Synthesis and Conformational Analysis of the Multidrug Resistance-Reversing Agent Hapalosin and Its Non-*N*-methyl Analog

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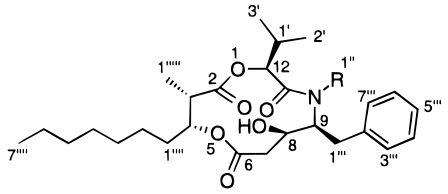
Hapalosin was initially synthesized by macrolactonization, and a second synthesis was achieved by cycloamidation. In both syntheses, three of the five stereocenters in hapalosin were established by two Brown allylboration reactions. The synthesis of the non-*N*-Me analog of hapalosin involved chelation-controlled reduction of a γ-amino-β-keto ester and cycloamidation. In $CDCl_3$ at 25 °C, synthetic hapalosin exists as a 2.3:1 mixture of conformers, while its non-*N*-Me analog exists only as a single conformer. $^1H,^1H$-NOESY and computation reveal that the configuration of the amide bond is responsible for the conformations of the two compounds. The major conformer of hapalosin is found to be an *s-cis* amide, the minor conformer an *s-trans* amide, and the non-*N*-Me analog an *s-trans* amide. Applying distance constraints to protons that exhibit NOESY correlations, computation shows that the major conformer of hapalosin and the non-*N*-Me analog have very different conformations. By contrast, the minor conformer of hapalosin and the non-*N*-Me analog have very similar conformations.

Introduction

Many cytotoxic drugs are quite effective during the initial stage of cancer chemotherapy. Over time, however, the tumor cells that survive the treatment often become resistant not only to the particular drugs employed but also to a broad spectrum of seemingly unrelated drugs. This phenomenon is known as multidrug resistance, or MDR.[1] The most consistent and dominant feature of MDR is reduced accumulation of cytotoxic drugs inside tumor cells. Higher doses of drugs must be used, with limited efficacy, while the side effects are more severe.

Of the various potential mechanisms of MDR, the most generally accepted one concerns the existence of a drug efflux pump called P-glycoprotein (P-gp). Indeed, tumor cells from chemotherapy patients often manifest increased P-gp expression and activity. P-gp is a 170−200 kDa transmembrane protein that is homologous with several bacterial transport ATPases. It utilizes ATP energy to extrude structurally diverse drugs such as taxol, *Vinca* alkaloids, and gramicidin D from the cytoplasm of tumor cells. An important strategy in chemotherapy is to use a chemosensitizer that blocks P-gp. The chemosensitizer is not necessarily cytotoxic itself. Rather, coadministration of the chemosensitizer with cytotoxic drugs allows the the intracellular concentrations of the drugs to remain high so that they can damage the cytoskeleton and DNA of tumor cells.

Hapalosin (**1**) is a novel cyclic depsipeptide that was recently isolated from the cyanobacterium *Hapalosiphon welwitschii*.[2] It exhibits a greater MDR-reversing activity than verapamil, which was one of the first drugs to be clinically tested for MDR reversal.[3] Hapalosin potentiates the cytotoxicity of drugs known to be transported by P-gp such as taxol, vinblastine, and actinomycin D. It achieves MDR reversal by elevating the intracellular concentrations of drugs putatively via inhibition of P-gp.[2]



1 R = Me, hapalosin
2 R = H, non-*N*-Me analog

Hapalosin was concurrently synthesized by our group[4] and by Ghosh et al.[5] Our synthetic hapalosin exists as a 2.3:1 mixture of conformers in $CDCl_3$ at 25 °C. Its $^1H,^1H$-NOESY spectrum showed a very strong correlation between H-9 and H-12 for the major conformer, indicative of an *s-cis* amide. On the other hand, there was a strong correlation between H-1″ and H-12 for the minor conformer, suggesting an *s-trans* amide, but there was none for the major conformer. Since secondary amides have a proclivity to exist in the *s-trans* conformation, we also wanted to synthesize the non-*N*-Me analog of hapalosin (**2**). It would be informative to relate the conformations of hapalosin and its non-*N*-Me analog to their MDR-reversing activity.

Results and Discussion

Synthesis. Hapalosin was initially synthesized by macrolactonization to form the O-5−C-6 ester bond (Scheme 1).[4] The synthesis converged at fragments **4** and **5**. Brown allylboration of an aldehyde derivative of L-Phe led to amino alcohol **4**. A second Brown allylboration of

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(1) For reviews on multidrug resistance, including the involvement of P-glycoprotein, see: (a) Simon, S. M.; Schindler, M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3497. (b) Gottesman, M. M.; Pastan, I. *Annu. Rev. Biochem.* **1993**, *62*, 385. (c) Ford, J. M.; Hait, W. N. *Pharmacol. Rev.* **1990**, *42*, 155. (d) Endicott, J. A.; Ling, V. *Annu. Rev. Biochem.* **1989**, *58*, 137.

(2) Stratmann, K.; Burgoyne, D. L.; Moore, R. E.; Patterson, G. M. L. *J. Org. Chem.* **1994**, *59*, 7219.

(3) Ozols, R. F.; Cunnion, R. E.; Klecker, R. W.; Hamilton, T. C.; Ostchega, Y.; Parrillo, J. E.; Young, R. C. *J. Clin. Oncol.* **1987**, *5*, 641.

(4) Dinh, T. Q.; Armstrong, R. W. *J. Org. Chem.* **1995**, *60*, 8118.

(5) Ghosh, A. K.; Liu, W.; Xu, Y.; Chen, Z. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 74.

