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Total Synthesis and Conformational Studies of Hapalosin, *N*-Desmethylhapalosin and 8-Deoxyhapalosin†

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Abstract—Hapalosin (**2**), a 12-membered cyclic depsipeptide possessing MDR-reversing activity, and analogues (**3**) and (**4**) have been synthesized using macrolactamization as an important ring-forming step. Three building blocks: (2*S*, 3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-methyl-decanoic acid (**13**), benzyl (*S*)-2-hydroxy-3-methylbutanate (**14**), and (4*S*,3*R*)-4-(benzyloxycarbonylmethylamino)-3-methoxymethoxy-5-phenyl-pentanoic acid (**28**) were prepared from Evans's chiral imide (**9**), L-valine, and L-*N*-Boc phenylalanine (**17**), respectively, and were assembled together by applying twice Yamaguchi's coupling methodology. A new and efficient selective *N*-methylation of γ-hydroxy-β-amino ester taking advantage of the vicinal amino alcohol function was uncovered in the course of this study. Thus, treatment of compound **19** with HCHO in the presence of catalytic amount of *p*TsOH followed by reduction ($NaBH_3CN$, TFA, CH_2Cl_2) of the so-formed oxazolidine **24** gave the *N*-methylated product **25**. Furthermore, a dual role of oxazolidine as protecting group of vicinal amino alcohol and latent *N*-methyl function was established which allowed synthesizing both hapalosin (**2**) and *N*-desmethylhapalosin (**3**) from the same linear precursor **32** in a step-efficient and atom economic way. In contrast to hapalosin (**2**) and *N*-desmethyl analogue (**3**), the amide bond of 8-deoxy hapalosin (**4**) exists at room temperature ($CDCl_3$) exclusively in *s-cis* conformation as evidenced by NOE studies. This observation has been explained on the basis of computational studies. No significant MDR reversing activity of 8-deoxy hapalosin (**4**) was observed in K562 R and S/Adriblastine against human erythroleucemic cell lines indicating thus the important contribution of hydroxy group to the bioactivity of hapalosin.

Introduction

One of the major problems in cancer therapy is the cellular resistance to a wide range of structurally unrelated cytotoxic drugs. This phenomenon, known as multidrug resistance (MDR),[1] is commonly developed following upon administration of a single anticancer agent. Reduced accumulation of the drug inside tumor cells appears to be one principal cause of this undesired scenario.[2] A wide variety of biochemical mechanisms have been identified in multidrug resistant cell lines, the most consistent of which is the increased expression of a drug efflux pump called P-glycoprotein (P-gp).[1,2] In fact, it has been observed that the level of P-gp expression correlates with the degree of drug resistance in a variety of different cell types. Since the first observation made at the beginning of the 1970s, the list of MDR drugs has grown progressively including tetracycline antibiotics (daunorubricin, adriamycin), vinca alkaloids (vinblastine, vincristine), cochicine, taxoides (taxol, taxotére) along with others such as mitomycin, topotecan, etoposides, to name a few. The ability of P-gp to bind and transport a range of structurally dissimilar compounds is truly remarkable and has provided impetus for the identification of new targets for chemotherapy.[1–3]

Among numerous prospects for circumventing MDR, one possibility is the use of chemosensitizers to block the action of P-gp,[3] thus potentiating the cytotoxicity of the anticancer agents. Verapamil (**1**, Fig. 1), a calcium channel blocker, was one of the first MDR reversing agents tested in clinic[4] and became the standard with which all other MDR modulators are compared.

Hapalosin (**2**), a 12-membered cyclic depsipeptide was recently identified by Moore and co-workers[5] from the screening extracts of blue-green algae (*cyanobacteria*). Hapalosin was mildly cytotoxic and was found to have better MDR reversing activity than verapamil, especially in promoting taxol accumulation in SKVLB1 cells. Shortly after its isolation, synthesis of hapalosin and analogues have been accomplished by Armstrong,[6,10] Ghosh,[7] Yamamura[8] and our group[9] employing both macrolactonization and macrolactamization methodology as key ring forming steps. We report herein in full details our previous route to

Key words: Hapalosin; anti-MDR; oxazolidine; selective *N*-methylation; macrolactamization.

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† Dedicated with affection and respect to the memory of Professor Sir Derek H. R. Barton in recognition of his distinguished contributions to science and in sincere gratitude for the immense degree of inspiration and encouragement he provided to one of us (J. Zhu).

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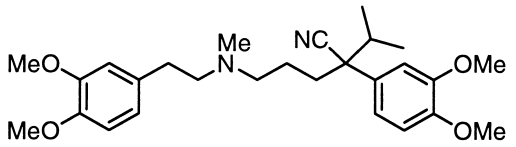
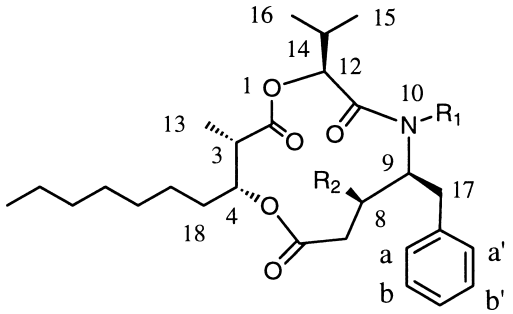
hapalosin and 8-deoxy-hapalosin, together with additional studies in which the dual role of oxazolidine as protecting group of vicinal amino alcohol and latent *N*-methyl group was established and exploited for the development of an improved synthesis of hapalosin and *N*-desmethyl analogues. The general strategy employing macrolactamization as a key ring forming step, similar to that employed by Ghosh[7] and Yamamura,[8] was depicted retrosynthetically in Scheme 1.

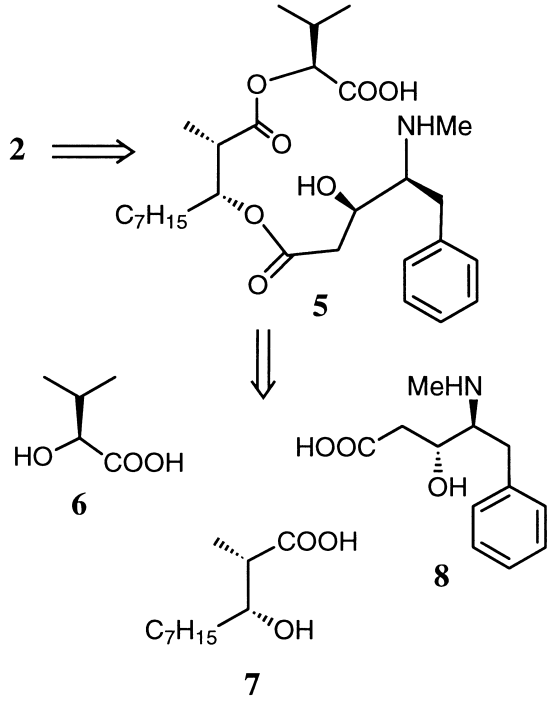


1 Verapamil



2 R_1 = Me, R_2 = OH, Hapalosin
3 R_1 = H, R_2 = OH, *N*-Desmethylhapalosin
4 R_1 = Me, R_2 = H, 8-Deoxyhapalosin

Figure 1.

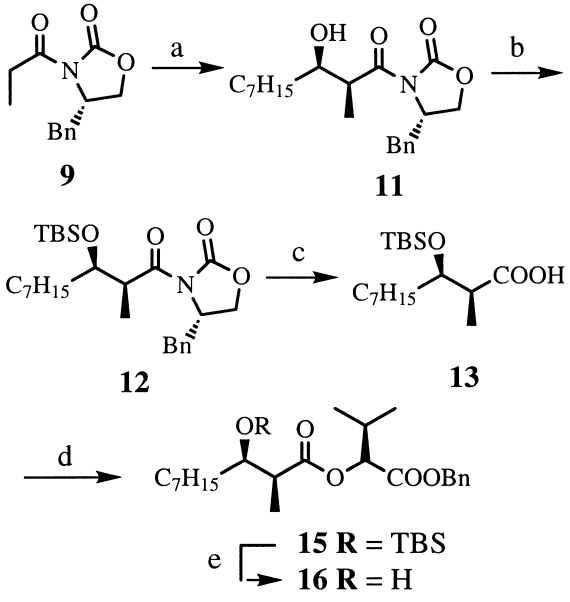


Scheme 1.

