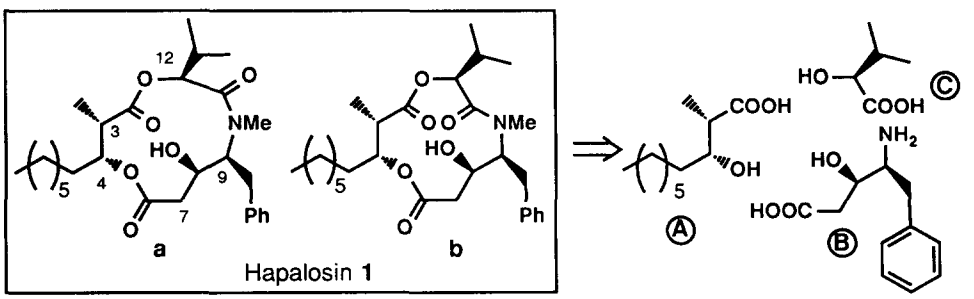
Abstract: Hapalosin **1** possessing a multidrug resistance reversing activity, has been synthesized from the corresponding hydroxy acids **A**, **C** and γ-amino acid **B**. The stereochemistry of the natural product **1** and N-demethylhapalosin **11** is discussed by means of spectroscopic manner as well as molecular modeling studies. Biological evaluation of **1** and **11** indicated that a cis-amide function is a crucial factor for the MDR reversing activity. Copyright © 1996 Elsevier Science Ltd

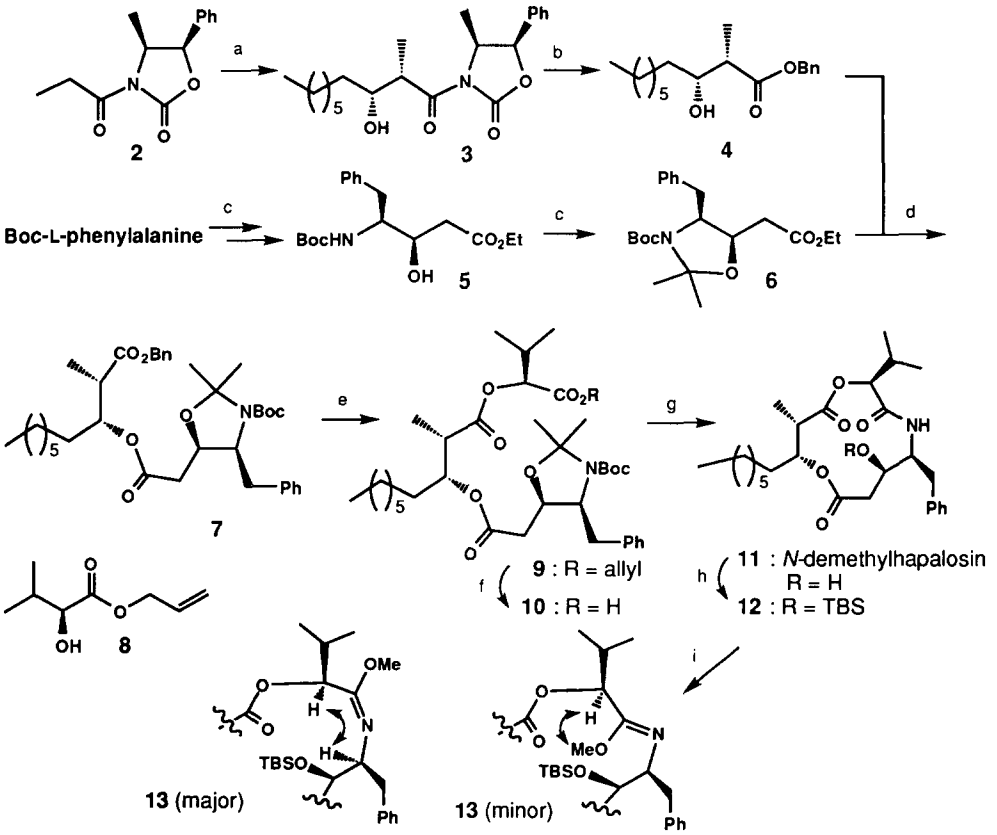
Chemotherapeutic treatment of cancer disease has encountered multidrug resistance (MDR) mediated by P-glycoprotein, which acts as a ATP-dependent drug efflux pump.[2] Among numerous natural and unnatural substances examined to solve this urgent problem, such agents as reserpine, quinidine, verapamil and related derivatives have been found to possess MDR reversing activities.[3] Recently, hapalosin **1**, a 12-membered cyclic depsipeptide isolated from the blue green alga *Hapalosiphon welwitschii* W. & G. S. West, was included as a new member of this family.[4] The cyclic structure bearing comparable activity to verapamil as well as low cytotoxicity (IC_{50} = 5 ~ 15 μM) might be a lead to develop new MDR reversing agents. From a stereochemical viewpoint, **1** was obtained as an inseparable mixture of conformers (**1a/1b** = ~3:1) around the amide function, and their gross stereostructures had not been elucidated. Accordingly, respective activities of both conformers had not been clarified. The chemical and biological interests in the molecule prompted us to initiate synthesis of **1** and its congeners.[5] The retrosynthetic analysis indicated that **1** might be constructed by a sequential coupling of 3-hydroxy-2-methylbutylic acid **A**, 3-hydroxy-4-amino acid **B** and 2-hydroxy acid



C. Final production of the amide linkage followed by the N-methylation, might set up congeners carrying diverse N-alkyl groups.

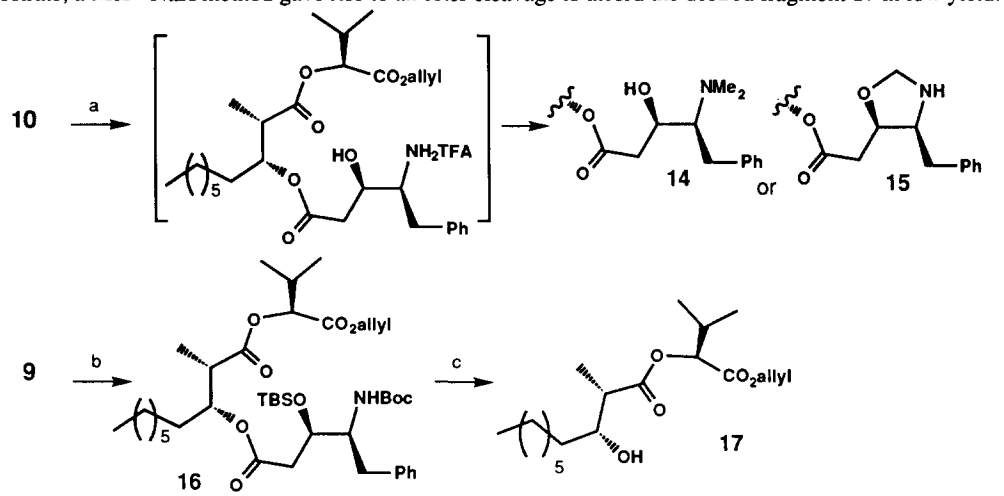
RESULTS AND DISCUSSION

According to the Evans aldol protocol,[6] the stereogenic centers of **A** were produced by coupling of *N*-propionyl-(4*S*,5*R*)-4-methyl-5-phenyl-2-oxazolidinone (**2**)[7] with octanal in the presence of Bu_2BOTf and Et_3N, leading to **3** in 90% yield, which on recrystallization contained no diastereomers detectable by the ^1H NMR spectrum. The chiral auxiliary was converted in 71% yield into the corresponding benzyl ester **4** by a standard procedure using BnOLi. Compound **4** was submitted to coupling with a carboxylic acid prepared from **6**[8] under the DCC conditions to give **7** in 86% yield based on **6**. After removal of the benzyl protective group of **7**, introduction of the third fragment **8** provided the fully functionalized seco derivative **9** in 81% yield from **7**. Treatment of **9** with $(Ph_3P)_4Pd$ furnished carboxylic acid **10** (80%), which underwent acid hydrolysis, followed by cyclization under the high dilution conditions (1 mmol/l) to yield desmethylhapalosin **11** in 83% yield. Transformation into the natural product **1** by employing the protected derivative **12** was troublesome, probably owing to low reactivity of the amide part under the usual N-methylation conditions examined (MeI - NaH, MeI - Ag_2O and MeI - LDA). During such attempts, exposure to Me_3OBF_4 and