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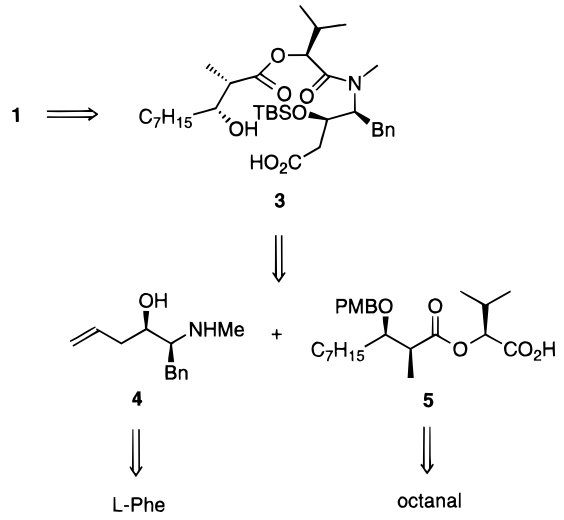
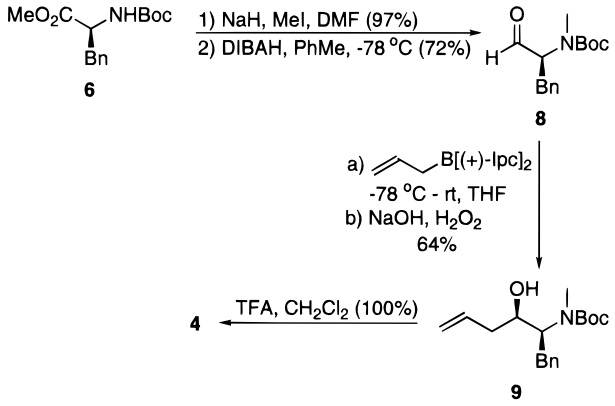
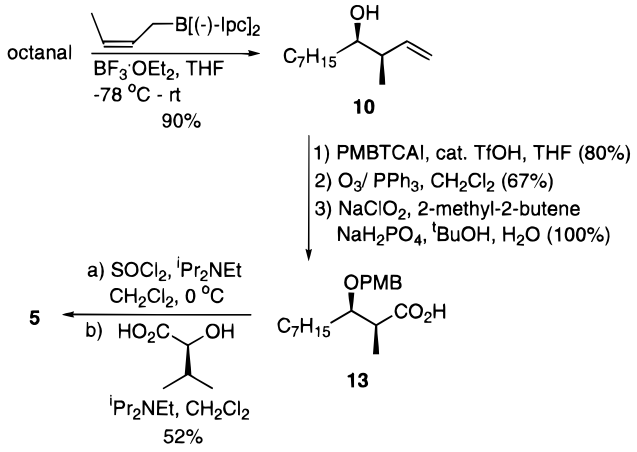
Scheme 1



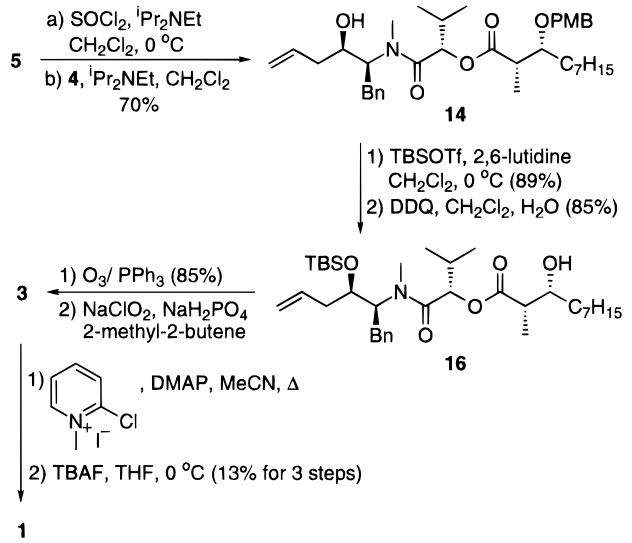
Scheme 2



Scheme 3



Scheme 4



octanal set the two contiguous stereocenters in acid **5**. A third key step was the macrolactonization of hydroxy acid **3**.

Amino alcohol **4** was synthesized in four steps starting with L-*N*-Boc-Phe-OMe **6** (Scheme 2). The amino ester was N-methylated with NaH and MeI[6] (97% yield) and reduced to the aldehyde with DIBAH (72% yield).[7] Aldehyde **8** underwent Brown allylboration[8] to give the desired diastereomer **9** in 64% yield and 90% de, the de being determined at the stage of amino alcohol **4**. All *N*,*N*-dialkylamides and -carbamates in Schemes 2 and 4 exist as rotamers in $CDCl_3$ at 25 °C. This characteristic complicates the interpretation of ^{1}H NMR spectra acquired at 25 °C of compounds more advanced than **8**.

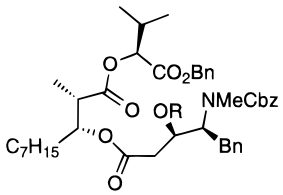
Synthesis of the second fragment, ester acid **5**, is illustrated in Scheme 3. Brown allylboration[9] of octanal furnished homoallylic alcohol **10** in 90% yield and 90% de.[10] The hydroxyl group was protected with *p*-methoxybenzyl 2,2,2-trichloroacetimidate (PMBTCAI)[11] in 80% yield, and the olefin was ozonized to the aldehyde (67% yield). Oxidation of the aldehyde with sodium chlorite[12] afforded acid **13** (100% yield). The acid was converted to the acid chloride, which reacted with (*S*)-α-hydroxyisovaleric acid to produce ester acid **5** (52% yield).

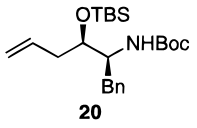
Six steps after coupling of fragments **4** and **5** provided hapalosin (Scheme 4). Amide **14** was formed by reacting amino alcohol **4** with the acid chloride derivative of ester acid **5** (70% yield). The hydroxyl group was protected[13] with TBSOTf (89% yield), and the PMB ether was deprotected with DDQ (85% yield). Alkenol **16** was ozonized (85% yield), and the aldehyde was oxidized to the acid with sodium chlorite. Macrolactonization of crude hydroxy acid **3** under modified Mukaiyama conditions[14] and deprotection of the TBS (*tert*-butyldimethylsilyl) ether of the resulting macrolide with TBAF produced hapalosin (**1**) in 13% yield for the final three steps.[15] Synthetic hapalosin was identical in all respects (^{1}H NMR, ^{13}C NMR, $[\alpha]_D$, IR, and HRMS) to the natural

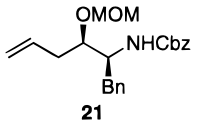
(6) Cheung, S. T.; Benoiton, N. L. *Can. J. Chem.* **1977**, *55*, 906.
(7) To minimize racemization of aldehyde **8**, solvents were removed from **8** at ≤30 °C and flash chromatography with silica gel was done in <30 min.
(8) Jadhav, P. K.; Bhat, K. S.; Perumal, P. T.; Brown, H. C. *J. Org. Chem.* **1986**, *51*, 432.
(9) (a) Brown, H. C.; Bhat, K. S. *J. Am. Chem. Soc.* **1986**, *108*, 5919. (b) Brown, H. C.; Jadhav, P. K.; Bhat, K. S. *J. Am. Chem. Soc.* **1988**, *110*, 1535.
(10) Subsequent formation of a single diastereomer of ester acid **5** proved that the ee of alcohol **10** was >95%.
(11) Nakajima, N.; Horita, K.; Abe, R.; Yonemitsu, O. *Tetrahedron Lett.* **1988**, *29*, 4139.
(12) Hillis, L. R.; Ronald, R. C. *J. Org. Chem.* **1985**, *50*, 470.
(13) Several attempts to macrolactonize the diolacid corresponding to **3** were unsuccessful.
(14) The original Mukaiyama conditions are in: Mukaiyama, T.; Usui, M.; Saigo, K. *Chem. Lett.* **1976**, *49*. The modification of Mukaiyama conditions is based on Evans' modification of Keck conditions for macrolactonization with DCC: Evans, D. A.; Kaldor, S. W.; Jones, T. K.; Clardy, J.; Stout, T. J. *J. Am. Chem. Soc.* **1990**, *112*, 7001.
(15) The low yield for the three steps was due to macrolactonization. The TBS ether of hapalosin could not be isolated in sufficient purity for the yield of the macrolactonization step to be accurately known.