Results and Discussion

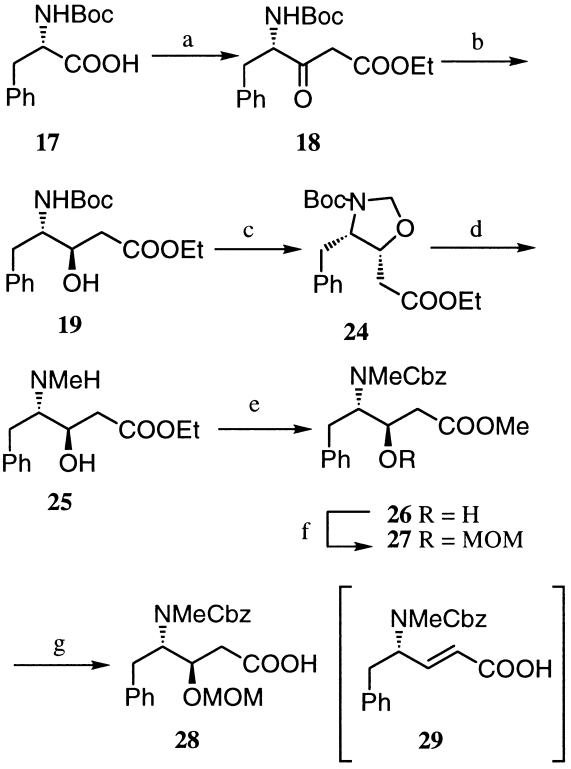
The β-hydroxy acid **13** was synthesized as shown in Scheme 2. Reaction of boron enolate of chiral imide (**9**) with *n*-octanaldehyde (**10**) under Evans' conditions[11] furnished *syn* aldol **11** in excellent yield and diastereoselectivity. Installation of TBS protecting group followed by removal of chiral auxiliary gave then the β-hydroxy acid **13** uneventfully. Esterification of **13** with benzyl (*S*)-(+)-2-hydroxy-3-methylbutanate (**14**), obtained in two straightforward steps from L-valine,[12] was carried out under different conditions and was best realized using Yamaguchi's reagent[13] to provide compound **15** in 88% yield. Removal of silyl ether was proved to be more difficult than expected due most probably to the presence of sensitive β-hydroxy ester entity. Among a range of reagents tested, only HF in MeCN[14] was found to be highly efficient in our hands to furnish **16** in 90% yield.

Synthesis of suitably protected β-hydroxy-γ-amino acid **28** was accomplished as shown in Scheme 3.[15] Treatment of *N*-Boc Phe (**17**) in THF with carbonyl diimidazole (CDI) gave the corresponding imidazolide which was reacted directly with lithium enolate of ethyl acetate to provide β-ketone ester **18** in 92% yield.[16] Reduction of **18** with $NaBH_4$ in ethanol[17] at −78 °C gave amino alcohol (de 80%) from which the diastereomerically pure *anti* product **19** could be isolated in 74% yield by simple recrystallization (ether/heptane). The diastereoselectivity of this reduction resulted from the chelation controlled process and the stereochemistry was confirmed by converting **19** and its *syn* diastereomer **20** (Fig. 2) into the corresponding oxazolidinone **21** and **22** (Fig. 2), respectively. From ^1H–^1H decoupling experiment, *J* values of 7.3 Hz and 4.6 Hz between protons H_a and H_b were determined for compounds **21** and **22**



Scheme 2. Reagents and conditions: (a) Bu_2BOTf, Et_3N, then *n*-$C_7H_{15}CHO$ (**10**), 92%; (b) TBSOTf, 2,6-lutidine, 95%; (c) LiOH, H_2O_2, 98%; (d) 2,4,6-trichlorobenzoyl chloride, (*S*)-(+)-benzyl 2-hydroxy-3-methylbutanate (**14**), Et_3N, 85%; (e) HF-MeCN, 90%.

respectively, in accord with the assigned stereostructure.[18,19] Furthermore, the significant NOE effect observed between protons H_a and H_b in the NOEDIFF spectra of oxazolidinone **21** reinforced the assignment of their *cis* orientation and, thus, the *anti* stereochemistry of amino alcohol **19**. The *N*-methylated keto ester **23** was also synthesized (Fig. 2) whose reduction under various conditions gave predominantely the undesired *syn* amino alcohol[20] (structure not shown) according to Felkin–Ahn model.[21]



Scheme 3. Reagents and conditions: (a) CDI, then lithium salt of ethyl acetate, 92%; (b) $NaBH_4$, EtOH, −78 °C, 74%; (c) HCHO, *p*TSOH, toluene, Dean–Stark, 77%; (d) $NaBH_3CN$, CH_2Cl_2, TFA, 81%; (e) CbzOSu, $NaHCO_3$, acetone, H_2O; (f) MOMBr, $^iPr_2NEt$, 87%; (g) K_2CO_3, MeOH, reflux, 90%.

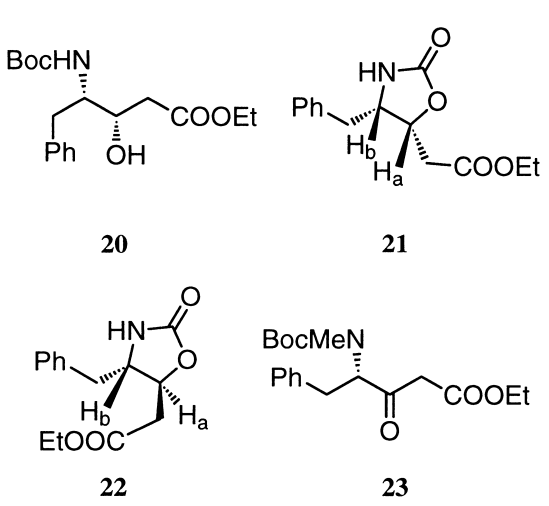


Figure 2.

Selective *N*-methylation of β-hydroxy-γ-amino ester of type **19** was reported to be troublesome[20,22] probably due to the competitive β-elimination, retro-Aldol and pyrrolidinone ring forming process. After several unsuccessful trials, we devised a new method taking advantage of the proximity of the amino alcohol function (Scheme 3). Thus, reaction of **19** with aqueous formaldehyde in the presence of a catalytic amount of *p*TsOH gave smoothly the corresponding oxazolidine **24**. It is worth noting that the formation of tetrahydroisoquinoline derivative via Pictet–Spengler reaction was not observed under these mild conditions.[23] Reduction of oxazolidine **24** with $NaBH_3CN$ in a mixture of solvents TFA–CH_2Cl_2 afforded selectively the *N*-methylated compound **25** in 81% yield. This two-step procedure is reminiscent of that developed by Freidinger et al. for the preparation of Fmoc protected *N*-alkyl amino acid.[24] It is worth noting that classic one-step reductive amination conditions failed to give the desired product even under recently modified conditions.[25] Selective *N*-acylation of **25** with CbzOSu[26] under Schotten–Baumann conditions afforded carbamate **26** whose secondary hydroxyl group was protected as MOM ether[27] to give compound **27** uneventfully. Finally, the ethyl ester function was hydrolyzed (K_2CO_3, MeOH, reflux, 3 h) to provide the appropriately protected acid **28**. A remarkable solvent effect was observed for this simple transformation as no hydrolysis occurred in EtOH under otherwise identical conditions. The reaction course had to be carefully monitored as prolonged reaction time led to the formation of a variable amount of inseparable β-elimination product **29** which was used for the synthesis of 8-deoxyhapalosin (vide infra).

The synthesis of hapalosin **2** and 8-deoxyhapalosin **4** was accomplished as shown in Scheme 4. Coupling of diester **16** with acid **28** using Yamaguchi's reagent gave the triester **30** which had been transformed into the hapalosin by Ghosh et al. in three steps.[7] On the other hand, the compound **4** was synthesized in 2 steps from fully protected amino ester **31**. Thus, simultaneous removal of benzyl ester, Cbz functions and 1,4-reduction of enoate by hydrogenolysis (Pearlmans catalyst, H_2, 1 atm) followed by standard DPPA mediated macrolactamization[28] afforded 8-deoxyhapalosin **4** in 45% overall yield.

At this stage, we wondered if we could take advantage of the oxazolidine function as a protecting group of vicinal amino alcohol to obtain the triester **32** (Fig. 3) from which both hapalosin and *N*-desmethyl hapalosin would be accessible. Moreover, if realizable, most of the redundant protection–deprotection steps found in Schemes 3 and 4 would then be avoided. The realization of this idea was shown in Scheme 5.

Selective hydrolysis of ester **24** to acid **33** was thought to be delicate due to the sensitivity of oxazolidine function. After several trials, the most classic method turned out to be the most efficient one. Thus, treatment of **24** with 1 M KOH in MeOH at room temperature for 10 min gives acid **33** in quantitative yield which was purified by

simple acid–base extraction. Coupling of acid **33** with hydroxy ester **16** under Yamaguchi's conditions gave the triester **32** in 76% yield. The synthesis was diverged at this point allowing access to both hapalosine (**2**) and *N*-desmethyl analogue (**3**). Thus, mild acidic hydrolysis of oxazolidine (TFA–CH_2Cl_2) gave the compound **34**. Hydrogenolysis followed by DPPA mediated macrolactamization afforded *N*-desmethyl analogue **3** in 48% yield. On the other hand, reduction of oxazolidine ($NaBH_3CN$, CH_2Cl_2, TFA) afforded the *N*-methylated derivative **35** which was transformed into hapalosin following the same two step sequence in 18% overall yield. The physical data of compounds **2** and **3** are identical with those described in the literatures.[5,6,8]