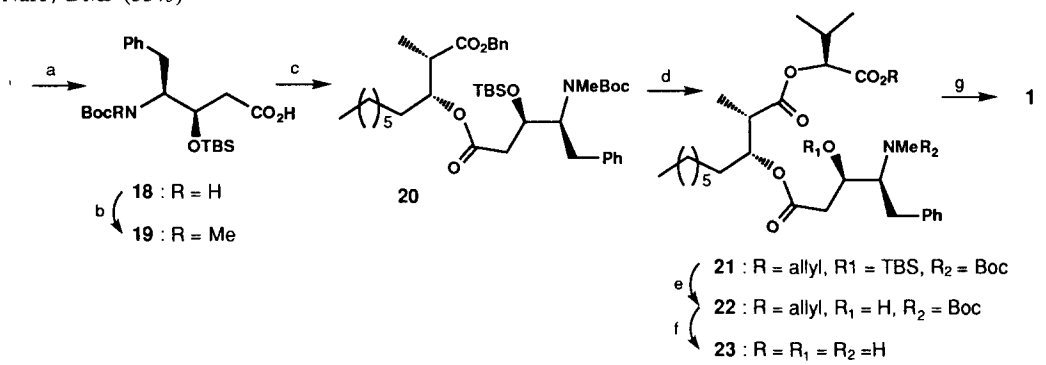


Scheme 1. *Reagents*: a. Bu_2BOTf, Et_3N / CH_2Cl_2, then $Me(CH_2)_6CHO$ (90%). b. nBuLi, BnOH / THF (71%). c. ref. 8. d. i) NaOH; ii) **4**, DCC, DMAP / CH_2Cl_2 (86% in two steps). e. i) H_2, $Pd(OH)_2$ / EtOH: ii) **8**, DCC, DMAP/CH_2Cl_2 (81% in two steps). f. $(Ph_3P)_4Pd$, morpholine/THF (80%). g. i) TFA / CH_2Cl_2: ii) DPPA, iPr_2NEt / DMF (1 mmol/l) (83% in two steps). h. TBSCl, Imd / DMF (92%). i. Me_3OBF_4, Proton Sponge™ / CH_2Cl_2 (66%).

Proton Sponge™,[9] produced **13**[10] as a 1.8:1 (cis / trans) mixture of geometrical isomers, whose structures were elucidated by the NOESY experiments, as depicted in Scheme 1. Interestingly, preference of the formation of the cis-type product is similar to the ratio of the natural conformers.[4] Additionally, N-methylation by using the primary amine was also unsuccessful; alkylation with HCHO - $NaBH_3CN$ gave dimethyl derivative **14** or acetal **15**, depending on the reaction conditions. Upon employing **16** as a substrate, a MeI - NaH method gave rise to an ester cleavage to afford the desired fragment **17** in low yield.



Scheme 2. *Reagents*: a. i) TFA / CH_2Cl_2: ii) HCHO, $NaBH_3CN$ / MeOH (**14**, 72%): i) TFA / CH_2Cl_2: ii) HCHO, then $NaBH_3CN$ (**15**, 67%). b. i) p-TsOH / MeOH (67%): ii) TBSCl, Imd / DMF (97%). c. MeI, NaH / DMF (35%)

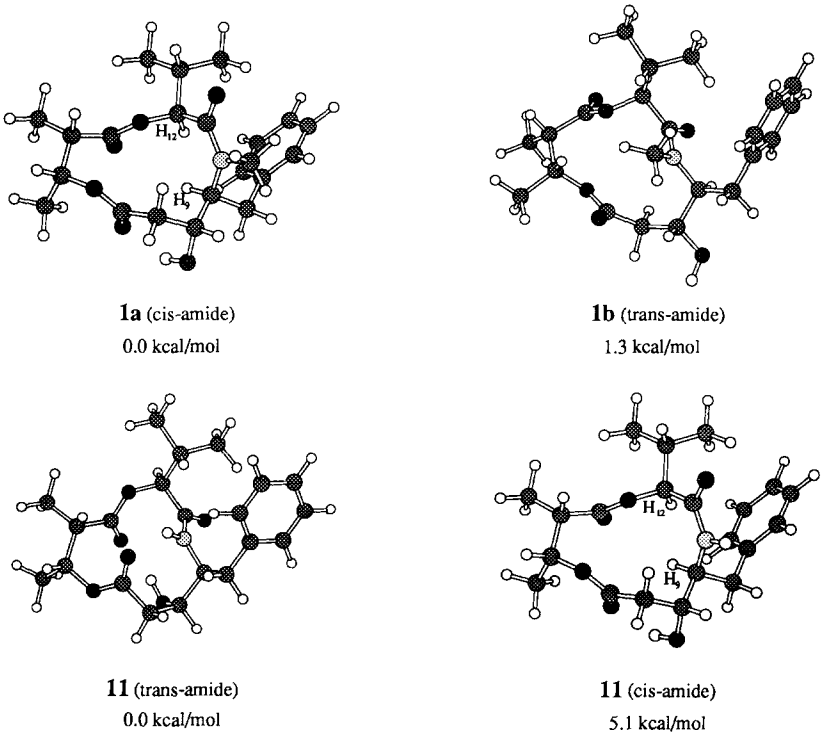


Scheme 3. *Reagents*: a. i) TBSCl, Imd / DMF (100%): ii) 2M NaOH (84%). b. MeI, NaH / THF (77%). c. **4**, DCC, DMAP / CH_2Cl_2 (77%). d. i) H_2, $Pd(OH)_2$ / EtOH: ii) **8**, DCC, DMAP / CH_2Cl_2 (96% in two steps). e. HF / pyridine (87%). f. i) $(Ph_3P)_4Pd$, morpholine / THF (97%): ii) TFA / CH_2Cl_2 (94%): g. DPPA, iPr_2NEt / DMF (1 mmol/l) (44%).