product. Other methods of macrolactonization did not give better results: (1) 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate, DMAP·HCl, DMAP,[16] (2) 2,2′-dipyridyl disulfide, PPh_3,[17] (3) 2,4,6-trichlorobenzoyl chloride, *N*,*N*-diisopropylethylamine (DIPEA), DMAP,[18] (4) pivaloyl chloride, DIPEA, DMAP,[19] and (5) bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl), DIPEA, and DMAP.[20]

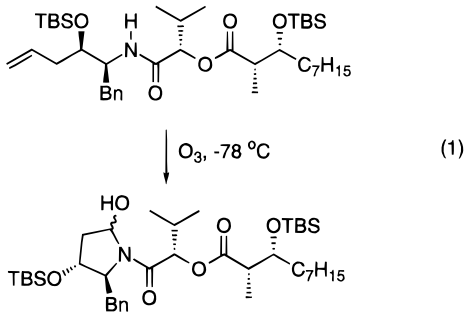
The TBS ether of hapalosin was also synthesized by cycloamidation. Bis-deprotection of TBS ether **18**[21] via hydrogenation over Pearlman's catalyst in MeOH and cyclization of the resulting amino acid with BOP-Cl and DIPEA in PhMe at 85 °C[22] produced the TBS ether of hapalosin in 16% yield for the two steps. The two corresponding steps provided the TBS ether of the non-*N*-Me analog of hapalosin in 60% yield (see Scheme 6). In Ghosh's synthesis of hapalosin, bis-deprotection of MOM ether **19** by hydrogenation over Pearlman's catalyst in EtOAc and cycloamidation with 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC), HOBt, and triethylamine in DMF at 60 °C afforded the MOM ether of hapalosin in 30−37% yield for the two steps.[5] Cycloamidation to form the TBS ether of hapalosin might have been sterically hindered by the TBS group, which is bulkier than MOM.[23] For example, bis-deprotection of TBS ether **18** was not complete after 15 h, whereas it was finished in 5 h for MOM ether **19**.[5] Furthermore, N-methylation of TBS ether **20** with NaH and MeI in DMF at 25 °C was poor while Ghosh could N-methylate MOM ether **21** with NaH and MeI in THF/DMF (10:1) at 25 °C in a good yield.[5]





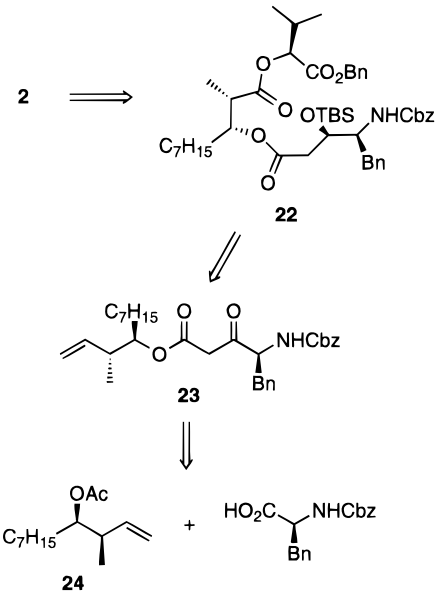


It was originally thought that synthesis of the non-*N*-Me analog of hapalosin could be achieved by macrolactonization as for hapalosin. The major problem, however, was that during ozonolysis, the amide nitrogen quickly cyclized onto the aldehyde group to form a pyrrolidinol (eq 1). The pyrrolidinol partially rearranged



(1)

Scheme 5



to a pyrrole overnight at 25 °C by losing the hydroxyl and ring TBS ether groups. Two possible ways to obviate pyrrolidinol formation were to protect the amide nitrogen or to convert the olefin to a carboxylic acid in one step. However, the simpler compound **20** was unreactive toward protection of the carbamate nitrogen with Cbz-Cl or Boc_2O and it could not be transformed into a carboxylic acid by $NaIO_4/KMnO_4$,[24] $NaIO_4/RuCl_3$,[25] or O_3/H_2O_2.[26]

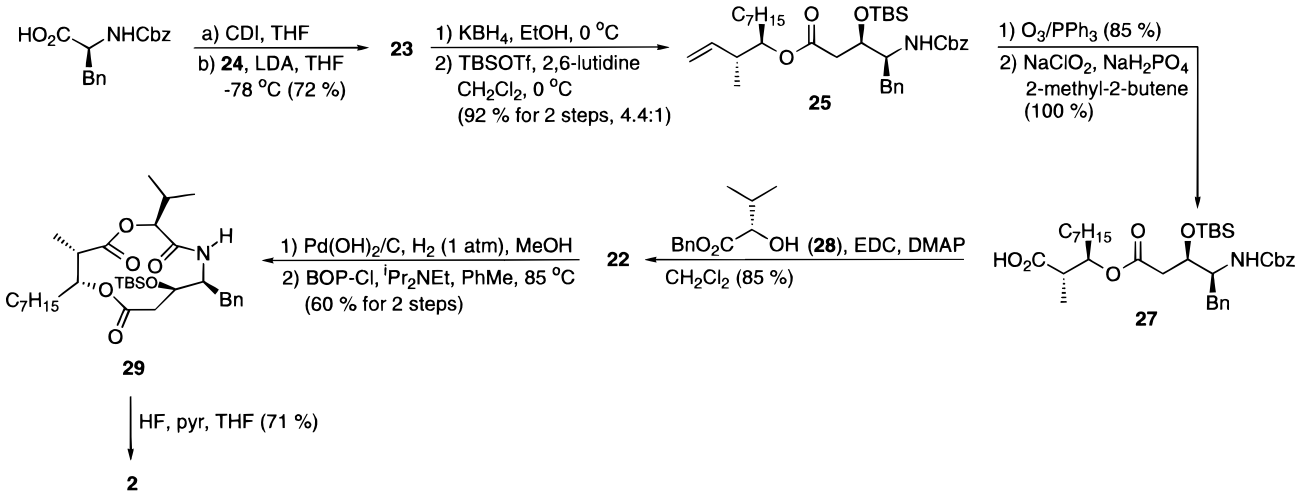
The non-*N*-Me analog of hapalosin was synthesized by cycloamidation after bis-deprotection of TBS ether **22** (Scheme 5). Compound **22** derived from chiral γ-amino-β-keto ester **23**. The necessary (*R*)-β-hydroxyl group was created by a chelation-controlled reduction of the amino ketone. Coupling of the enolate of acetate **24** to an activated L-*N*-Cbz-Phe-formed γ-amino-β-keto ester **23**. The chemistry concerning **23** was based on the work of Joullie et al. in the synthesis of the didemnins.[27]