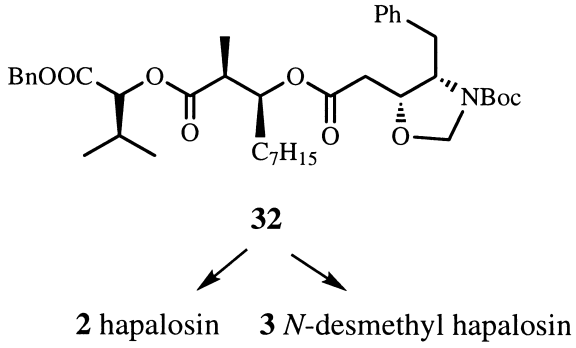
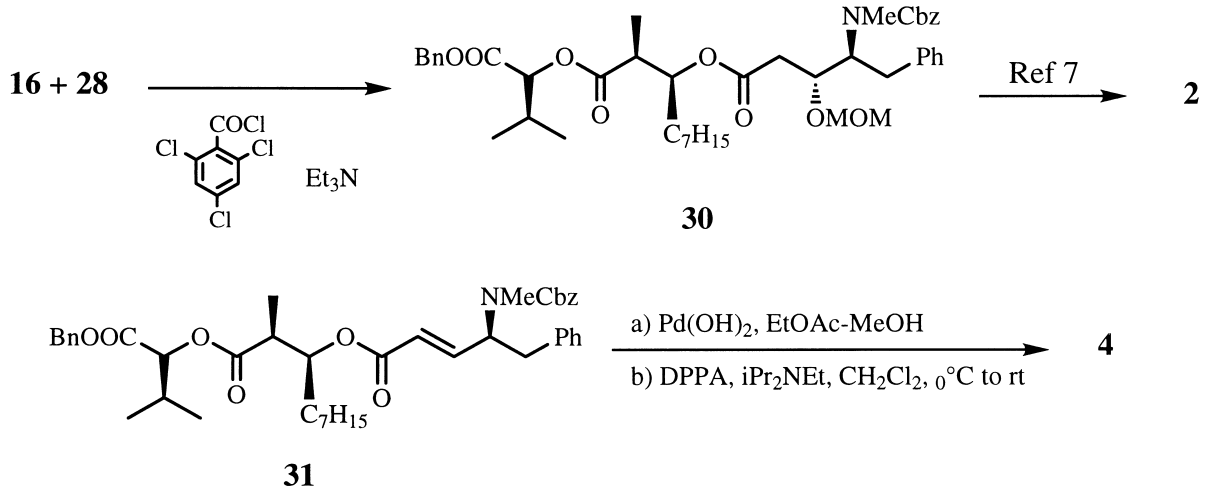


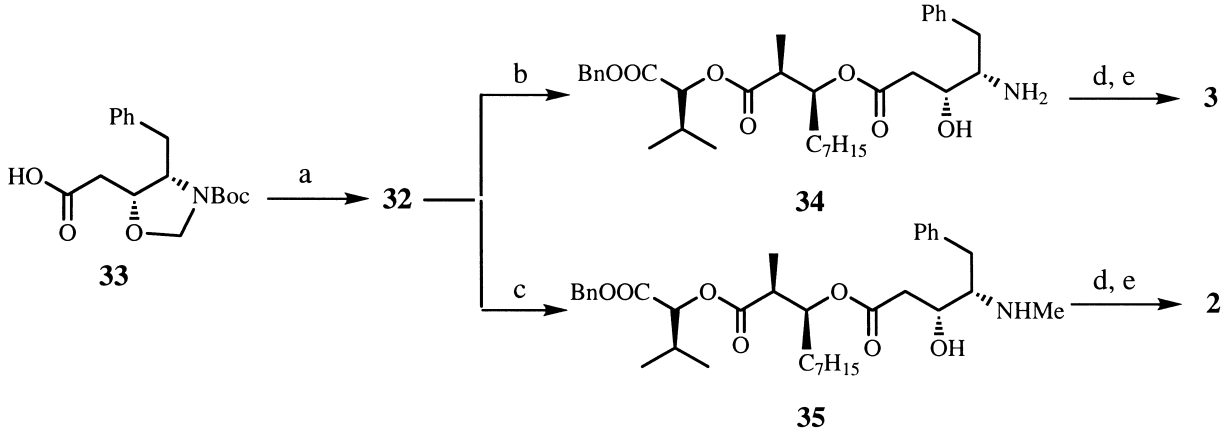
Figure 3.

Conformational studies

The conformation of both hapalosin (**2**)[5–8] and its *N*-desmethyl analogue (**3**) has been carefully determined by way of NMR and computational studies.[5,6,8] While the natural product exists at room temperature ($CDCl_3$) as a mixture of two rotamers in favor of *s-cis* amide linkage, the *N*-desmethyl analogue exists only as a single *s-trans* conformer. Interestingly, we found that the 8-deoxyhapalosin (**4**) exists exclusively as a *s-cis* conformer at room temperature in $CDCl_3$ as evidenced by the presence of a strong NOE correlation between protons H_9 and H_{12} in NOESY spectra. Other characteristic NOE cross peaks were listed as follows: H_{12}–H_{15}, NMe–H_{17}, NMe–H_8, H_{12}–H_a ($H_{a'}$), H_9–H_a ($H_{a'}$), NMe–H_a ($H_{a'}$), H_{17}–H_a ($H_{a'}$).



Scheme 4.



Scheme 5. Reagents and conditions: (a) 2,4,6-trichlorobenzoyl chloride, **16**, Et_3N, 76%; (b) TFA, CH_2Cl_2, 0 °C; (c) TFA, CH_2Cl_2, $NaBH_3CN$, 0 °C; (d) Pd/C, EtOH; (e) DPPA, $^iPr_2NEt$.

To understand this conformational preference, a computational study of compound **4** was performed. For the purpose of reducing the number of available conformations as well as the computational time, the heptyl side chain was replaced by a methyl group. Three thousand conformations of 8-deoxy-hapalosin (**4**) were generated by random search Monte Carlo method[29] and optimized by TNCG Truncated Newton minimization method[30] using the Macromodel (Version 5.5) program[31] with the MM2* force field.[32] (All of the computations discussed below were also conducted using the MM2* force field and GB/SA water solvation which led to similar results). Both *s-cis* amide **4a** and *s-trans* amide **4b** were used as the starting geometry and the search was carried out on blocks of 1000 Monte Carlo steps until no additional conformation was found to be of lower energy than the current minimum. Duplicated conformations as well as those that had chirality changes were discarded.

From this search, nine conformations were found within 3 kcal/mol from the global minimum. Seven of them have an *s-cis* amide bond and two have an *s-trans* amide bond. The lowest energy conformation of the *s-cis* amide form of 8-deoxy-hapalosin **4** was calculated to be 1.6 kcal/mol more stable than the *s-trans* amide form. Assuming the Boltzman distribution[33] and taking into account the energy of the seven possible conformations of *s-cis* amide conformers (E_1,...,E_7 = 115.9, 121.7, 123.3, 124.5, 125.1, 126.8, 127.8 kJ/mol) as well as the two possible *s-trans* amide isomer (E_1, E_2 = 122.6, 122.7 kJ/mol), the calculated population of *s-cis* amide conformers were greater than 90% which correlated nicely with our experimental observations. The calculated steric energies (MM2) as well as the most relevant geometrical parameters of these structures are summarized in Table 1. The distance between the H9 and H_{12} hydrogens (the dotted line) of the two lowest *s-cis* amide conformers was 2.2 Å (Fig. 4) which was consistent with a strong NOE correlation observed between these two protons in NOESY spectrum. A distance of 4.5 Å between H_9 and H_{12} was found in the most stable *s-trans* amide form of compound **4**.

The two lowest energy conformations of *s-cis* and the lowest *s-trans* amide conformers of compound **4** together with that of natural hapalosin (**2**)[34] were shown in Figure 4. Careful analysis of these structures revealed that the ring conformation of the most stable *s-trans* amide form of 8-deoxy-hapalosin (**4**) was very close to that of hapalosin (**2**). However, a slight conformational difference of the lowest energy *s-cis* conformers between compounds **4** and **2** became also evident from this examination. Thus, a C_8–C_7–C_6–O_5 torsion angle of 19.3° was found for the most stable (E = 115.9 kJ/mol) conformation of *s-cis* amide form of the 8-deoxy-hapalosin (**4**) which was not the most stable one in the *s-cis* amide form of the hapalosin. The most stable conformation of *s-cis* amide form of the hapalosin corresponded in fact to the second lowest energy conformer (E = 121.7 kJ/mol) of 8-deoxy-hapalosin, in which the C_8–C_7–C_6–O_5 torsion angle was −73.6° (Table 1). Minimizing the electrostatic repulsion between the oxygen of the hydroxyl group borne by the C_8 carbon atom and that of the C_6 ester function in hapalosin may account for the deviation of this conformational preference. As a matter of fact, the *pro R* proton of C_8 in the second lowest *s-cis* amide conformation of compound **4** was equidistant to the two oxygen atoms of C_6 ester (3.2 Å), while in the lowest energy conformer, the distance between this *pro R* proton and one of the ester oxygen atom was reduced to 2.6 Å disfavoring thus this conformer in the case of hapalosin because of the electrostatic repulsion.

Biological activity

The ability of 8-deoxy-hapalosin (**4**) to reverse P-gp-mediated multidrug resistance was evaluated in drug accumulation assays using K562 R and S/Adriblastine against human erytholeucemic cell lines. At 10 μM concentration of compound **4**, the IC_{50} value of adriblastine

Table 1

	8-Deoxy-hapalosin (*cis*-amide) **4a** 1st conf	8-Deoxy-hapalosin (*cis*-amide) **4a** 2nd conf	8-Deoxy-hapalosin (*trans*-amide) **4b**
E (kJ/mol)	115.9	121.7	122.6
ΔE (kJ/mol)	0	5.8	6.7
ΔE (kcal/mol)	0	1.4	1.6
CH_8–O_5	2.6	3.2	4.5
CH_8–O_6	4.1	3.2	3.9
H_{12}–H_9	2.2	2.2	4.5
C_{12}–C_{11}–N_{10}–C_9	−3.3	−3.3	175.0
C_{11}–N_{10}–C_9–C_8	−129.1	−133.1	−115.6
N_{10}–C_9–C_8–C_7	50.2	53.9	52.8
C_9–C_8–C_7–C_6	64.8	84.4	−84.3
C_8–C_7–C_6–O_5	19.3	−73.6	141.5
C_7–C_6–O_5–C_4	−177.7	−170.2	−174.1
C_6–O_5–C_4–C_3	70.0	171.4	151.5
O_5–C_4–C_3–C_2	44.5	49.3	−52.8
C_4–C_3–C_2–O_1	−103.4	−89.9	−31.2
C_3–C_2–O_1–C_{12}	−179.3	177.8	169.7
C_2–O_1–C_{12}–C_{11}	−140.7	−140.6	−106.4
O_1–C_{12}–C_{11}–N_{10}	74.8	80.9	−64.2

[a]The distances are in Å and the torsion angles are in degrees.