Successful transformation into the target molecule was achieved by employing **19** carrying the N-methyl group as fragment **C** equivalent. Thus, a hydroxyl group of **5** was protected as a siloxy ether (100%), followed by alkaline hydrolysis, leading to **18** in 84% yield. The desired N-methylation of **18** was effected under MeI – NaH conditions[11] to provide **19** in 77% yield. Compound **19** in hand was directly coupled with **4**, followed by reductive abstraction of a benzyl group, and further coupling with **8** to give **20** in a moderate overall yield. After successive removal of a siloxy (87%) and an allyl (97%) protective groups of **20**,

hydrolysis with TFA (94%) provided a pair of a free carboxylic acid and an ammonium salt. Final cyclization as described in the case of **11** afforded hapalosin **1** in 44% yield, exhibiting superimposable spectral data to those reported for the natural product.[4] Detailed ^{1}H NMR study indicated that synthetic **1** at room temperature existed as a ~3:1 (**1a** / **1b**) mixture of the conformers as reported by Moor. The ratio was reversibly changed to 1.7:1 at 100 °C (DMSO-d_6). The NOE experiments indicated the major **1a** exhibited a correlation between H_9 and H_{12} ascribed to a cis-amide, while the minor **1b** adopted a trans-amide without a NOE effect at the same position.[12] Interestingly, contrary to the case of **1**, the ^{1}H NMR spectrum of N-demathylhapalosin **11** showed the presence of a trans-amide as a single isomer.

Molecular Modeling Study. Monte Carlo conformational analysis was carried out using Macromodel[13a] with MM2*[13b] force field. The heptyl group was replaced with a methyl group in order to reduce the number of conformations. The lowest energy conformation for the cis-amide form of hapalosin (**1a**) was calculated to be 1.3 kcal/mol more stable than the trans-amide form (**1b**), which is consistent with our experimental findings. The preference for the cis-amide form is mainly due to the steric repulsion between the N-methyl and the isopropyl groups. The H_9-H_{12} distance in the force field optimized structures are 2.08 Å and 4.49 Å, respectively. Similar procedure was carried out for N-demethylhapalosin **11**. The lowest energy conformation of the cis-amide was found to be 5.1 kcal/mol higher than that of the trans-amide. The relative energy between trans- and cis-N-methyl acetamide was calculated to be 1.9 kcal/mol in favor of the trans conformation. Calculation on **11** indicated that the trans conformation has additional 3 kcal/mol preference.



1a (cis-amide)
0.0 kcal/mol

1b (trans-amide)
1.3 kcal/mol

11 (trans-amide)
0.0 kcal/mol

11 (cis-amide)
5.1 kcal/mol

Figure 1. Lowest Energy Conformations of the Cis- and Trans-amides of Hapalosin (**1**) and N-Demethylhapalosin (**11**) Optimized by MM2* Force Field Calculations.

The extra preference is probably due to an additional intramolecular hydrogen bondings in trans-**11** which may not exist in a polar solvent. Monte Carlo conformational search with GB/SA solvation (water) model calculations was performed to take this effect into account. As a result, the cis-preference for **1** stayed same, while the trans-preference for **11** was reduced to 3.7 kcal/mol. The computational results clearly explain why only the trans-amide was observed in N-demethylhapalosin (**11**).

Biological Activity. To elucidate structure - activity relationship, MDR reversing activities of **1** and **11** were examined by the human breast cancer cell lines MCF-7 and Adr^R (adriamycin resistant). As can be seen in Fig. 2, **1** during 10 ~ 2.5 μM concentration exhibited a similar activity to those of verapamil used as a positive control, although **11** indicated no remarkable activities. Our results could not reproduce the observation by Moore et al. that natural **1** has a more potent activity than verapamil.[4] However, it should be mentioned that **11** possessing a trans-amide exhibited few MDR reversing activity. Accordingly, the cis-amide conformation assisted by the N-methyl group may be a crucial factor for expression of the MDR activity. Evaluation of relationship between size of the N-alkyl group and the activity is in progress.

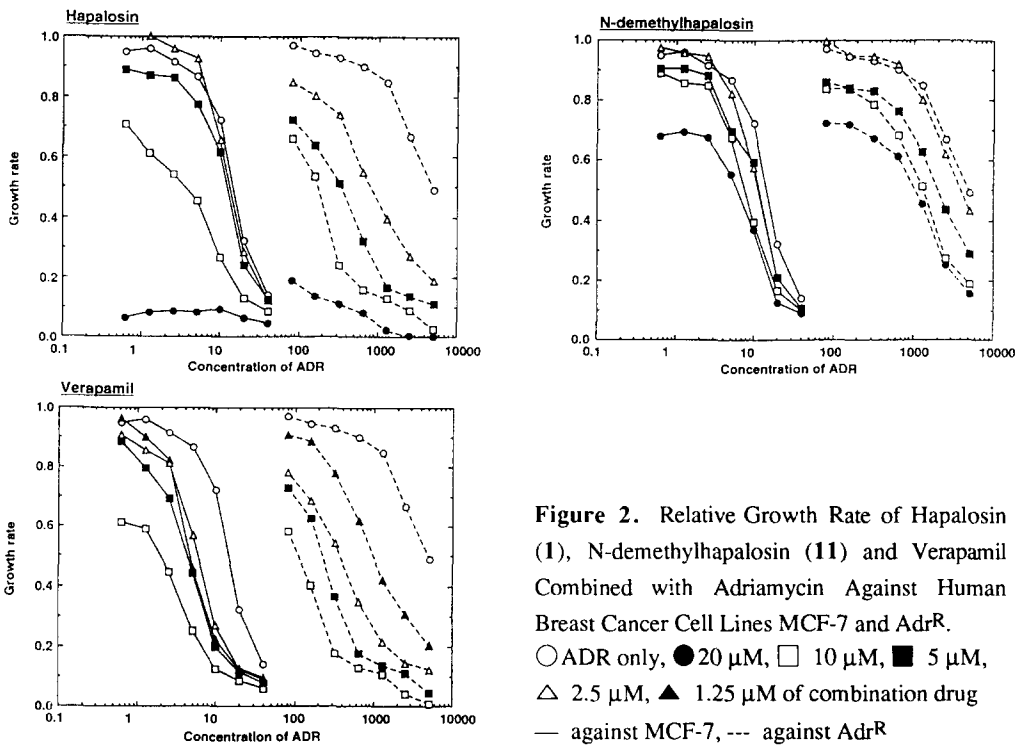


Figure 2. Relative Growth Rate of Hapalosin (**1**), N-demethylhapalosin (**11**) and Verapamil Combined with Adriamycin Against Human Breast Cancer Cell Lines MCF-7 and Adr^R. ○ADR only, ●20 μM, □ 10 μM, ■ 5 μM, △ 2.5 μM, ▲ 1.25 μM of combination drug — against MCF-7, --- against Adr^R

EXPERIMENTAL

All of the melting points were obtained on a Yanaco MP-S3 melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO Model A-202 spectrophotometer. ^1H NMR and ^{13}C NMR spectra were obtained on a JEOL JNM EX-270, a JEOL JNM GX-400 or a JNM ALPHA-400 NMR spectrometer in a

deuteriochloroform ($CDCl_3$) solution using tetramethylsilane as an internal standard, unless otherwise stated. Optical rotations were recorded on a JASCO DIP-360 digital polarimeter. High-resolution mass spectra were obtained on a Hitachi M-80 GC-MS spectrometer operating at the ionization energy of 70 eV. Preparative and analytical TLC were carried out on silica-gel plates (Kieselgel 60 F_{254}, E. Merck A. G., Germany) using UV light and/or 5% molybdophosphoric acid in ethanol for detection. Katayama silica-gel (K 070) was used for column chromatography.