An efficient synthesis of the non-*N*-Me analog of hapalosin is illustrated in Scheme 6. Addition of the enolate of acetate **24**[28] to the imidazolide of L-*N*-Cbz-Phe furnished γ-amino-β-keto ester **23** (72% yield).[27] Chelation-controlled KBH_4 reduction of the keto group resulted in two inseparable diastereomers.[27] The alcohols were separated as TBS ethers in a 4.4:1 diastereomeric ratio (92% yield for two steps).

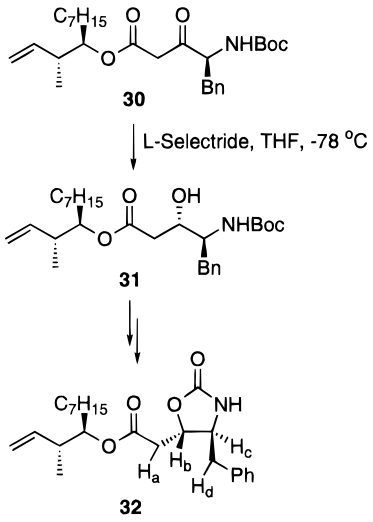
The prediction that KBH_4 reduction of γ-amino-β-keto ester **23** would favor the chelation-controlled product was verified by experiments with the very similar compound

(16) The Evans paper in ref 14.
(17) Corey, E. J.; Nicolaou, K. C. *J. Am. Chem. Soc.* **1974**, *96*, 5614.
(18) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989.
(19) Roush, W. R.; Blizzard, T. A. *J. Org. Chem.* **1984**, *49*, 1772.
(20) Corey, E. J.; Hua, D. H.; Pan, B.-C.; Seitz, S. P. *J. Am. Chem. Soc.* **1982**, *104*, 6818.
(21) Characterization data of **18** are in the Experimental Section and its ^1H and ^{13}C NMR spectra are in the supporting information.
(22) Evans; D. A.; Miller, S. J.; Ennis, M. D. *J. Org. Chem.* **1993**, *58*, 471.
(23) In a work published after this paper was submitted, cycloamidation of the amino acid with a free β-hydroxyl group using diphenyl phosphorazidate and DIPEA in DMF generated hapalosin in 44% yield: Ohmori, K.; Okuno, T.; Nishiyama, S.; Yamamura, S. *Tetrahedron Lett.* **1996**, *37*, 3467.
(24) Aristoff, P. A.; Johnson, P. D.; Harrison, A. W. *J. Am. Chem. Soc.* **1985**, *107*, 7967.
(25) Carlsen; P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936.
(26) Bailey, P. S. *Ind. Eng. Chem.* **1958**, *50*, 993.
(27) (a) Li, W.-R.; Ewing, W. R.; Harris, B. D.; Joullie, M. M. *J. Am. Chem. Soc.* **1990**, *112*, 7659. (b) Harris, B. D.; Joullie, M. M. *Tetrahedron* **1988**, *44*, 3489.
(28) Acetate **24** was quantitatively obtained by acetylation of alcohol **10** with AcCl, pyridine, and catalytic DMAP in CH_2Cl_2 at 0−25 °C.

Scheme 6



Scheme 7



30 (Scheme 7). KBH_4 reduction of γ-amino-β-keto ester **30** produced two separable diastereomers in a 4.4:1 ratio. L-Selectride (Aldrich) reduction of **30**[16] resulted in a substantially greater diastereomeric excess, but the major diastereomer in this reaction was the minor diastereomer in the KBH_4 reaction. The major diastereomer in the L-Selectride reaction, **31**, was converted to oxazolidinone **32**. $^1H,^1H$-NOESY of oxazolidinone **32** showed that the two ring protons, H_b and H_c, were *trans*. For instance, there were crosspeaks correlating H_b to both H_d protons and to the *o*-phenyl protons, and there was no crosspeak correlating either of the H_a protons to either of the H_d protons. The *trans* relationship in **32** proved that L-Selectride reduction of **30** favored the Felkin−Anh product **31**. These results agreed with Joullie's findings that KBH_4 reductions of similar chiral *N*-Boc- and *N*-Cbz-γ-amino-β-keto esters favored chelation-controlled products.[27,29]

The desired TBS ether, **25**, was ozonized to the aldehyde (85% yield), which was then oxidized to the acid with sodium chlorite (100% yield). Acid **27** was coupled to hydroxybenzyl ester **28**[30] with EDC and DMAP to furnish tris-protected **22** (85% yield). Bis-deprotection of **22** by hydrogenation over Pearlman's catalyst and cyclization of the resulting amino acid with BOP-Cl and DIPEA in PhMe at 85 °C[22] generated the TBS ether of the non-*N*-Me analog of hapalosin, **29**, in 60% yield for the two steps.[31] As stated before, the two corresponding reactions for **18** produced the TBS ether of hapalosin in only 16% yield. Cyclization of the non-*N*-Me amino acid with BOP-Cl was superior to that with 2-chloro-*N*-methylpyridinium iodide/DIPEA (55% yield),[14] 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate/DMAP/DMAP·HCl (33% yield),[16] or 2-mesitylenesulfonyl chloride/DIPEA (40% yield).[32] Deblocking of TBS ether **29** with 2.0 equiv of TBAF in THF at 0 °C for 0.5 h resulted in about 50% epimerization of the non-*N*-Me analog of hapalosin, **2** (the site of epimerization is unknown). This was a surprising observation considering that TBAF did not cause any epimerization in the formation of hapalosin. Removal of the TBS group for the non-*N*-Me analog was effected by HF/pyridine without any epimerization (71% yield).

Conformational Analysis. In $CDCl_3$ at 25 °C, synthetic hapalosin exists as a 2.3:1 mixture of conformers whereas its non-*N*-Me analog exists only as a single conformer. Their conformations in $CDCl_3$ at 25 °C were analyzed by $^1H,^1H$-NOESY at 500 MHz over three different mixing times—1.15, 2.30, and 3.45 s (0.80 s was too short for hapalosin). The two compounds tumbled rapidly in the positive NOE regime. Crosspeaks of at least medium strength that were used in the conformational computation of the major conformer of hapalosin and the non-*N*-Me analog are listed in Table 1. Due to peak overlap of H-1‴b/H-7b and H-1″/H-7a for the major conformer of hapalosin, some potential correlations involving these protons were not considered. Attempts to crystallize the two compounds were unsuccessful.