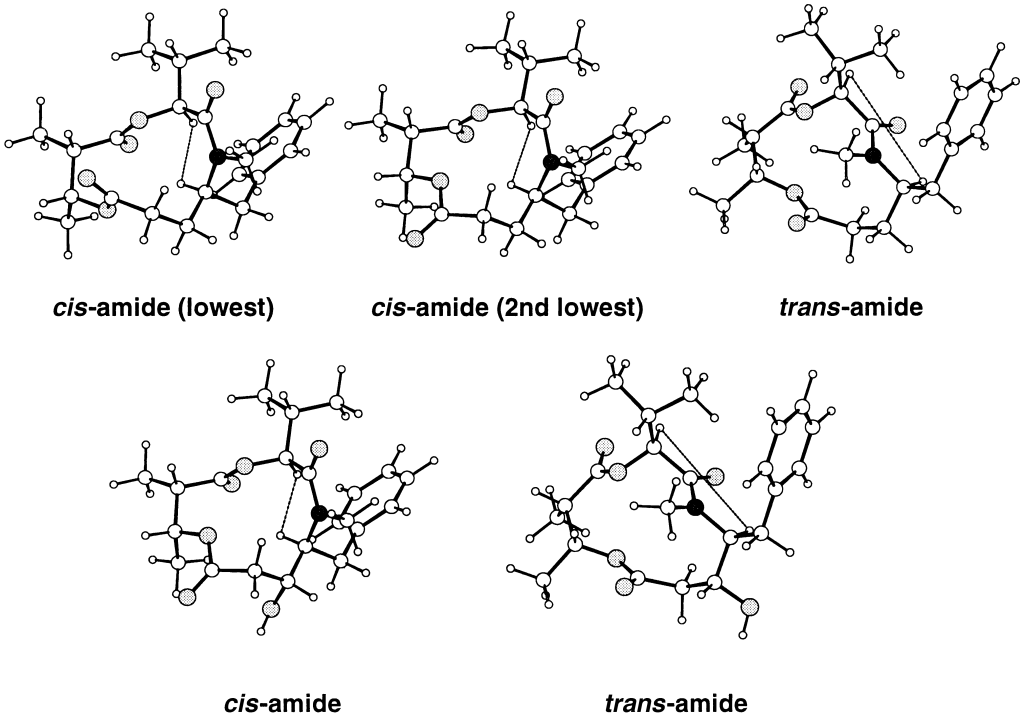


Figure 4. Conformations of the lowest *cis*- and *trans*-amides of 8-deoxyhapalosin (top) and of hapalosin (bottom) optimized by MM2* force field calculations.

(5×10^{-6} M) against resistance K562 R cell lines remained almost unchanged indicating the lack of significant anti MDR activity of **4**. This result may infer that hydroxy group, in addition to the conformation of the amide bond (*s-cis* versus *s-trans*), played an important role in the bioactivity of the natural product (**2**).

Conclusion

A concise synthesis of hapalosin and its 8-deoxy, *N*-desmethyl analogues have been developed. The dual role of oxazolidine as protecting group of vicinal amino alcohol and latent *N*-methyl function was uncovered in the course of this study. While the generality of this methodology remains to be fully exploited, its application to the synthesis of other complex natural products[20,22] might be rewarding. The conformational studies and the bioassay of synthetic analogue **4** indicated that the *s-cis* amide conformation is necessary but not sufficient to account for the bioactivity of hapalosin. The hydroxy group is indispensable to the anti MDR activity of hapalosin-type compounds.

Experimental

Melting points were determined with a Kofler apparatus and were uncorrected. Infrared (IR) spectra were recorded on a Nicolet-205 spectrometer. ^{1}H NMR spectra were measured on Brucker AC-200 (200 MHz), Bruker AC-250 (250 MHz), Bruker (300 MHz), and Bruker WM-400 (400 MHz) spectrometers with tetramethylsilane as the internal standard (δ ppm). Flash chromatography was performed using Kieselgel 60 (230–400 mesh, E. Merck) and usually employed a stepwise solvent polarity gradient, correlated with TLC mobility. Solvents and reagents were purified according to standard laboratory techniques. Optical rotations were determined on a Perkin–Elmer automatic polarimeter at room temperature. Mass spectra were run on AEI MS-50 (EI), AEI MS-9 (CI), and Kratos MS-80 (FAB), respectively. All reactions requiring anhydrous conditions or inert atmosphere were conducted under Argon.

(4*S*)-3[(2′*S*,3′*R*)-2′-Methyl-3′-hydroxydecanoyl)-4-phenylmethyl-2-oxazolidinone (11). To a cooled solution of **9** (1.24 g, 5.32 mmol) in anhydrous dichloromethane (15 mL) were added triethylamine (0.97 mL, 6.96 mmol) followed by *n*-Bu_2BOTf (6.1 mL, 1 M solution in dichloromethane). The reaction mixture was then cooled down to −78 °C and *n*-hexanal **10** (0.91 mL, 5.85 mmol) was added dropwise over a period of 5 min. After being stirred at −78 °C for 20 min and at 0 °C for 1 h, the reaction mixture was quenched by addition of saturated NH_4Cl solution and methanol (20 mL). To this cloudy solution was added H_2O_2 (30% solution, 10 mL) at such a rate as to keep the internal temperature below 10 °C. After stirring for one additional hour at 0 °C, the volatile was removed under reduced pressure. The resulting mixture was extracted with dichloromethane. The combined organic extracts were washed with aqueous $NaHCO_3$ solution and then brine, dried over Na_2SO_4 and concentrated to afford a light yellow oil. Purification by flash chromatography (SiO_2, eluent: Et_2O:heptane = 3:2) gave the *syn* aldol **11** (1.77 g, 92%): $[\alpha]_D = +36.6$ (*c*

1.6, $CHCl_3$); IR ($CHCl_3$) ν 2931, 2853, 1771, 1679, 1384 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.90 (t, $J=7.0$ Hz, 3H), 1.25 (m, 15H), 2.78 (dd, $J=9.4$, 13.4 Hz, 1H), 2.91 (d, $J=2.9$ Hz, 1H), 3.24 (dd, $J=3.3$, 13.4, 1H), 3.76 (qd, $J=2.7$ Hz, 7.0 Hz, 1H), 3.94 (m, 1H), 4.20 (m, 2H), 4.70 (m, 1H), 7.1–7.4 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 10.7, 14.4, 22.9, 26.3, 29.5, 29.8, 32.1, 34.2, 38.1, 42.4, 55.4, 66.4, 71.8, 127.6, 129.5, 129.8, 135.3, 153.3, 177.8; MS (CI) m/z 362 (M + H), 234. Anal. calcd for $C_{21}H_{31}NO_4$: C, 69.77; H, 8.64; N, 3.87. Found: C, 69.61; H, 8.48; N, 3.95.

(4*S*)-3[(2′*S*,3′*R*)-2′-Methyl-3′-(*tert*-butyldimethylsilyloxy)-decanoyl)-4-phenylmethyl-2-oxazolidinone (12). To a solution of compound **11** (880.00 mg, 2.43 mmol) and 2,6-lutidine (0.55 mL, 4.70 mmol) in anhydrous dichloromethane was added dropwise TBDMSOTf (0.82 mL, 3.60 mmol) at 0 °C. After being stirred at 0 °C for 1 h and then at room temperature for 2.5 h, the reaction mixture was diluted with CH_2Cl_2 and the organic phase was washed with 5% aqueous citric acid followed by a saturated NH_4Cl solution and dried over Na_2SO_4. After evaporation of the combined organic phases, the crude product was purified by flash chromatograhy (SiO_2, heptane:EtOAc = 9:1) to afford compound **12** (1.06 g, 95%): mp 42–46 °C; $[\alpha]_D = +59$ (c 0.8, $CHCl_3$); IR ($CHCl_3$) δ 2938, 2868, 1771, 1693, 1468, 1391, 1110, 821 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.01 (s, 3H), 0.02 (s, 3H), 0.90 (br s, 12H), 1.2–1.7 (m, 15H), 2.55 (dd, $J=9.8$, 13.2 Hz, 1H), 3.30 (dd, $J=3.0$, 13.2 Hz, 1H), 3.85 (m, 1H), 4.02 (q, $J=5.2$ Hz, 1H), 4.20 (m, 2H), 4.61 (m, 1H), 7.1–7.3 (m, 5H); ^{13}C ($CDCl_3$) δ −4.8, −4.1, 11.5, 14.1, 18.1, 22.6, 25.0, 25.8, 29.2, 29.8, 31.8, 35.6, 37.6, 42.8, 55.8, 66.0, 72.9, 127.3, 128.9, 129.5, 135.4, 153.1, 175.3; MS (CI) m/z 476 (M + H). Anal. calcd for $C_{27}H_{45}NO_4Si$: C, 68.17; H, 9.53; N, 2.94. Found: C, 68.19; H, 9.33; N, 3.12.