(4*S*,5*R*)-3-[(2*S*,3*R*)-3-Hydroxy-2-methyl-1-oxodecanyl]-4-methyl-5-phenyl-2-oxazolidinone (3). To a precooled solution of *N*-propionyl-(4*S*,5*R*)-4-methyl-5-phenyl-2-oxazolidinone (**2**, 451 mg, 1.9 mmol) in CH_2Cl_2 (10 ml) was added Et_3N (0.33 ml, 2.4 mmol), followed by the dropwise addition of 1M solution of Bu_2BOTf in CH_2Cl_2 (2.2 ml, 2.2 mmol) at 0 °C. The mixture was stirred at the same temperature for 30 min. Octanal (0.33 ml, 2.1 mmol) was then added dropwise at -78 °C. The solution was stirred at -78 °C for 30 min, then at room temperature for 2 h. The reaction was quenched with phosphate buffer (pH 7.0, 10 ml), and the mixture was extracted with CH_2Cl_2. The organic extracts were combined, dried (Na_2SO_4), then concentrated in vacuo. The residue was dissolved in MeOH (10 ml), and 30% aqueous H_2O_2 (6 ml) was added at 0 °C. After being stirred at 0 °C for 1 h, the reaction was quenched by the addition of 10% aq. $NaHSO_4$. The mixture was extracted with EtOAc, and the organic extracts were washed with sat.aq. $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography (50 g, hexane/EtOAc = 3:1), then recrystallized from EtOAc - hexane to afford **3** (629 mg, 90%) as needles: mp 107 - 108 °C: $[\alpha]_D^{21}$ -16.2° (*c* 1.00, $CHCl_3$); IR (film) 3470, 1760, and 1680 cm^{-1}; δ_H 0.9 (3H, br. d, *J*= 6 Hz), 1.22 - 1.29 (18H, complex), 2.86 (1H, d, *J*= 3 Hz), 3.77 (1H, m), 3.96 (1H, m), 4.79 (1H, m), 5.68 (1H, d, *J*= 7.2 Hz), and 7.25 - 7.46 (5H, complex); δ_C 10.1, 14.0, 14.3, 22.5, 25.9, 29.1, 29.4, 31.7, 33.8, 42.1, 54.0, 71.5, 78.8, 125.5, 128.6, 128.7, 133.0, 152.5, and 177.3. Found: C, 69.65; H, 8.94; N, 3.90%. Calcd for $C_{21}H_{31}NO_4$: C, 69.78; H, 8.64; N, 3.87%.

Benzyl (2*S*,3*R*)-3-Hydroxy-2-methyldecanoate (4). To a solution of **3** (243 mg, 0.67 mmol) in THF (5 ml) at -78 °C was added nBuOLi in THF (5 ml) prepared from nBuLi (2 ml, 3.4 mmol, 1.7M hexane solution) and BnOH (0.35 ml, 3.4 mmol); the mixture was stirred at the same temperature for 30 min, then at 0 °C for 1h. After the addition of aq. NH_4Cl, The resulting mixture was extracted with EtOAc. The organic extracts were washed with brine, dried (Na_2SO_4), and evaporated to dryness. The residue was purified by silica gel column chromatography (15 g, hexane/EtOAc = 10:1 → 3:1) to give **4** (140 mg, 71%) as an oil: $[\alpha]_D^{24}$ +3.7° (*c* 1.00, $CHCl_3$); IR (film) 3450 and 1715 cm^{-1}; δ_H 0.88 (3H, t, *J*= 7 Hz), 1.16 - 1.46 (15H, complex), 2.56 (2H, complex), 3.90 (1H, m), 5.14 (2H, s), and 7.31 (5H, complex); δ_C 10.7, 14.0, 22.6, 25.9, 29.1, 29.4, 31.7, 33.8, 44.4, 66.3, 71.2, 128.1, 128.2, 128.5, 135.8, and 175.8. Found: C, 73.67; H, 9.94%. Calcd for $C_{18}H_{28}O_3$: C, 73.93; H, 9.65%.

Benzyl (2*S*,3*R*)-3-[(4*S*,5*R*)-4-Benzyl-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-5-oxazolidineacetoxy]-2-methyldecanoate (7). Ethyl ester **6** (5.07 g, 13 mmol) was hydrolyzed according to the known procedure.[8] A mixture of the resulting carboxylic acid, **4** (3.90 g, 13.4 mmol), DCC (4.5 g, 22 mmol), and DMAP (0.23 g, 1.9 mmol) in CH_2Cl_2 (70 ml) was stirred at 0 °C for 2 h, then at room temperature for 40 h. The reaction mixture was diluted with 10% aq. citric acid, and extracted with CH_2Cl_2. The organic extracts were washed with sat.aq. $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by column chromatography (200 g, hexane/EtOAc = 10:1 → 3:1) to give **7** (7.22 g, 86% in two steps) as an oil: $[\alpha]_D^{16}$ -16.4° (*c* 1.00, $CHCl_3$); IR (film) 1740 and 1695 cm^{-1}; δ_H 0.87 (3H, t, *J*= 6.6 Hz), 1.14 (3H, d, *J*= 6.9 Hz), 1.22-1.93 (26H, complex), 2.35 (2H, complex), 2.56 - 2.86 (3H, complex), 3.19 (1H, m), 4.25 (1H, m), 4.40 (1H, m), 5.09 (2H, s), 5.16 (1H, m), and 7.21 - 7.33

(10H, complex); δ_C 12.0, 14.0, 22.5, 23.6, 25.3, 27.4, 28.1, 28.3, 29.0, 29.2, 31.7, 31.8, 35.0, 36.4, 43.1, 60.0, 66.4, 73.1, 74.6, 77.2, 93.1, 126.1, 128.2, 128.3, 128.5, 129.3, 135.7, 138.4, 151.6, 169.8, and 173.4. Found: C, 71.14; H, 8.74; N, 2.26%. Calcd for $C_{37}H_{53}NO_7$: C, 71.24; H, 8.56; N, 2.25%.

2-Propenyl (*S*)-2-Hydroxy-3-methylbutanoate (8). A mixture of 2-hydroxy acid prepared by the known procedure[14] from L-valine (13.7 g, 0.12 mol), allyl bromide (80 ml, 0.92 mol), and $NaHCO_3$ (52.5 g, 0.62 mol) in DMF (100 ml) was stirred at room temperature for 20 h. The reaction mixture was partitioned between EtOAc and H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (300 g, hexane/EtOAc = 5:1) to give **8** (9.1 g, 49% in two steps) as an oil: $[\alpha]_D^{19}$ -0.6° (*c* 1.00, $CHCl_3$); IR (film) 3510 and 1725 cm^{-1}; δ_H 0.84 (3H, d, *J*= 6.8 Hz), 0.99 (3H, d, *J*= 6.8 Hz), 2.06 (1H, m), 2.85 (1H, d, *J*= 3.6 Hz), 4.03 (1H, m), 4.63 (2H, complex), 5.24 (1H, br. d, *J*= 10.2 Hz), 5.31 (1H, dd, *J*= 1.3, 17 Hz) and 5.89 (1H, m); δ_C 15.9, 18.7, 32.1, 66.0, 75.0, 119.1, 131.4, and 174.6.