Since rotational isomerism about the amide bond is common for tertiary *N*,*N*-dialkylamides and secondary amides tend to favor the *s-trans* configuration, these characteristics were conjectured to be responsible for the conformations of hapalosin and its non-*N*-Me analog.

(29) KBH_4 reduction of the *N*-Me variant of **23** for the synthesis of hapalosin would probably demonstrate insignificant stereoselectivity since this was so when MOM-protected *trans*-4-hydroxy-L-proline was used in lieu of the L-phenylalanine moiety in **23**.

(30) Hydroxybenzyl ester **28** was made in 87% yield by reaction of (*S*)-α-hydroxyisovaleric acid, BnBr, and DBU in MeCN at 25 °C. The protocol was culled from: Rao, C. G. *Org. Prep. Proc. Int.* **1980**, *12*, 225.

(31) Since it was best not to purify the zwitterionic amino acid resulting from bis-deprotection of **22**, the yield of this step is not specifically known but is probably good.

(32) Baker, R.; Castro, J. L. *J. Chem. Soc. Perkin Trans. 1* **1990**, 47.

Table 1. NOESY Correlations between Protons[a]

hapalosin (**1**)[b]		non−N−Me analog (**2**)	
9−12	2′−3‴/7‴	1′−1″	7b−1‴a
8−1″	2′−4‴/6‴	2′−1″	7b−1‴b
1″−1‴b	2′−5‴	3′−1″	1⁗a−3
3−1⁗	1″−3‴/7‴	12−1″	1⁗a−1⁗′
1⁗a−1⁗′	9−3‴/7‴	1‴a−1″	1⁗b−1⁗′
12−3‴/7‴		1‴b−1″	3′−3‴/7‴
		8−1″	1′−3‴/7‴
		OH−9	1″−3‴/7‴
		7a−9	9−3‴/7‴
		8−1‴a	8−3‴/7‴
		8−1‴b	

[a] ^{1}H,^{1}H-NOESY of the two compounds in $CDCl_3$ was conducted at 25 °C at 500 MHz over three different mixing times−1.15, 2.30, and 3.45 s. All the correlations are of at least medium strength. [b] The major conformer.

Indeed, the NOESY spectra showed prima facie that the major conformer of hapalosin is an *s-cis* amide, the minor conformer is an *s-trans* amide, and the non-*N*-Me analog is an *s-trans* amide. There was a very strong correlation between H-9 and H-12 for the major conformer of hapalosin, suggesting an *s-cis* amide. By contrast, there was a strong correlation between H-1″ and H-12 for the minor conformer, implying an *s-trans* amide, but no such correlation existed for the major conformer. The non-*N*-Me analog of hapalosin appeared to be an *s-trans* amide due to the presence of crosspeaks correlating all four types of isobutyl protons (H-12, -1′, -2′, and -3′) to H-1″. Computation corroborated the NOESY interpretations.

Molecular modeling studies were performed with Macromodel (v. 4.5)[33] using the AMBER* force field and GB/SA chloroform solvation.[34] (All of the computations discussed below were also conducted using the MM3* force field, and the results are similar to those with the AMBER* force field.) Conformational searches were performed using Still's internal coordinate Monte Carlo protocol.[35] The search was done on blocks of 1000 Monte Carlo steps until no lower-energy conformation was found compared to the current global minimum. For hapalosin, different *cis* and *trans* conformations were used as the starting geometry, and no lower energy minimum was found. To simplify the number of conformations found in the computation, the carbon atoms of the heptyl side chain were not compared; i.e., if two conformations had exactly the same coordinates on all atoms except for the heptyl side chain, they were considered to be the same conformation. Duplicate conformations as well as those that had chirality change were discarded. Internuclear distance constraints for protons that have NOESY correlations were set between 1.5 and 5.0 Å. Table 1 contains the pairs of protons used in the distance constraints for the major conformer of hapalosin and the non-*N*-Me analog. Clustering of conformations of the same structure was done employing the Xcluster program implemented with Macromodel. The clustering was based on atomic root mean square displacement between pairs of structures following rigid-body superimposition. Atoms C-11, the oxygen attached to C-11, N-10, C-1″, the hydroxyl oxygen, C-2, and C-6 were compared during clustering for hapalosin and atoms C-11, the oxygen attached to C-11, N-10, the hydroxyl oxygen, C-2, and C-6 for the non-*N*-Me analog. Superimposition of the *s-trans* conformation of hapalosin with the non-*N*-Me analog was done by Biosym's INSIGHT II (v. 2.3.5). The carbonyl atoms C-2, C-6, and C-11 were superimposed.

From the conformational search of hapalosin, 32 conformations were found within 5 kcal/mol from the global minimum. Twenty-one of them have an *s-cis* amide bond, and 11 have an *s-trans* amide bond. The lowest-energy conformation found has an *s-cis* amide bond. The distance between H-9 and H-12 for all *s-cis* conformations ranges from 2.0 to 3.0 Å except for one of 3.3 Å, while that for all *s-trans* conformations is above 4.4 Å. This is in accordance with the very strong NOESY correlation observed between H-9 and H-12 for the major conformer of hapalosin. These results strongly suggest that the major conformer has an *s-cis* amide bond and the minor conformer has an *s-trans* amide bond. With regard to the non-*N*-Me analog of hapalosin, 12 conformations were found within 5 kcal/mol from the global minimum. All have an *s-trans* amide bond. This agrees with the NOESY data and the fact that only one conformer exists in $CDCl_3$ at 25 °C.

Though hapalosin is not a big molecule, it is quite flexible. Twelve clusters could be found among all conformations.[36] They have different ring shapes and different carbonyl orientations (Figure 1a). Ring shapes 1−7 belong to the *s-cis* amide configuration and ring shapes 8−12 to the *s-trans* amide configuration. There are four very different combinations of the relative positions of the carbonyl groups: uuu, uud, pdu, and udu in the order of C-11, C-6, and C-2 (with respect to the ring, "u" denotes up, "d" down, and "p" parallel). Most of the conformations do not have any hydrogen bonding between the hydroxyl group and a carbonyl oxygen. By contrast, the non-*N*-Me analog of hapalosin is much less variable due to the intramolecular hydrogen bonds formed. In every conformer, the amide H-1″ hydrogen bonds with O-1 and the oxygen attached to C-6 and the hydroxyl group hydrogen bonds with at least one carbonyl oxygen. The three clusters of the non-*N*-Me analog[37] vary in their ring shape but have similar carbonyl orientations (Figure 1b).