(2*S*,3*R*)-3-(*tert*-Butyldimethylsilyloxy)-2-methyl-decanoic acid (13). To a cooled (0 °C) solution of **12** (1.24 g, 2.61 mmol) in THF/H_2O (4/1, 50 mL) was added H_2O_2 (30% in water, 5.46 mL, 53.4 mmol) and then LiOH hydrate (1.12 g, 26.7 mmol) in one portion. The temperature was allowed to come to room temperature and stirring was continued overnight. After evaporation of the volatile, the residue was suspended in water, and the mixture was acidified with citric acid and extracted several times with dichloromethane. The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated to dryness. Purification by flash chromatography (SiO_2, eluent: heptane:ether = 9:1) afforded pure compound **13** (823.0 mg, 98%): $[\alpha]_D = +25$ (c 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 0.10 (s, 3H), 0.11 (s, 3H), 0.90 (s, 9H, tBu), 0.91 (t, $J=7.1$ Hz, 3H), 1.13 (d, $J=7.0$ Hz, 3H), 1.2–1.3 (m, 12H), 2.60 (m, 1H), 3.95 (m 1H); ^{13}C NMR ($CDCl_3$) −4.2, −4.6, 10.9, 14.2, 18.1, 22.8, 25.5, 25.9, 29.3, 29.8, 31.9, 34.5, 44.5, 73.6, 179.3; MS (CI) m/z 317 (M + H). Anal. calcd for $C_{17}H_{36}O_3Si$: C, 64.50; H, 11.46. Found: C, 64.74; H, 11.65.

(*S*)-1-Carboxy-2-methylpropyl(2*S*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-methyl-decanoate (15). To a solution of acid **13** (637.0 mg, 2.01 mmol) in anhydrous THF (5 mL) were added triethylamine (0.33 mL, 2.41 mmol) and 2,4,6-trichlorobenzoyl chloride (0.31 mL, 2.00 mmol), successively. After being stirred at room temperature for 30 min, a solution of *S*-benzyl 2-hydroxy-3-methyl butanate **14** (1.00 g, 4.81 mmol) and DMAP (490.00 mg, 4.02 mmol) in toluene (15 mL) were added. The reaction mixture was stirred at room temperature for another 18 h and was then diluted with aqueous NH_4Cl solution. The volatile was removed under reduced pressure and the remaining aqueous solution was extracted with CH_2Cl_2. The combined organic extracts were washed with a saturated NH_4Cl solution, dried, and evaporated. Purification by flash chromatography (SiO_2, eluent: heptane:ether = 40:1) afforded **15** (870 mg, 85%): $[\alpha]_D = -20$ (c 0.9, $CHCl_3$); IR ($CHCl_3$) ν 2925, 2843, 1737, 1450, 1250, 1106, 1050 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.02 (s, 3H), 0.05 (s, 3H), 0.86 (br s, 12H), 0.96 (d, $J=6.9$ Hz, 3H), 0.98 (d, $J=6.9$ Hz, 3H), 1.18 (d, $J=7.0$ Hz, 3H), 1.2–1.4 (m, 12H), 1.50 (m, 1H), 2.63 (d of septet, $J=4.5$, 6.9 Hz, 1H), 3.99 (q, $J=5.2$ Hz, 1H), 4.84 (d, $J=4.5$ Hz, 1H), 5.14, 5.20 (AB system, $J=12.3$ Hz, 2H), 7.34 (m, 5H); ^{13}C NMR($CDCl_3$) δ −4.3, −4.0, 12.7, 14.4, 17.6, 19.1, 23.7, 25.6, 26.0, 26.6, 30.3, 32.9, 34.7, 36.0, 45.8, 67.8, 74.4, 78.1, 129.3, 129.4, 129.5, 137.0, 170.8, 176.1; MS (CI) m/z 507 $(M+H)^+$. Anal. calcd for $C_{29}H_{50}O_5Si$: C, 68.73; H, 9.94. Found: C, 69.03; H, 10.06.

(*S*)-1-Carboxy-2-methylpropyl(2*S*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-methyl-decanoic acid (16). A solution of compound **15** (363.0 mg, 0.72 mmol) in a mixture of solvent acetonitrile:aqueous HF (95:5, 10 mL) was stirred at room temperature for 1 h. The reaction mixture was diluted with water and the volatile was removed under reduced pressure. The remaining aqueous solution was extracted with CH_2Cl_2 and the combined organic extracts were washed with a saturated NH_4Cl solution, dried and evaporated. The crude product was purified by flash chromatography (SiO_2, eluent: heptane:ether = 1:1) to afford compound **16** (253 mg, 90%): $[\alpha]_D = -11.0$ (c 0.2, $CHCl_3$); IR ($CHCl_3$) ν 3500, 2925, 2862, 1725, 1443, 1181, 1131 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.90 (t, $J=6.9$ Hz, 3H), 0.94 (d, $J=6.9$ Hz, 3H), 1.00 (d, $J=6.9$ Hz, 3H), 1.17 (d, $J=7.1$ Hz, 3H), 1.3–1.7 (m, 12H), 2.28 (m, 1H), 2.69 (dq, $J=3.1$, 7.1 Hz, 1H), 2.90 (d, $J=4.8$ Hz, 1H), 4.08 (m, 1H), 5.00 (d, $J=4.0$ Hz, 1H), 5.14, 5.25 (AB system, $J=12.2$ Hz, 2H), 7.34 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 9.6, 14.2, 18.9, 22.8, 26.3, 29.4, 29.7, 30.1, 31.9, 33.6, 44.6, 67.4, 72.0, 76.5, 128.6, 128.5, 128.6, 137.2, 170.4, 175.3; MS (CI) m/z 393 $(M+H)^+$, 333, 285, 209, 203. Anal. calcd for $C_{23}H_{36}O_5$: C, 70.38; H, 9.24. Found: C, 70.18; H, 9.16.

(4*S*)-4-*tert*-Butoxycarbonyl amino-3-oxo-5-phenyl-pentanoic acid ethyl ester (18). To a solution of L-*N*-Boc-phenylalanine **17** (7.35 g, 27.73 mmol) in dry THF (150 mL) was added carbonyldiimidazole (4.95 g, 30.51 mmol) in one portion at 0 °C. The solution was stirred at 0 °C for 30 min and at room temperature for 1.5 h. The formation of the amide can be monitored by TLC (EtOAc:heptane = 1:2). To another flask, butyllithium (1.6 M solution in hexane, 78.00 mL, 124.8 mmol) was added

dropwise to diisopropylamine (18.37 mL, 131.04 mmol) in THF (50 mL) at 0 °C. The light yellow suspension was stirred for 15 min and was then cooled down to −78 °C. Ethyl acetate (12.2 mL, 124.8 mmol) was added and stirring was continued for 30 min. The so formed light-yellow solution of the lithium enolate of ethyl acetate was then added during 5 min to a precooled solution of the imidazolide. After stirring at −78 °C for 1.5 h, the cooling bath was removed and the reaction temperature was raised to ambient. The suspension was then quenched by addition of saturated NH_4Cl solution. After evaporation of the volatile, the aqueous solution was extracted with dichloromethane. The combined organic phases were washed with 1 N HCl and saturated NH_4Cl solution, dried over Na_2SO_4, and evaporated to dryness. Purification by flash chromatography (SiO_2, EtOAc:heptane = 1:4) give compound **18** (8.55 g, 92%): mp 62–64 °C; $[\alpha]_D = -2.5$ (*c* 1.1, $CHCl_3$); IR ($CHCl_3$) ν 3012, 1728, 1506, 1362, 1325, 1162 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.25 (t, *J* = 7.1 Hz, 3H), 1.42 (s, 9H), 2.94 (dd, *J* = 7.6, 14.0 Hz, 1H), 3.16 (dd, *J* = 5.8, 14.0 Hz, 1H), 3.42, 3.52 (AB system, *J* = 16.0 Hz, 2H), 4.16 (q, *J* = 7.1 Hz, 2H), 4.55 (br q, *J* = 7.8, 1H), 5.22 (d, *J* = 7.8 Hz, 1H), 7.1–7.4 (m, 5H); ^{13}C ($CDCl_3$) δ 14.0, 28.2, 36.7, 46.7, 60.4, 61.3, 80.0, 126.8, 128.6, 129.2, 136.3, 155.2, 166.8, 201.9; MS (CI) *m*/*z* 336 (M + H), 280. Anal. calcd for $C_{18}H_{25}NO_5$: C, 64.46; H, 7.51; N, 4.17. Found: C, 64.38; H, 7.22; N, 4.21.