2-Propenyl (2*S*)-2-[(2*S*,3*R*)-3-[(4*S*,5*R*)-4-Benzyl-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-5-oxazolidineacetoxy]-2-methyldecanoyloxy]-3-methylbutanoate (9). A solution of **7** (52 mg, 0.084 mmol) in EtOH (1.5 ml) in the presence of $Pd(OH)_2$ (6 mg) was stirred at room temperature for 2 h under a hydrogen atmosphere. The reaction mixture was filtered, and the filtrate was evaporated to give a carboxylic acid, which was dissolved in CH_2Cl_2 (1.5 ml). After the addition of **8** (20 mg, 0.13 mmol), DCC (30 mg, 0.15 mmol) and DMAP (1.8 mg, 0.015 mmol), the mixture was stirred at 0 °C for 2 h, then at room temperature for 12 h. The reaction mixture was filtered, and the filtrate was partitioned between CH_2Cl_2 and 10% aq. citric acid. The organic extracts were washed with sat.aq. $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated in vacuo. A crude product was purified by silica gel column chromatography (5 g, hexane/EtOAc = 4:1) to yield **9** (45 mg, 81% from **7**) as an oil: $[\alpha]_D^{19}$ -25° (*c* 1.00, $CHCl_3$); IR (film) 1740, 1695, and 1465 cm^{-1}; δ_H 0.84 (3H, t, *J*= 6.6 Hz), 0.95 (6H, complex), 1.13 - 1.90 (30H, complex), 2.20 (1H, m), 2.43 (1H, m) 2.60 - 2.77 (3H, complex), 3.16 (1H, m), 4.25 (1H, m), 4.44 (1H, m), 4.57 (2H, d, *J*= 5.6 Hz), 4.78 (1H, d, *J*= 4.6 Hz), 5.10 - 5.31 (3H, complex), 5.82 (1H, m), and 7.16 - 7.24 (5H, complex); δ_C12.5, 14.0, 17.2, 18.7, 22.6, 24.6, 25.3, 25.4, 27.4, 28.1, 28.3, 29.1, 29.3, 30.0, 31.7, 31.8, 34.9, 36.4, 43.0, 55.7, 60.0, 65.6, 74.6, 76.8, 77.2, 93.1, 118.7, 126.1, 128.3, 128.4, 129.4, 131.6, 151.7, 168.9, 170.0, and 173.2. Found: C, 67.82; H, 9.18; N, 2.45%. Calcd for $C_{38}H_{59}NO_9$: C, 67.73; H, 8.82; N, 2.08%.

(2*S*)-2-[(2*S*,3*R*)-3-[(4*S*,5*R*)-4-Benzyl-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-5-oxazolidineacetoxy]-2-methyldecanoyloxy]-3-methylbutanoic Acid (10). To a solution of **9** (194 mg, 0.29 mmol) in THF (5 ml) under an argon atmosphere were added $(Ph_3P)_4Pd$ (0) (22 mg, 5 mol%) and morpholine (0.25 ml, 2.9 mmol). After being stirred at room temperature for 4 h, the mixture was partitioned between EtOAc and 10% aq. citric acid. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was separated by silica gel column chromatography (5 g, $CHCl_3$/MeOH = 10:1) to give **10** (146 mg, 80%) as an oil: $[\alpha]_D^{21}$ -18° (*c* 1.00, $CHCl_3$); IR (film) 3450, 1740, 1695, and 1590 cm^{-1}; δ_H 0.83-0.92 (9H, complex), 1.13 - 1.66 (30H, complex), 2.22 (1H, m), 2.42 (1H, m), 2.62 - 2.84 (4H, complex), 4.26 - 4.49 (2H, complex), 4.70 (1H, m), 5.21 (1H, m), and 7.18 - 7.24 (5H, complex); δ_C 12.6, 14.5, 17.5, 19.7, 23.1, 24.1, 25.9, 27.9, 28.6, 28.8, 29.7, 30.2, 32.2, 33.0, 35.7, 36.9, 60.5, 73.7, 75.4, 77.7, 80.0, 80.6, 93.6, 126.7, 128.7, 128.9, 129.9, 152.1, 171.1, 176.4, and 186.7.

***N*-Demethylhapalosin (11).** To a solution of **10** (146 mg, 0.23 mmol) in CH_2Cl_2 (5 ml) was added TFA (1 ml); the mixture was stirred at room temperature for 3 h. After evaporation to dryness, the residue was dissolved in DMF (200 ml, 1 mmol/l), and DPPA (0.15 ml, 0.69 mmol) and iPr_2NEt (0.24 ml, 1.4 mmol) were dropwisely added at 0 °C. After being stirred at 0 °C for 3 h followed by at room temperature for 13 h, the reaction mixture was partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. Purification of the residue by silica gel column chromatography (5 g, $CHCl_3$/MeOH = 50:1 → 10:1) to give **11** (91 mg, 83% from **10**) as an amorphous solid: $[\alpha]_D^{22}$ -32° (*c* 0.50, $CHCl_3$); IR (film) 3320, 1735, 1665, 1595, and 1490 cm^{-1}; δ_H 0.66 (3H, d, *J*= 6.8 Hz), 0.85 - 0.92 (6H, complex), 1.20 - 1.81 (16H, complex), 2.57 (1H, dd, *J*= 5.6, 14 Hz), 2.65 (1H, dd, *J*= 3.6, 14 Hz), 2.87 - 3.04 (3H, complex), 4.08 (1H, br. s), 4.53 (1H, d, *J*= 8 Hz), 4.63 (1H, m), 5.46 (1H, d, *J*= 10.8 Hz), 5.52 (1H, m), and 7.24 (5H, complex); δ_C 9.2, 14.1, 17.7, 18.5, 22.6, 25.5, 29.1, 29.3, 29.9, 31.0, 31.7, 37.5, 39.0, 41.2, 54.0, 70.6, 75.8, 81.8, 126.7, 128.6, 129.0, 136.9, 169.8, 173.4, and 174.1. Found: m/z 475.2945. Calcd for $C_{27}H_{41}NO_6$: M, 475.2932.

8-*tert*-Butyldimethylsilyl-*N*-demethylhapalosin (12). A mixture of **11** (16 mg, 0.034 mmol), TBSCl (29 mg, 0.19 mmol), and imidazole (50 mg, 0.74 mmol) in DMF (2 ml) was stirred at room temperature for 15 h. The reaction mixture was partitioned between EtOAc and H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (3 g, hexane/EtOAc = 20:1 → 5:1) to afford **12** (19 mg, 92%) as an oil: $[\alpha]_D^{21}$ -44.2° (*c* 1.00, $CHCl_3$); IR (film) 3400, 1735, 1665, and 1545 cm^{-1}; δ_H 0.13 (3H, s), 0.14 (3H, s), 0.56 (3H, d, *J*= 6.8 Hz), 0.76 (3H, d, *J*= 6.8 Hz), 0.86 - 1.73 (27H, complex), 2.48 (2H, d, *J*= 5.6 Hz), 2.66 (1H, dd, *J*= 9.4, 13.9 Hz), 3.07 (1H, m), 3.22 (1H, dd, *J*= 4.3, 13.9 Hz), 4.21 - 4.41 (2H, complex), 4.80 (1H, d, *J*= 8.2 Hz), 5.16 (1H, m), 5.55 (1H, d, *J*= 10.2 Hz), and 7.2 (5H, complex); δ_C -4.5, -4.7, 10.7, 14.0, 17.8, 18.2, 22.6, 25.7, 29.1, 29.3, 29.5, 30.4, 31.7, 38.8, 40.5, 40.9, 55.7, 71.7, 75.7, 80.0, 126.5, 128.3, 129.2, 137.7, 169.0, 171.7, and 173.0.