Conformational searches with distance constraints for protons that have correlations in the NOESY of the major conformer of hapalosin and the non-*N*-Me analog (Table 1) were also conducted. Nine conformations were found for the major conformer of hapalosin. They all have an *s-cis* amide bond, indicating again that the major conformer of hapalosin has an *s-cis* amide bond. The nine conformations can be separated into four clusters, and their ring shapes can be represented by ring shapes 1, 2, 4, and 7 of hapalosin in Figure 1a.[38] The superimposition of the nine conformations is shown in Figure 2a. The distance constraints helped to locate the isopropyl group and the benzyl group very well, and their positions remain relatively unchanged through all nine conformations. Four conformations were found for the non-*N*-Me analog, separated into two clusters represented by ring

(33) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.

(34) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127.

(35) Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379.

(36) The number of conformations contained in clusters 1−12 of hapalosin is six, four, five, two, two, one, one, five, two, two, one, and one, respectively.

(37) The number of conformations contained in clusters 1−3 of the non-*N*-Me analog is three, seven, and two, respectively.

(38) The number of conformations belonging to ring shapes 1, 2, 4, and 7 for the major conformer of hapalosin is four, two, one, and two, respectively.

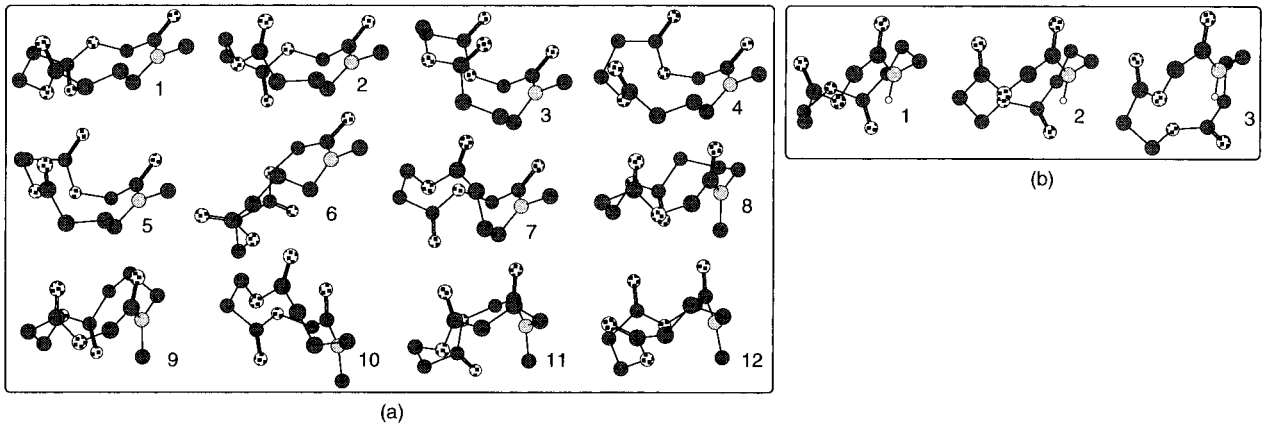


Figure 1. Ring conformations of the clusters for hapalosin (a) and for the non-*N*-Me analog (b) within 5 kcal/mol of the found global minimum. All the side chains and protons on the ring carbon atoms are removed for clarity. Key: dark, C; spotted, O; gray, N; white, H.

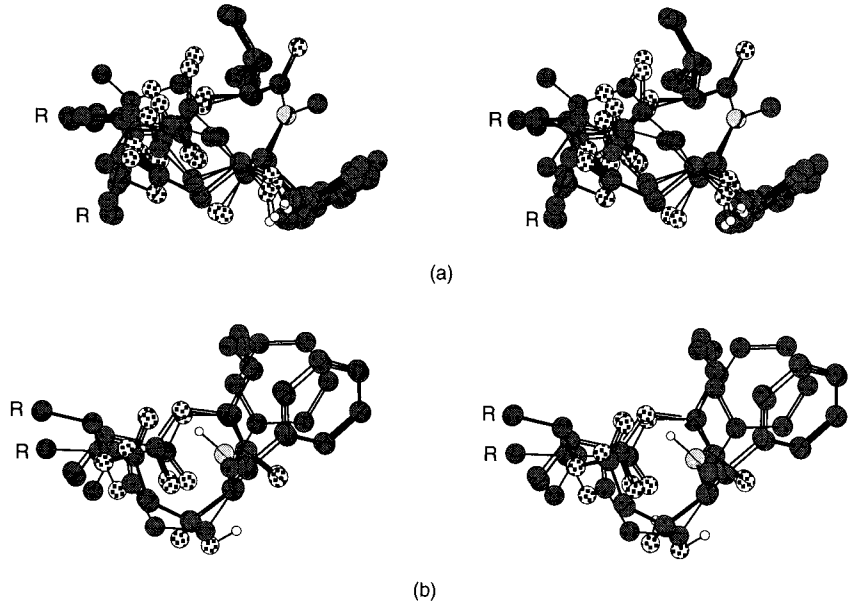


Figure 2. Stereoviews: superimposition of the nine conformations of the major conformer of hapalosin (a) and superimposition of the four conformations of the non-*N*-Me analog (b) that satisfy the distance constraints, with the four atoms of the amide bond superimposed. The heptyl group is labeled as R. The protons on carbon atoms are removed for clarity. Key: dark, C; spotted, O; gray, N; white, H.

shapes 1 and 2 in Figure 1b.[39] The four conformations are superimposed in Figure 2b. The lowest-energy conformation from the major cluster of *s-cis* hapalosin and that for the non-*N*-Me analog are displayed in Figure 3a,b, respectively. Comparison of Figure 3a to b shows that the major conformer of hapalosin and the non-*N*-Me analog have different orientations of side chains and very different ring shapes as a result of the difference in amide bond geometry. Considering such dissimilar conformations, the major conformer of hapalosin and the non-*N*-Me analog may have very different biological actvities. The non-*N*-Me analog is currently undergoing biological evaluation for its ability to reverse MDR.

In contrast, there are many similarities between the lowest energy conformation from the major cluster of the *s-trans* amide conformations of hapalosin and the lowest energy, distance-constrained conformation from the major cluster of the non-*N*-Me analog (Figure 4). The two rings superimpose quite well on the left half from C-12 to C-6 as exhibited in Figure 4. Both amide bonds are *s-trans*, and all three carbonyl groups are in similar orientations. The isopropyl and benzyl groups are also in similar orientations. The difference on the right side of the ring is a result of the orientation of the hydroxyl group. In the *s-trans* conformer of hapalosin, the hydroxyl group is sticking out while in the non-*N*-Me analog, the hydroxyl group rotates inward to form a hydrogen bond with the carbonyl oxygen attached to C-2. The computation suggests that the non-*N*-Me analog and minor conformer of hapalosin, having similar conformations, may share similar biological activities.