(3*R*,4*S*)-4-*tert*-Butoxycarbonyl amino-3-hydroxy-5-phenylpentanoic acid ethyl ester (19). To a solution of **18** (2.02 g, 6.03 mmol) in ethanol (50 mL) was added $NaBH_4$ (573.0 mg, 15.07 mmol). After being stirred at −78 °C for 2 h, the reaction mixture was quenched by addition of water (10 mL) and 1 N HCl (10 mL). After evaporation of the volatile, the suspension was extracted with dichloromethane. The combined organic phases were washed with a saturated NH_4Cl solution, dried over Na_2SO_4 and evaporated to dryness. Recrystallization from EtOAC/hexane gave **19** (1.27 g). The filtrate was evaporated and purified by flash chromatography (SiO_2, eluent: EtOAC:hexane = 1:4) to afford another portion of compound **19** (237.0 mg, total yield of **19**: 74%) and its (3*S*,4*S*) diastereomer **20** (158.0 mg, 7.8%). Compound **19**: mp 143–144 °C; $[\alpha]_D$ +3.8° (*c* 0.9, $CHCl_3$); IR ($CHCl_3$) ν 3347, 1778, 1714, 1510, 1194, 864 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.25 (t, *J* = 7.1 Hz, 3H), 1.40 (s, 9H), 2.50 (m, 2H), 2.82 (dd, *J* = 7.5, 13.8 Hz, 1H), 2.96 (dd, *J* = 4.5, 13.8 Hz, 1H), 3.60 (br s, 1H), 3.7–3.9 (m, 2H), 4.15 (q, *J* = 7.1 Hz, 2H), 4.62 (br d, *J* = 9.2 Hz, 1H), 7.1–7.4 (m, 5H); ^{13}C ($CDCl_3$) α 13.7, 27.8, 35.4, 37.8, 54.8, 60.4, 69.7, 79.8, 125.9, 127.9, 129.0, 137.2, 155.3, 172.5; MS (CI) *m*/*z* 338 (M + H), 282. Anal. calcd for $C_{18}H_{27}NO_5$: C, 64.07; H, 8.06; N, 4.15. Found: C, 63.96; H, 7.95; N, 4.25. The *syn* diastereomer **20**: mp 87 °C; $[\alpha]_D = 20.6$ (*c* 0.2, $CHCl_3$); IR ($CHCl_3$) ν 3550, 3443, 2981, 1720, 1605, 1506, 1368 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.20 (t, *J* = 7.2 Hz, 3H), 1.49 (s, 9H), 2.48 (dd, *J* = 2.9, 16.8 Hz, 1H), 2.60 (m, 1H), 2.8–3.0 (m, 2H), 3.46 (br s, 1H), 3.71 (m, 1H), 3.90 (m, 1H), 4.10 (q, *J* = 7.2 Hz, 2H), 4.98 (br d, *J* = 9.5 Hz, 1H), 7.1–7.4 (m, 5H); ^{13}C ($CDCl_3$) δ 14.1, 28.3, 38.6, 55.4, 60.8, 67.1, 70.1, 80.2, 126.4, 128.5, 129.4, 138.2, 155.5, 173.0; MS (CI) *m*/*z* 338 (M + H), 238, 282. Anal. calcd for $C_{18}H_{27}NO_5$: C, 64.07; H, 8.06; N, 4.15. Found: C, 63.79; H, 8.11; N, 4.09.

(4*S*,5*R*)-(4-Benzyl-2-oxo-oxazolidin-5-yl) acetic acid ethyl ester (21). To a solution of **19** (56.0 mg, 0.16 mmol) in dichloromethane (0.5 mL) was added TFA (0.5 mL) at 0 °C. After being stirred at room temperature for 10 min, the reaction mixture was diluted with 20 mL ether and concentrated in vacuo. The remaining TFA was removed azeotropically with ether, toluene, and dried under high vacuum. To the solution of crude amino alcohol in dry dichloromethane (1.5 mL) were added at 0 °C, diisopropylethylamine (72.0 μL, 0.41 mmol) and carbonyldiimidazole (67.00 mg, 0.41 mmol), successively. After being stirred at room temperature for 24 h, the reaction mixture was diluted with dichloromethane and washed with aqueous citric acid solution (10%) and saturated NH_4Cl solution. The collected organic phases were dried over Na_2SO_4 and evaporated to dryness. The residue was purified by preparative TLC (SiO_2, heptane: ethyl acetate = 1:1) to afford compound **21** (28.1 mg, 67%). $[\alpha]_D = -38$ (*c* 1.8, $CHCl_3$); IR ($CHCl_3$) ν 3437, 2975, 1768, 1506, 1406, 1181 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.31 (t, *J* = 7.1 Hz, 3H), 2.6–2.90 (m, 3H), 2.92 (dd, *J* = 6.9, 16.7 Hz, 1H), 4.12 (m, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 4.85 (br s, 1H), 5.12 (q, *J* = 7.3 Hz, 1H), 7.1–7.4 (m, 5H); ^{13}C ($CDCl_3$) δ 14.5, 34.9, 36.7, 56.7, 61.7, 75.8, 127.8, 129.3, 129.5, 136.5, 155.5, 169.9; MS (CI) *m*/*z* 264 (M + H). Anal. calcd for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32. Found: C, 63.45; H, 6.61; N, 5.66; In NOEDIFF spectrum, irradiation at O_2 = 4135.422 Hz (250 MHz), corresponding to the OC*H* proton (δ = 5.12 ppm) leading to the 8% enhancement of the signal of NC*H* proton at δ = 4.12 ppm. The ^1H-^1H decoupling experiment allowed the calculation of coupling constant between H_a and H_b (*J* = 7.3) which is also indicative of *cis* relationship between these two protons.

(4*S*,5*S*)-(4-Benzyl-2-oxo-oxazolidin-5-yl)acetic acid ethyl ester (22). $[\alpha]_D = -89$ (*c* 1.5, $CHCl_3$); δ 1.31 (t, *J* = 7.1 Hz, 3H), 2.60 (dd, *J* = 6.9, 16.3 Hz, 1H), 2.65 (dd, *J* = 6.2, 16.3 Hz, 1H), 2.83 (dd, *J* = 8.1, 13.4 Hz, 1H), 2.96 (dd, *J* = 5.4, 13.4 Hz, 1H), 3.80 (m, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 4.70 (dt, *J* = 4.8, 6.5 Hz, 1H), 5.20 (br s, 1H), 7.1–7.4 (m, 5H); ^{13}C ($CDCl_3$) δ 14.7, 39.7, 42.3, 59.5, 61.9, 78.0, 127.9, 129.5, 129.7, 136.4, 158.7, 169.8; MS (CI) *m*/*z* 264 (M + H); The ^1H–^1H decoupling experiment allows the calculation of coupling constant between H_a and H_b (*J* = 4.6), indicative of *trans* relationship between these two protons.

(4*S*,5*R*)-4-Benzyl-3-(*tert*-butoxycarbonyl)-oxazolidin-5-yl)-acetic acid ethyl ester (24). To a solution of **19** (1.22 g, 3.62 mmol) in toluene (40 mL) were added aqueous HCHO solution (30% in water, 3.62 mL, 36.2 mmol) and *p*-toluenesulfonic acid (34.4 mg, 0.18 mmol). The mixture was heated to reflux using a Dean–Stark apparatus for removing water. After being refluxed for 20 h, the reaction mixture was diluted with aqueous NH_4Cl solution, the volatile was evaporated in vacuo and the aqueous residue was extracted with dichloromethane.

The combined organic phases were washed with brine, dried, and evaporated to dryness. The crude product was purified by flash chromatography (silica, EtOAc: heptane = 1:4) to afford **24** (973 mg, 77%): mp 68 °C; $[\alpha]_D = -32$ (*c* 0.9, $CHCl_3$); IR ($CHCl_3$) ν 2980, 1743, 1693, 1393 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): due to the presence of two rotamers, signals in ^1H NMR spectrum were broadened δ 1.1–1.3 (m, 12H), 2.5–2.7 (m, 4H), 4.00 (m, 2H), 4.40 (m, 2H), 4.90 (m, 2H), 7.1–7.4 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 28.2, 34.8, 35.2, 59.1, 61.0, 77.1, 77.9, 80.3, 126.3, 128.4, 129.5, 138.0, 170.3; MS (CI) *m*/*z* 350 (M + H), 294. Anal. calcd for $C_{19}H_{27}NO_5$:H, 7.79; N, 4.01. Found: C, 65.69; H, 7.62; N, 3.91.

(4*S*,3*R*)-4-(Benzyloxycarbonyl-methylamino)-3-hydroxy-5-phenyl-pentanoic acid ethyl ester (26). To a solution of CH_2Cl_2 (10 mL) and TFA (10 mL), cooled at 0 °C, was added $NaBH_3CN$ (2.07 g, 32.81 mmol) in a small portion. A solution of compound **21** (2.29 g, 6.56 mmol) in CH_2Cl_2 (10 mL) was then added in such a rate as the internal temperature did not exceed 0 °C. After stirring at the same temperature for 1 h, the volatile was removed and the residue was redissolved in CH_2Cl_2 and aqueous $NaHCO_3$ solution. The aqueous phase was extracted with CH_2Cl_2, the combined organic extracts were washed with H_2O, brine, dried and evaporated to give crude compound **25** which was used directly for the next step. A small amount of pure **25** was obtained by preparative TLC (SiO_2, CH_2Cl_2:MeOH = 9:1): $[\alpha]_D = -3.22$ (*c* 1.2, $CHCl_3$); IR ($CHCl_3$) ν 3612, 3012, 2968, 1750, 1681, 1443, 1412, 1125, 1043 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.25 (t, *J* = 7.1 Hz, 3H), 2.50 (m, 2H), 2.70 (s, 3H), 3.10 (d, *J* = 7.3 Hz, 2H), 3.60 (m, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 4.45 (m, 1H), 7.1–7.4 (m, 5H), 7.6 (br s, 2H); ^{13}C NMR ($CDCl_3$) δ 13.9, 32.1, 32.8, 36.9, 61.7, 65.0, 65.6, 128.0, 128.9, 129.5, 134.6, 172.5. To the solution of crude amino alcohol **25** in H_2O (30 mL) and acetone (30 mL) was added CbzOSu (2.45 g, 9.84 mmol) and $NaHCO_3$ (827 mg, 9.84 mmol). After being stirred at room temperature for 16 h, the reaction mixture was diluted with H_2O (30 mL), the volatile was removed under reduced pressure and the remaining aqueous phase was extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried, and evaporated. Purification by flash chromatography (SiO_2, eluent: EtOAc:heptane = 1:4 then 1:3) afforded the compound **26** (1.74 g, 69% in two steps): $[\alpha]_D = -33$ (*c* 1.5, $CHCl_3$); IR ($CHCl_3$) ν 3400, 2980, 1740, 1681, 1468, 1325, 1137 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.28 (t, *J* = 7.2 Hz, 3H), 2.42 (dd, *J* = 8.6, 17.0 Hz, 1H), 2.60 (m, 1H), 2.61 (s, 3H), 3.00 (dd, *J* = 11.6, 14.4, 1H), 3.21 (dd, *J* = 4.0, 14.4 Hz, 1H), 3.45 (d, *J* = 4.4 Hz, 1H), 4.12 (m, 1H), 4.18 (q, *J* = 7.2 Hz, 2H), 4.28 (m, 1H), 5.10 (s, 2H), 7.0–7.4 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 14.2, 33.2, 38.2 (38.8), 60.8, 64.5, 66.9 (70.5), 69.2, 70.1, 126.5, 127.5, 127.9, 128.4, 128.9, 139.0, 159.5, 172.5; MS (CI) *m*/*z* 386 (M + H). Anal. calcd for $C_{22}H_{27}NO_5$: C, 68.55; H, 7.06; N, 3.63. Found: C, 68.03; H, 7.04; N, 3.78.