Reaction of 12 with Proton Sponge™ and Me_3OBF_3 (13a and 13b). A mixture of **12** (30 mg, 0.051 mmol), Proton Sponge™ (163 mg, 0.76 mmol), and Me_3OBF_3 (113 mg, 0.77 mmol) in CH_2Cl_2 (3 ml) was stirred at room temperature for 20 h. The resulting mixture was partitioned between EtOAc and 5% aq. citric acid. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated to dryness. The residue was purified by preparative TLC (hexane/EtOAc = 5:1) to give **13** (7 mg, 23%) as a 1.8:1 mixture of **13a** (*cis*) and **13b** (*trans*), along with unreacted **12** (20 mg, 67%). **13a**: δ_H 0.02 - 0.19 (6H, complex), 0.52 (3H, d, *J*= 6.8 Hz), 0.86 - 0.92 (6H, complex), 0.95 (9H, s), 1.17 (3H, d, *J*= 7.6 Hz), 1.20 - 1.49 (11H, complex), 1.95 - 2.05 (2H, complex), 2.30 (1H, dd, *J*= 10.0, 12.8 Hz), 2.54 (1H, dd, *J*= 5.6, 16.2 Hz), 2.76 (1H, dd, *J*= 2.4, 16.2 Hz), 2.87 (1H, m), 3.34 (1H, dd, *J*= 2.1, 12.8 Hz), 3.72 (1H, ddd, *J*= 2.1, 7.9, 10.0 Hz), 3.88 (1H, ddd, *J*= 2.4, 5.6, 7.9 Hz), 4.22 (1H, d, *J*= 8.8 Hz), 5.16 (1H, m), and 7.08 - 7.31 (5H, complex)

Conversion of 10 into the *N*,*N*-Dimethyl Derivative 14. To a solution of **10** (99 mg, 0.16 mmol) in CH_2Cl_2 (3 ml) was added TFA (0.6 ml); the reaction mixture was stirred at room temperature for 4 h. The resulting mixture was concentrated in vacuo, and the residue was dissolved in MeOH (3 ml). After the addition of 3.5% HCHO in aq. MeOH (0.16 ml, 0.20 mmol, prepared by 10-fold dilution of 35% aq. HCHO with MeOH) and $NaBH_3CN$ (9.8 mg, 0.16 mmol), the mixture was stirred at 0 °C for 5 h, and partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), then evaporated. The residue was purified by preparative TLC ($CHCl_3$/MeOH = 10:1) to yield **14** (53 mg, 64%) and unreacted **10** (10 mg, 10%). **14** as an oil: IR (film) 3400, 1730, and 1600 cm^{-1}; δ_H 0.87 (3H, t, *J*= 6.8 Hz), 0.95 (3H, d, *J*= 7 Hz), 0.99 (3H, d, *J*= 7 Hz), 1.17 (3H, d, *J*= 7.6 Hz), 1.25 (10H, complex), 1.52

(1H, m), 1.75 (1H, m), 2.25 (3H, complex), 2.40 (1H, m), 2.70 (1H, m), 2.82 (1H, m), 3.01 (1H, dd, *J*= 6.8, 14.8 Hz), 3.24 (1H, dd, *J*= 6.8, 14.8 Hz), 3.50 (1H, m), 4.63 (1H, m), 4.71 (1H, m), 5.23 (1H, m), and 7.25 (5H, complex); δ_C 10.8, 14.1, 17.6, 19.3, 22.6, 25.7, 29.1, 29.3, 29.5, 29.7, 29.9, 30.4, 31.7, 41.1, 41.3, 66.8, 69.2, 74.8, 79.8, 126.9, 128.8, 129.2, 137.8, 170.6, 173.8, and 175.2.

Conversion of 10 into Acetal 15. To a solution of **10** (106 mg, 0.16 mmol) in CH_2Cl_2 (3 ml) was added TFA (0.6 ml); the reaction mixture was stirred at room temperature for 4 h. The resulting mixture was evaporated. A mixture of the residue and 3.5% HCHO in aq. MeOH (0.093 ml, 0.10 mmol, prepared by 10-fold dilution of 35% aq. HCHO with MeOH) in MeOH (4 ml) was stirred at 0 °C for 1 h, then $NaBH_3CN$ (9.8 mg, 0.16 mmol) was added. After being stirred at 0 °C for 2 h, the mixture was partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. Chromatographic purification ($CHCl_3$/MeOH = 10:1) of the residue provided 15 (49 mg, 55%) and unreacted **10** (18 mg, 17%). **15** as an oil: δ_H 0.88 (3H, t, *J*= 7.2 Hz), 0.97 (3H, d, *J*= 6.6 Hz), 1.16 (3H, d, *J*= 6.9 Hz), 1.2 - 1.3 (10H, complex), 1.35 (3H, d, *J*= 6.9 Hz), 1.52 (1H, m), 1.86 (1H, m), 2.29 (1H, m), 2.51 (2H, complex), 2.87 (1H, m), 3.08 (2H, complex), 3.41 (1H, m), 4.37 (1H, br. s), 4.75 (1H, d, *J*= 3.9 Hz), 5.24 (1H, m), 5.90 (1H, broad signal), and 7.29 (5H, complex).

Conversion of 9 into the Fragment 17. A solution of **9** (210 mg, 0.31 mmol) in MeOH (5 ml) in the presence of TsOH (3 mg) was stirred at room temperature for 3 days. The reaction mixture was partitioned between EtOAc and H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (10 g, hexane/EtOAc = 4:1 → 2:1, then $CHCl_3$/MeOH = 20:1) to give a deacetonide derivative (72 mg, 36%), along with unreacted **9** (33 mg, 16%) and N-deprotected derivative (40 mg, 24%).

A mixture of the deacetonide derivative (72 mg, 0.11 mmol), TBSCl (34 mg, 0.23 mmol), and imidazole (23 mg, 0.34 mmol) in DMF (3 ml) was stirred at room temperature for 20 h. The reaction mixture was partitioned between EtOAc and H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by preparative TLC (hexane/EtOAc = 4:1) to give **16** (82 mg, 97%).

A mixture of **16** (16 mg, 0.021 mmol), NaH (2.5 mg, 0.063 mmol, 60% dispersion in mineral oil), and CH_3I (0.026 ml) in DMF (3 ml) was stirred at 0 °C for 3 h, then at room temperature for 12 h. The reaction mixture was partitioned between EtOAc and H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by preparative TLC (hexane/EtOAc = 5:1) to give **17** (2.6 mg, 35%). **17** as an oil: δ_H 0.88 (3H, t, *J*= 6.9 Hz), 1.01 (3H, d, *J*= 3.3 Hz), 1.04 (3H, d, *J*= 3.3 Hz), 1.25 - 1.50 (15H, complex), 2.14 - 2.35 (3H, complex), 4.65 (2H, complex), 4.88 (1H, d, *J*= 4.6 Hz), 5.24 (1H, br. d, *J*= 10.6 Hz), 5.33 (1H, dd, *J*= 1.4, 17 Hz), and 5.95 (1H, m).