Conclusion

NOESY and computation demonstrate that the major conformer of hapalosin has an *s-cis* amide bond, the minor conformer has an *s-trans* amide bond, and the non-*N*-Me analog has an *s-trans* amide bond. With the aid of the NOESY data, computation shows that the major

(39) The number of conformations belonging to ring shapes 1 and 2 for the non-*N*-Me analog is three and one, respectively.

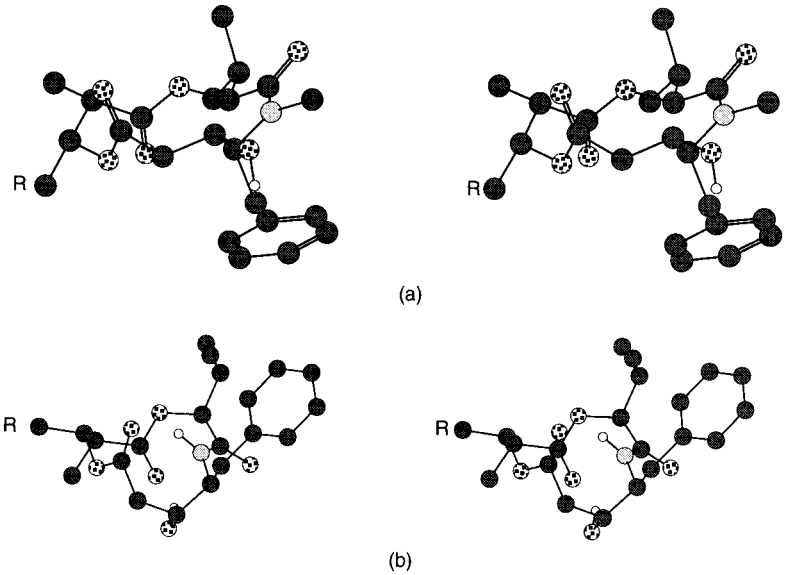


Figure 3. Stereoviews: the lowest-energy conformation from the major cluster of hapalosin with an *s-cis* amide (a) and that for the non-*N*-Me analog (b) that satisfy the distance constraints. The heptyl group is labeled as R. The protons on carbon atoms are removed for clarity. Key: dark, C; spotted, O; gray, N; white, H.

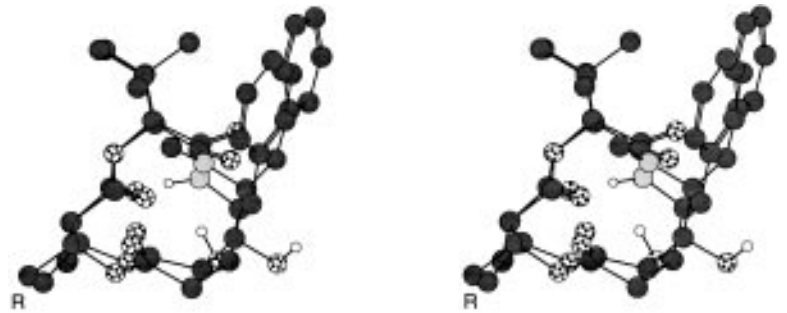


Figure 4. Stereoview: superimposition of the lowest-energy conformation from the major cluster of the non-*N*-Me analog that satisfies the distance constraints and the lowest-energy conformation from the major cluster of hapalosin with an *s-trans* amide. The carbonyl atoms C-2, C-6, and C-11 are superimposed. The heptyl group is labeled as R. The protons on carbon atoms are removed for clarity. Key: dark, C; spotted, O; gray, N; white, H.

conformer of hapalosin and the non-*N*-Me analog have very different conformations. On the other hand, the minor conformer of hapalosin and the non-*N*-Me analog exhibit very similar conformations. Knowledge of the MDR-reversing activity of the non-*N*-Me analog may reveal whether the major or minor conformer of hapalosin accounts for its MDR-reversing property.

Experimental Section

General Procedures. ^{1}H and ^{13}C NMR spectra were obtained at 25 °C with a Bruker ARX-400 or -500 spectrometer. ^{1}H NMR chemical shifts are referenced to TMS (0.00 ppm) and those for ^{13}C NMR to $CDCl_3$ (77.0 ppm). IR spectra were recorded with a Nicolet 510P FT-IR spectrometer. Optical rotations were measured with a Perkin-Elmer 241MC polarimeter, and concentrations (c) are reported in g/mL. High-resolution mass spectroscopy (HRMS) was performed with a VG Autospec instrument for the EI and CI methods and a VG ZAB-SE instrument for the FAB method.

All water-sensitive reactions were conducted in oven- or flame-dried glassware under a nitrogen atmosphere. The starting materials were azeotroped two or three times with benzene before the reactions. Solvents were distilled immediately prior to use: CH_2Cl_2 from P_2O_5, PhMe from CaH_2, MeOH from magnesium metal, and THF from sodium metal/benzophenone ketyl. Anhydrous DMF and MeCN and (*S*)-α-hydroxyisovaleric acid were purchased from the Aldrich Chemical Co. and utilized without further purification. L-*N*-Boc-Phe-OMe (**6**) can be bought from Aldrich. Most commercially available reagents were distilled before use. Thin-layer chromatography (TLC) was performed on silica gel-coated plates (Merck, Kieselgel 60 F_{254}, 0.25 mm thickness for analytical and 0.5 mm for preparative TLC) and visualized by UV light and/or *p*-anisaldehyde, ninhydrin, or bromocresol green (for carboxylic acids) staining. After all aqueous extractions of crude reaction products, the combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo* before further treatment.