(4*S*,3*R*)-4-(Benzyloxycarbonyl-methylamino)-3-methoxymethoxy-5-phenyl-pentanoic acid ethyl ester (27). To a solution of compound **26** (1.50 g, 3.9 mmol) in CH_2Cl_2 (30 mL) was added $MeOCH_2Br$ (1.59 mL, 19.52 mmol) and $^iPr_2NEt$ (3.73 mL, 21.47 mmol) at 0 °C. After being stirred at room temperature for 2 days, the reaction mixture was diluted with aqueous NH_4Cl solution and extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried, and evaporated. Purification by flash chromatography (SiO_2, eluent: EtOAc: heptane = 1:4) afforded compound **27** (1.45 g, 87%): $[\alpha]_D = -39$ (*c* 0.4, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): two rotamers δ 1.25 (t, *J* = 7.1 Hz, 3H), 2.60, 2.70 (s, 3H), 2.5–3.0 (m, 3H), 3.20 (m, 1H), 3.41, 3.42 (s, 3H), 4.10–4.35 (m, 4H), 4.75 (m, 2H), 4.90 (m, 1H), 5.10 (s, 1H), 7.0–7.4 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 14.4, 34.2 (32.4), 38.3 (38.5), 56.1 (56.2), 60.5 (60.7), 61.9, 66.7, 67.4, 77.1, 97.4, 126.2 (126.4), 127.4 (127.8), 128.1 (128.2), 128.5 (128.6), 129.0 (129.1), 136.5 (136.9), 138.3 (138.5), 156.3 (156.4), 171.0 (171.3); MS (CI) *m*/*z* 430 (M + H), 398. Anal. calcd for $C_{24}H_{31}NO_6$: C, 67.11; H, 7.27; N, 3.26. Found: C, 67.26; H, 7.22; N, 3.15.

(4*S*,3*R*)-4-(Benzyloxycarbonyl-methylamino)-3-methoxymethoxy-5-phenyl-pentanoic acid (28). To a solution of ester **27** (1.28 g, 2.98 mmol) in MeOH (40 mL) and water (10 mL) was added K_2CO_3 (824.8 mg, 5.98 mmol). After being refluxed for 3 h, the reaction mixture was diluted with aqueous NH_4Cl solution, the volatile was removed and the remaining aqueous phase was extracted with hexane to remove any neutral materials. The aqueous phase was then acidified with 2 N HCl and extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried, and evaporated to give acid **28** which was used without further purification (1.08 g, 90%): $[\alpha]_D = -30$ (*c* 1.3, $CHCl_3$); IR ($CHCl_3$) ν 3068, 2981, 1706, 1425 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ two rotamers 2.5–3.0 (m, 6H), 3.20 (m, 1H), 3.38, 3.40 (s, 3H), 4.2 (m, 1H), 4.75 (m, 1H), 4.9–5.2 (m, 4H) 7.0–7.4 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 33.9 (34.1), 36.9, 55.8 (55.9), 57.3 (57.5), 66.7 (66.9), 67.3, 76.4 (76.5), 96.8, 126.0–129.1 (complex), 135.9 (136.3), 137.9 (138.0), 156.3 (156.4), 175.0 (175.5); MS (CI) *m*/*z* 402 (M + H), 370, 340.

Compound 30 and 31. To a solution of **28** (141.00 mg, 0.35 mmol) in anhydrous toluene (5 mL) was added Et_3N (59.00 μL, 0.42 mmol) and 2,4,6-trichlorobenzoylchloride (55.00 μL, 0.35 mmol) at room temperature. After stirring at room temperature for 1.5 h, a solution of **16** (264.00 mg, 0.67 mmol) and 4-dimethylaminopyridine (428.00 mg, 3.51 mmol) was added at room temperature and stirring was continued for 1.5 h. The reaction mixture was diluted with NH_4Cl solution and the organic phase was separated. The aqueous phase was extracted with dichloromethane. The combined organic phases were washed with brine, dried, and evaporated. Purification by flash chromatography (SiO_2, heptane:hexane = 6:1) afforded **30** (186.2 mg, 68%) and a variable amount of β-elimination product **31** (the yield was variable depending on the purity of acid **28** as well as the reaction time of this coupling step). Prolonged reaction time increased the yield of **31** at the expense of that of compound **30**. Compound **30**: $[\alpha]_D = -25.7$ (*c* 0.7, $CHCl_3$); IR ($CHCl_3$) ν 2937, 2856, 1743, 1693, 1456, 1143 cm^{-1}; ^1H NMR (250 MHz,

$CDCl_3$) δ two rotamers 0.90–1.60 (m, 24H), 2.25 (m, 1H), 2.40–3.00 (m, 8H), 3.00 (m, 1H), 3.39, 3.40 (m, 3H), 4.30 (m, 1H), 4.60–5.30 (m, 7H), 7.00–7.40 (m, 15H); ^{13}C ($CDCl_3$): δ two rotamers 12.3, 12.6, 14.0, 14.2, 17.1, 18.7, 22.6, 25.3, 29.1, 29.3, 29.7, 30.1, 31.7, 31.9, 34.3, 37.4, 37.7, 37.9, 42.8, 43.0, 56.2, 57.4, 60.6, 66.6, 66.8, 67.2, 74.4, 74.5, 76.6, 76.8, 97.2, 97.4, 122.4, 126.2, 126.3, 126.7, 127.4, 127.7, 127.9, 128.3, 128.4, 128.5, 128.8, 137.2, 138.5, 145.4, 166.5, 170.6, 177.5; MS (CI) m/z 776 $(M+H)^+$. Compound **31**: $[\alpha]_D = -10.0$ (c 0.8, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ two rotamers 0.80–1.70 (m, 24H), 2.25 (m, 1H), 2.60–3.00 (m, 6H), 3.9–4.1 (m, 1H), 4.8–5.3 (m, 6H), 5.87 (dd, $J=1.8$, 15.8 Hz, 1H), 6.92 (dd, $J=4.6$, 15.8 Hz, 1H), 7.10–7.40 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 12.6, 14.0, 17.1, 18.7, 22.6, 25.4, 29.1, 29.3, 29.4, 29.7, 30.1, 31.7, 31.9, 37.4, 42.9, 43.2, 57.3, 66.8, 67.1, 74.4, 76.3, 122.3, 126.3, 126.7, 127.5, 127.7, 127.9, 128.2, 128.3, 128.4, 128.5, 128.8, 129.2, 135.4, 136.9, 145.8, 165.5, 169.1, 173.3; MS (FAB) m/z 720 $(M+Li)^+$. Anal. calcd for $C_{43}H_{55}NO_8$: C, 72.34; H, 7.77; N, 1.96 Found: C, 72.41; H, 7.72; N, 1.98.