(3*R*,4*S*)-4-[*N*-(*tert*-Butoxycarbonyl)amino]-3-(*tert*-butyldimethylsilyloxy)-5-phenylpentanoic Acid (18). A mixture of **5** (1.62 g, 4.8 mmol), TBSCl (1.45 g, 9.62 mmol), and imidazole (1.00 g, 14.7 mmol) in DMF (30 ml) was stirred at room temperature for 40 h. The resulting mixture was partitioned between EtOAc and H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), then evaporated to give a crude product , which was chromatographed on a silica gel column (40 g, hexane/EtOAc = 10:1 → 3:1) to yield a siloxy ether (2.27 g, quantitative yield) as an oil: $[\alpha]_D^{21}$ -20° (*c* 1.00, $CHCl_3$); IR (film) 1710 cm^{-1}; δ_H 0.06 (3H, s), 0.11 (3H, s), 0.91 (9H, s), 1.25 - 1.32 (12H, complex), 2.51 - 2.80 (3H, complex), 2.97 (1H, dd, *J*= 4.6, 14.2 Hz), 3.87 (1H, br. s), 4.15 (2H, complex), 4.29 (1H, br. s), 4.47 (1H, br. s), and 7.18 - 7.31 (5H, complex); δ_C -4.8, -4.7, 14.1, 18.0, 25.8, 35.8, 39.9, 56.1, 60.6, 71.2, 77.2, 79.0, 126.2, 128.3, 129.1, 138.3, 155.1, and 171.2.

To a solution of the ether (41 mg, 0.12 mmol) in EtOH (1 ml) was added 2M NaOH (1 ml, 2 mmol) at 0 °C; the mixture was stirred at room temperature for 2 h. The reaction mixture was acidified to pH 5 by the

addition of 10% aq. citric acid, and partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by preparative TLC ($CHCl_3$/MeOH = 10:1) to give **18** (33 mg, 84%) as an oil: $[\alpha]_D^{20}$ -31° (*c* 1.00, $CHCl_3$); IR (film) 3450 and 1720 cm^{-1}; δ_H 0.07 - 0.16 (6H, complex), 0.90 (9H, s), 1.14 - 1.31 (9H, complex), 2.37 - 2.59 (3H, complex), 3.07 (1H, m), 3.64 - 4.58 (2H, complex), and 7.16 - 7.28 (5H, complex). Found: C, 61.35; H, 9.11; N, 3.53%. Calcd for $C_{22}H_{37}NO_5Si \cdot 1/2H_2O$: C, 61.08; H, 8.85; N, 3.24%.

(3*R*,4*S*)-4-[*N*-(*tert*-Butoxycarbonyl)-*N*-methylamino]-3-(*tert*-butyldimethylsilyloxy)-5-phenylpentanoic Acid (19). A mixture of **18** (164 mg, 0.39 mmol), CH_3I (0.6 ml, 10 mmol), and NaH (116 mg, 2.9 mmol, 60% dispersion in mineral oil) in THF (8 ml) was stirred at 0 °C for 3 h, then at room temperature for 40 h. The reaction mixture was acidified by the addition of 10% aq. citric acid, and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was subjected to preparative TLC ($CHCl_3$/MeOH = 10:1) to give **19** (142 mg, 83%) as an oil: $[\alpha]_D^{22}$ -41.2° (*c* 1.00, $CHCl_3$); IR (film) 3450 and 1700 cm^{-1}; δ_H 0.13 - 0.16 (6H, complex), 0.92 - 0.94 (9H, complex), 1.17 - 1.32 (9H, complex), 2.47 - 2.61 (6H, complex), 3.18 (1H, br. d, *J*= 11.9 Hz), 4.30 (2H, complex), and 7.12 - 7.28 (5H, complex). Found: C, 62.16; H, 9.33; N, 3.09%. Calcd for $C_{23}H_{39}NO_5Si \cdot 1/2H_2O$: C, 61.85; H, 9.03; N, 3.14%.

Benzyl (2*S*,3*R*)-3-[(3*R*,4*S*)-4-[*N*-(*tert*-Butoxycarbonyl)-*N*-methylamino]-3-(*tert*-butyldimethylsilyloxy)-5-phenylpentanoyloxy]-2-methyldecanoate (20). A mixture of **19** (481 mg, 1.1 mmol), **4** (335 mg, 1.2 mmol) and DCC (384 mg, 1.9 mmol) in CH_2Cl_2 (8 ml) in the presence of DMAP (21 mg, 0.17 mmol) was stirred at 0 °C for 2 h, then at room temperature for 10 h. The reaction mixture was filtered, and the filtrate was partitioned between CH_2Cl_2 and 10% aq. citric acid. The organic layer was washed with sat.aq. $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated. A crude product was chromatographed on silica gel column (20 g, hexane/EtOAc = 10:1 → 2:1) to give **20** (597 mg, 77%) as an oil: $[\alpha]_D^{21}$ -21.9° (*c* 1.00, $CHCl_3$); IR (film) 1745 and 1700 cm^{-1}; δ_H 0.01 - 0.07 (6H, complex), 0.76 - 0.85 (12H, complex), 1.07 (3H, d, *J*= 6.9 Hz), 1.10 - 1.48 (21H, complex), 2.30 - 2.80 (7H, complex), 3.07 (1H, m), 4.01 (1H, m), 4.30 (1H, m), 5.05 (2H, s), 5.06 (1H, m), and 7.02 - 7.28 (10H, complex); δ_C -4.1, -4.0, 12.4, 12.5, 14.0, 17.9, 18.0, 22.5, 25.4, 25.8, 25.9, 28.2, 29.1, 29.3, 31.7, 31.8, 43.1, 66.4, 70.1, 74.7, 77.2, 79.1, 128.1, 128.18, 128.24, 128.3, 128.5, 128.9, 135.8, 139.2, 155.7, 170.7, and 173.5. Found: C, 68.91; H, 9.37; N, 2.14%. Calcd for $C_{41}H_{65}O_7NSi$: C, 69.16; H, 9.20; N, 1.97%.

2-Propenyl (2*S*)-2-[(2*S*,3*R*)-3-[(3*R*,4*S*)-4-[*N*-(*tert*-Butoxycarbonyl)-*N*-methylamino]-3-(*tert*-butyldimethylsilyloxy)-5-phenylpentanoyloxy]-2-methyldecanoyloxy]-3-methylbutanoate (21). A solution of **20** (63 mg, 0.09 mmol) in EtOH (2 ml) in the presence of catalytic amounts of $Pd(OH)_2$ was stirred at room temperature for 1 h under a hydrogen atmosphere. The reaction mixture was filtered, and the filtrate was evaporated. A mixture of the residue, **8** (23 mg, 0.15 mmol), DCC (30 mg, 0.15 mmol), and DMAP (1.5 mg, 0.012 mmol) in CH_2Cl_2 (2 ml) was stirred at 0 °C for 2 h, then at room temperature for 30 h. The resulting mixture was filtered, and the filtrate was partitioned between CH_2Cl_2 and 10% aq. citric acid. The organic layer was washed with sat.aq. $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated. The residue was purified by preparative TLC (hexane/EtOAc = 4:1) to yield **21** (64 mg, 96% in two steps) as an oil: $[\alpha]_D^{21}$ -25.6° (*c* 1.00, $CHCl_3$); IR (film) 1740 and 1695 cm^{-1}; δ_H 0.08 - 0.15 (6H, complex), 0.86 (3H, t, *J*= 6.8 H), 0.91 - 1.02 (15H, complex), 1.18 - 1.37 (22H, complex), 1.62 (2H, complex), 2.23 (1H, m), 2.43 - 2.80 (7H, complex), 3.16 (1H, m), 4.12 (1H, m), 4.40 (1H, m), 4.61 (1H, m), 4.81 (1H, m), 5.14 (1H, m), 5.23 (1H, m), 5.23 (1H, d, *J*= 10.8 Hz), 5.32 (1H, dd, *J*= 16.8, 1.6 Hz), 5.88 (1H, m), and 7.11 - 7.29 (5H, complex); δ_C -4.1, -3.9, 12.9, 13.2, 14.0, 17.2, 18.8, 22.6, 25.4, 25.9, 26.0, 28.2, 28.3, 29.1, 29.4, 30.0, 31.7, 31.8, 34.6, 40.0, 42.9, 43.3, 65.6, 70.1,

70.3, 74.4, 74.6, 76.8, 79.1, 79.7, 118.7, 126.0, 126.1, 129.0, 131.6, 139.3, 155.8, 168.9, 170.9, and 173.3.

2-Propenyl (2*S*)-2-[(2*S*,3*R*)-3-[(3*R*,4*S*)-4-[*N*-(*tert*-Butoxycarbonyl)-*N*-methylamino]-3-hydroxy-5-phenylpentanoyloxy]-2-methyldecanoyloxy]-3-methylbutanoate (22). To a solution of **21** (181 mg, 0.24 mmol) in THF (5 ml) was added. 70% HF - pyridine (1 ml). After being stirred at room temperature for 4 h, the reaction mixture was diluted with aq. $NaHCO_3$, and extracted with EtOAc. The organic extracts were washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by preparative TLC (hexane/EtOAc = 4:1) to give **22** (132 mg, 87%) as an oil: $[\alpha]_D^{21}$ -24.8° (*c* 1.00, $CHCl_3$); IR (film) 3480, 1745, and 1700 cm^{-1}; δ_H 0.87 (3H, t, *J*= 6.9 Hz), 0.94-1.02 (6H, complex), 1.22-1.69 (24H, complex), 2.20 - 3.30 (9H, complex), 3.76 - 4.24 (3H, complex), 4.64 (2H, d, *J*= 5.9 Hz), 4.84 (1H, d, *J*= 4.3 Hz), 5.23 - 5.37 (3H, complex), 5.90 (1H, m), and 7.14 - 7.27 (5H, complex); δ_C 11.8, 14.0, 17.2, 18.8, 22.6, 25.5, 25.6, 28.1, 28.3, 29.1, 29.3, 30.0, 31.1, 31.4, 31.7, 33.2, 39.3, 39.4, 42.1, 42.5, 65.7, 65.8, 70.0, 74.3, 118.9, 119.0, 126.1, 128.2, 129.0, 131.4, 169.3, 171.9, 172.0, and 173.6.

(2*S*)-2-[(2*S*,3*R*)-3-[(3*R*,4*S*)-4-*N*-Methylamino-3-hydroxy-5-phenylpentanoyloxy]-2-methyldecanoyloxy]-3-methylbutanoic Acid (23). To a solution of **22** (68 mg, 0.11 mmol) in THF (5 ml) under an argon atmosphere were added $(Ph_3P)_4Pd$ (0) (6.2 mg, 5 mol%) and morpholine (0.094 ml, 1.1 mmol); the mixture was stirred at room temperature for 3 h. The reaction mixture was partitioned between EtOAc and 10% aq. citric acid. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by preparative TLC ($CHCl_3$/MeOH = 10:1) to give a carboxylic acid (62 mg, 97%) as an oil: $[\alpha]_D^{21}$ -24° (*c* 1.00, $CHCl_3$); IR (film) 3500, 1740, and 1705 cm^{-1}; δ_H 0.84 - 1.65 (33H, complex), 2.30 - 3.30 (9H, complex), 4.08 - 4.23 (2H, complex), 4.94 (1H, br. s), 5.40 (1H, br. s), and 7.15 - 7.27 (5H, complex).

A mixture of the carboxylic acid (44 mg, 0.073 mmol), and TFA (0.3 ml) in CH_2Cl_2 (1.5 ml) was stirred at room temperature for 2 h. After removal of volatile materials, the residue was subjected to preparative TLC ($CHCl_3$/MeOH = 10:1) to give **23** (35 mg, 94%) as an oil: $[\alpha]_D^{22}$ -2.4° (*c* 1.00, $CHCl_3$); IR (film) 3470, 1735, and 1680 cm^{-1}; δ_H 0.84 - 1.71 (24H, complex), 2.17 - 2.57 (6H, complex), 2.82 (1H, br. d, *J*= 6.6 Hz), 3.00 - 3.19 (2H, complex), 3.41 (1H, br. s), 4.57 (2H, complex), 5.23 (1H, br. s), and 7.26 - 7.35 (5H, complex).

Hapalosin (1). To a solution of **23** (34 mg, 0.067 mmol) in DMF were dropwisely added DPPA (0.043 ml, 0.12 mmol) and iPr_2NEt (0.069 ml, 0.4 mmol) at 0 °C; the mixture was stirred at the same temperature for 3 h, then at room temperature for 3 days. The resulting mixture was partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. Purification of the residue by silica gel column chromatography ($CHCl_3$/MeOH = 50:1 → 10:1) yielded **1** (14.5 mg, 44%) as a white solid: $[\alpha]_D^{18}$ -41° (*c* 1.00, CH_2Cl_2) [-40.9° (*c* 0.11, CH_2Cl_2),[5b] -48° (*c* 0.0029, CH_2Cl_2),[5a] -49.2° (*c* 0.35, CH_2Cl_2)[4]]; IR (film) 3430, 1735, and 1635 cm^{-1}; δ_H (a major isomer) 0.23 (3H, d, *J*= 6 Hz), 0.57 (3H, d, *J*= 6 Hz), 0.88 - 0.92 (3H, complex), 1.14 - 1.40 (13H, complex), 1.50 - 2.05 (3H, complex) 2.61 (1H, m), 2.65 (1H m), 2.86 (3H, complex), 2.92 (1H, dd, *J*= 18, 5 Hz), 3.22 (1H, m), 3.41 (1H, dd, *J*= 2.6, 14 Hz), 3.69 (1H, dt, *J*= 2.6, 10 Hz), 3.85 (1H, m), 4.31 (1H, d, *J*= 8.4 Hz), 5.12 (1H, m), and 7.17 - 7.35 (5H, complex); δ_C 12.1, 14.1, 17.5, 18.3, 22.6, 26.0, 28.0, 28.2, 28.8, 29.1, 29.2, 31.7, 36.4, 37.0, 40.7, 61.4, 70.2, 73.8, 76.5, 127.0, 128.9, 129.7, 137.4, 168.5, 168.7, and 172.7. Found: m/z 489.3079. Calcd for $C_{28}H_{43}NO_6$: M, 489.3087.

Bioassay. Human breast carcinoma MCF-7 and adriamycin resistant (Adr^R) cells were obtained from the National Cancer Institute (U. S. A.), and these cell lines were maintained at 37 °C in RPMI 1640 with 10% fetal bovine serum in humidified atmosphere with 5% CO_2. Samples such as **1**, **11** and verapamil were dissolved in DMSO, and diluted with saline. Adriamycin (ADR) was used as a saline solution, prepared before use. Drug cytotoxicity assay was performed in a 96-well microplates. Cell solutions (0.2 ml/well, $1x10^4$ cells/ml for MCF-7, $2x10^4$ cells/ml for Adr^R) were preincubated for 24 h (37 °C, 5% CO_2) with the medium. After the addition of a solution of a sample and ADR into each well, the incubation was continued for 72 h. The cells were stained with methylene blue, and the growth rates were obtained from absorbance at 660 nm. Each experimental point was determined in triplicate.

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