8-Deoxyhapalosin (4). A solution of compound **30** (40 mg, 56.0 μmmol) in EtOAc (2 mL) and MeOH (1 mL) was hydrogenated at 1 atm in the presence of Pearman's catalyst. After being stirred at room temperature for 1 h, the reaction mixture was filtered through a short pad of Celite. The filtrate was evaporated to give crude seco acid (28 mg): MS (CI) m/z 492 $(M+1)^+$; IR ($CHCl_3$) ν 3600, 3468, 2937, 1731, 160, 1193 cm^{-1}. To the solution of this crude seco acid in DMF (56 mL) were added at 0 °C, DPPA (24 μL, 114 μmol) and Hunig's base (20 μL, 114 μmol), successively. After being stirred at 0 °C for 24 h, the reaction mixture was diluted with EtOAc (200 mL) and washed with H_2O, brine successively. The organic phase was then dried and evaporated. Purification by preparative TLC (SiO_2, EtOAc:heptane = 1:3) afforded compound **4** (12 mg, 45%): $[\alpha]_D = +12.7$ (c 1.4, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.46 (d, $J=6.7$ Hz, 3H), 0.65 (d, $J=6.9$ Hz, 3H), 0.89 (t, $J=6.5$ Hz, 3H), 1.13 (d, $J=7.1$, 3H), 1.20–1.40 (m, 10H), 1.55–1.72 (m, 3H), 1.95 (m, 1H), 2.17 (m, 1H), 2.27 (dd, $J=2.2$, 12.5 Hz, 1H), 2.48 (ddd, $J=2.4$, 5.7, 18.5 Hz, 1H), 2.74 (d, $J=7.2$ Hz, 2H), 2.84 (s, 3H), 3.24 (quintet, $J=7.0$ Hz, 1H), 3.95 (m, 1H), 4.57 (d, $J=8.3$ Hz, 1H), 4.87 (ddd, $J=3.0$, 6.3, 16.3, 1H), 7.15–7.35 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 12.8, 14.2, 17.8, 18.7, 22.8, 25.9, 27.4, 29.4, 29.3, 29.7, 31.9, 40.4, 40.8, 55.5, 74.3, 76.1, 127.1, 128.5, 128.9, 129.2, 129.5, 169.5, 170.4, 175.3; MS (CI) m/z 474 $(M+H)^+$; HRMS (CI) m/z 474.3224 ($C_{28}H_{43}NO_5$ + H requires 474.3220).

(4*S*,5*R*)-4-Benzyl-3-(*tert*-butoxycarbonyl)-oxazolidin-5-yl)-acetic acid (33). A solution of ester **24** (182 mg, 0.52 mmol) in EtOH (4 mL) and 1 N NaOH (4 mL) was stirred at room temperature for 10 min. The reaction was diluted with H_2O (10 mL) and the volatile was removed under reduced pressure. The aqueous phase was extracted with Et_2O to remove any neutral species. The aqueous phase was then acidified with citric acid and extracted with EtOAc. The combined organic extracts were washed with brine, dried, and evaporated to give acid **33** which was used directly for the next step without further purification (166 mg, 100%): IR ($CHCl_3$) ν 3450, 3075, 2981, 2943, 1730, 1693, 1406, 1200, 1187 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ two rotamers 1.47 (s, 9H), 2.21 (dd, $J=2.5$, 14.8 Hz, 1H), 2.42 (dd, $J=8.5$, 14.8, 1H), 2.80 (m, 2H), 3.20 (m, 1H), 3.94 (m, 1H), 4.60, 4.90 (m, 2H), 7.0–7.4 (m, 5H); MS (CI) m/z 322 $(M+H)^+$.

Compound 32. To a solution of acid **33** (112.00 mg, 0.35 mmol) in THF (4 mL) were added Et_3N (48.00 μL, 0.35 mmol) and Yamaguchi's reagent (54.00 μL, 0.35 mmol). The resulting reaction mixture was stirred at room temperature for 20 min and then a solution of compound **16** (109.00 mg, 0.28 mmol) in THF was introduced. After being stirred at room temperature for 3 h, the reaction mixture was diluted with aqueous NH_4Cl solution and extracted with EtOAc. The combined organic extracts were washed with brine, dried, and evaporated. Purification by flash chromatography (SiO_2, eluent: heptane:EtOAc = 10:1 then 4:1) afforded compound **32** (149.00 mg, 76%): $[\alpha]_D = -16.0$ (c 0.3, $CHCl_3$); IR ($CHCl_3$) ν 2945, 2860, 1735, 1454, 1293, 1208, 1131, 983 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) two rotamers δ 0.80 (d, $J=6.9$ Hz, 3H), 0.85 (t, $J=7.0$ Hz, 3H), 0.90 (d, $J=6.9$ Hz, 3H), 1.20 (d, $J=7.0$ Hz, 3H), 1.25–1.60 (m, 21H), 2.20–2.90 (m, 6H), 4.05 (m, 1H), 4.40 (m, 1H), 4.95 (d, $J=4.0$ Hz, 1H), 5.10–5.30 (m, 4H), 7.1–7.4 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 9.6, 14.2, 17.0, 17.2, 18.9, 22.7, 25.3, 26.3, 28.4, 29.6, 30.2, 31.8, 32.0, 33.6, 38.4, 44.6, 66.9, 67.4, 72.1, 76.5, 77.0, 78.1, 80.6, 126.4, 126.8, 128.4, 128.5, 128.7, 129.7, 137.5, 137.6, 170.4, 172.5, 175.3; MS (CI) m/z 696 $(M+H)^+$.

***N*-Desmethyl hapalosin (3).** A solution of compound **32** (32.00 mg, 46.00 μmol) in CH_2Cl_2 (2 mL) and TFA (0.50 mL) was stirred at 0 °C for 2 h. The volatile was removed and the residue, dissolved in EtOH (2.0 mL), was hydrogenated at 1 atm in the presence of Pd/C. After being stirred at room temperature for 2 h, the reaction mixture was filtered through a short pad of Celite. The filtrate was evaporated under reduced pressure and the residue was redissolved in DMF (40 mL, 10^{-3} M). To this solution cooled at 0 °C were added DPPA (30.00 μL, 0.14 mmol) and diisopropylethylamine (48.00 μL, 0.28 mmol) dropwise. After being stirred at 0 °C for 5 h and at room temperature for 18 h, the reaction mixture was diluted with EtOAc (200 mL) and washed with water, brine successively. The organic phase was then dried and evaporated. Purification by flash chromatography gave the *N*-desmethyl hapalosin **3** (10.5 mg, 48%): $[\alpha]_D = -54$ (c 0.2, $CHCl_3$);{Lit [6], $[\alpha]_D = -66$ (c 0.0061, $CHCl_3$); Lit. 7, $[\alpha]_D = -32$ (c 0.5, $CHCl_3$)}; IR ($CHCl_3$) ν 3425, 2932, 2856, 1742, 1675, 1650, 1519, 1460, 1272, 1179, 1085, 991 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 0.66 (d, $J=6.8$ Hz, 3H), 0.85 (d, $J=6.6$ Hz, 3H), 0.88 (t, $J=6.7$ Hz, 3H), 1.19 (d, $J=7.0$ Hz, 3H), 1.20–1.24 (m, 10H), 1.50–1.90 (m, 3H), 2.30 (m, 1H), 2.55 (dd, $J=5.7$, 13.9 Hz, 1H), 2.62 (dd, $J=3.7$, 13.9 Hz, 1H), 2.88–3.02 (m, 3H), 4.10 (m, 1H), 4.55 (d, $J=8.0$ Hz, 1H), 4.65 (m, 1H), 5.45 (d, $J=10.5$ Hz, 1H), 5.52 (dt, $J=3.6$, 7.0 Hz, 1H), 7.1–7.4 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 9.4, 14.2, 17.8, 18.6, 22.7,

25.7, 29.2, 29.4, 29.9, 31.1, 31.9, 37.7, 39.2, 41.4, 54.2, 70.8, 76.0, 81.8, 126.9, 128.6, 129.3, 137.2, 169.9, 173.9, 174.2; MS (CI) m/z 476 $(M+H)^+$; HRMS (CI) m/z 476.3018 ($C_{27}H_{42}NO_6$ requires 476.3012).

Hapalosin 2. To a mixture of solvent TFA (0.5 mL) and CH_2Cl_2 (0.5 mL) cooled at 0 °C were added $NaBH_3CN$ (12.60 mg, 0.20 mmol) followed by a solution of compound **29** (28.00 mg, 40.00 μmol) in CH_2Cl_2 (1 mL). After stirring at 0 °C for 2 h, the volatile was removed and the residue, dissolved in EtOH (2 mL), was hydrogenated at 1 atm in the presence of Pd/C for 2 h. The reaction mixture was filtered through a short pad of Celite. The filtrate was evaporated under reduced pressure to give the seco acid: MS (CI) m/z 508 $(M+H)^+$. To this crude seco acid, redissolved in DMF (40 mL, 10^{-3} M) and cooled at 0 °C, DPPA (26.00 μL, 0.12 mmol) and diisopropylethylamine (42.00 μL, 0.24 mmol) were added dropwise. After being stirred at 0 °C for 5 h and then at room temperature for 72 h, the reaction mixture was diluted with EtOAc (200 mL) and washed with water, brine successively. The organic phase was then dried and evaporated. Purification by flash chromatography gave the hapalosin **2** (3.5 mg, 18%): $[\alpha]_D = -45$ (c 0.1, CH_2Cl_2); {Lit. 5, $[\alpha]_D = -49.2$ (c 0.35, CH_2Cl_2)}; IR ($CHCl_3$) ν 3435, 2950, 1735, 1638 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 0.22 (d, $J = 6.9$ Hz, 3H), 0.57 (d, $J = 6.9$ Hz, 3H), 0.90 (d, $J = 7.0$ Hz, 3H), 1.2–1.4 (m, 13H), 1.60–2.0 (m, 3H), 2.61 (m, 1H), 2.65 (m, 1H), 2.85 (s, 3H), 2.91 (dd, $J = 17.8$, 5.1 Hz, 1H), 3.22 (m, 1H), 3.41 (dd, $J = 13.9$, 2.5 Hz, 1H), 3.70 (dt, $J = 10.1$, 2.5 Hz, 1H), 3.85 (m, 1H), 4.30 (d, $J = 8.4$ Hz, 1H), 5.12 (m, 1H), 7.2–7.4 (m, 5H); MS (CI) m/z 490 $(M+H)^+$; HRMS (CI) m/z 490.3179 ($C_{28}H_{44}NO_6$ requires 490.3169).

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