L-*N*-(ᵗButyloxycarbonyl)-*N*-methylphenylalanine Methyl Ester (7). A solution of L-*N*-Boc-Phe-OMe (**6**) (10.00 g, 35.8 mmol) and MeI (4.5 mL, 72 mmol) in DMF (130 mL) was cooled to 0 °C. NaH (1.12 g, 46.5 mmol) was added in 200 mg portions, and more was added when bubbling subsided. After all the NaH was added, the ice bath was removed, and the mixture was stirred at 25 °C for 15 h. The mixture was quenched with saturated aqueous NH_4Cl (5 mL), and much DMF was removed *in vacuo*. Extraction in EtOAc (3 × 200 mL) with H_2O (250 mL) gave pure **7** (10.18 g, 97% yield) as an oil: $[\alpha]^{22}_D$ −54° (c 0.043, $CHCl_3$); IR (neat) 1144, 1393, 1699, 1746, 2977 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, 1.3:1 rotamers) major rotamer δ 1.33 (s, 9 H), 2.72 (s, 3 H), 3.01 (dd, J = 14.2, 10.9 Hz, 1 H), 3.27 (m, 1 H), 3.75 (s, 3 H), 4.54 (dd, J = 10.7, 4.4 Hz, 1 H), 7.12−7.24 (m, 3 H), 7.28 (m, 2 H), minor rotamer δ 1.37 (s, 9 H), 2.70 (s, 3 H), 3.01 (dd, J = 14.2, 10.9 Hz, 1 H), 3.31 (m, 1 H), 3.73 (s, 3 H), 4.94 (dd, J = 10.5, 5.3 Hz, 1 H), 7.12−7.24 (m, 3 H), 7.28 (m, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) both rotamers δ 28.1, 28.2, 31.8, 32.5, 35.0, 35.5, 52.1, 59.4,

61.6, 79.9, 80.2, 126.4, 126.6, 128.3, 128.5, 128.9, 129.0, 137.3, 137.6, 154.9, 155.7, 171.5, 171.9; HRFAB calcd for $C_{16}H_{24}O_4N$ [$(M + H)^+$] 294.1705, found 294.1710.

L-*N*-(ᵗButyloxycarbonyl)-*N*-methylphenylalaninal (8). A solution of methyl ester **7** (9.64 g, 32.9 mmol) in PhMe (100 mL) was cooled to −78 °C. DIBAH (49.6 mL in hexanes, 49.6 mmol) was added over 15 min, and the solution was stirred for 2.5 h at −78 °C and then quenched with −78 °C MeOH (20 mL). After successive extractions in CH_2Cl_2 (3 × 200 mL) with cold aqueous 1.2 M HCl (300 mL) and brine (300 mL) and concentration of the combined organic layers *in vacuo* at 30 °C, flash chromatography with silica gel (gradient to 50% EtOAc/hexanes) was performed in <30 min to minimize racemization. Aldehyde **8** (6.26 g, 72% yield) was obtained as a colorless oil: $[\alpha]^{22}_D$ −83° (*c* 0.023, $CHCl_3$); IR (neat) 1152, 1368, 1686, 1740, 2977 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 1.2:1 rotamers), major rotamer δ 1.43 (s, 9 H), 2.68 (s, 3 H), 2.89 (dd, *J* = 14.1, 10.4 Hz, 1 H), 3.30 (dd, *J* = 14.2, 4.5 Hz, 1 H), 4.01 (dd, *J* = 10.4, 4.1 Hz, 1 H), 7.16 (d, *J* = 7.3 Hz, 2 H), 7.23 (m, 1 H) 7.30 (m, 2 H), 9.66 (s, 1 H), minor rotamer δ 1.37 (s, 9 H), 2.62 (s, 3 H), 3.00 (dd, *J* = 14.2, 10.3 Hz, 1 H), 3.30 (dd, *J* = 14.2, 4.5 Hz, 1 H), 4.19 (dd, *J* = 10.3, 4.9 Hz, 1 H), 7.19 (d, *J* = 7.6 Hz, 2 H), 7.23 (m, 1 H), 7.30 (m, 2 H), 9.66 (s, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$), both rotamers δ 28.1, 28.2, 32.7, 33.5, 34.7, 34.9, 68.3, 69.5, 80.5, 81.2, 126.5, 126.7, 128.5, 128.7, 129.1, 137.6, 154.7, 155.8, 198.9, 199.3; HRFAB calcd for $C_{15}H_{22}NO_3$ [$(M + H)^+$] 264.1600, found 264.1603.

(2*S*,3*R*)-2-[*N*-(ᵗButyloxycarbonyl)-*N*-methylamino]-1-phenylhex-5-en-3-ol (9). To a solution of (+)-*B*-methoxydiisopinocampheylborane (7.93 g, 25.1 mmol) in THF (65 mL) at −78 °C was slowly added allylmagnesium bromide (23.6 mL in Et_2O, 23.6 mmol), and the mixture was stirred at −78 °C for 15 min and then at 25 °C for 1 h. The reaction solution was cooled to −78 °C, and a solution of amino aldehyde **8** (5.50 g, 20.9 mmol, azeotroped with PhMe under high vacuum at <25 °C) in THF (40 mL) was added slowly. The solution was stirred at −78 °C for 2 h and then for 1.5 h without the dry ice/acetone bath. Oxidation of the boronate with aqueous 3 M NaOH (21 mL) and aqueous 30% H_2O_2 (9 mL) went overnight at 25 °C. A great deal THF was removed *in vacuo* before extraction in EtOAc (3 × 200 mL) with brine (300 mL). Much isopinocampheyl alcohol was removed under high vacuum at 90 °C. Flash chromatography with silica gel (gradient to 50% EtOAc/hexanes) afforded **9** (4.07 g, 64% yield) as a colorless oil: $[\alpha]^{22}_D$ −26° (*c* 0.067, $CHCl_3$); IR (neat) 1169, 1366, 1667, 2977, 3428 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) both rotamers δ 1.25 (broad s, 9 H), 1.39 (s, 9 H), 2.10−2.42 (m, 2 H), 2.47 (s, 3 H), 2.72 (broad s, 3 H), 2.96−3.24 (m, 2 H), 3.60−3.97 (broad m, 2 H), 5.08−5.21 (m, 2 H), 5.87 (m, 1 H), 7.10−7.30 (m, 5 H); ^{13}C NMR (101 MHz, $CDCl_3$) both rotamers δ 27.9, 28.1, 32.4, 34.1, 34.7, 38.8, 39.3, 71.4, 72.9, 79.2, 79.5, 117.7, 118.1, 125.8, 128.0, 128.8, 134.2, 134.7, 139.0, 155.4, 156.3; HRFAB calcd for $C_{18}H_{28}NO_3$ [$(M + H)^+$] 306.2069, found 306.2071.

(2*S*,3*R*)-2-(Methylamino)-1-phenylhex-5-en-3-ol (4). To a solution of **9** (615 mg, 2.01 mmol) in CH_2Cl_2 (14 mL) was added TFA (3.9 mL, 50 mmol), and the stirring transpired for 1.5 h at 25 °C. TFA was removed *in vacuo*, and extraction in EtOAc (3 × 75 mL) with saturated aqueous Na_2CO_3 (100 mL) provided pure **4** (413 mg, 100% yield) as a white solid: $[\alpha]^{22}_D$ +6° (*c* 0.009, $CHCl_3$); IR (neat) 698, 914, 2946, 3069, 3291 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.29 (m, 1 H), 2.29 (s, 3 H), 2.40 (m, 1 H), 2.58 (dd, *J* = 13.8, 10.2 Hz, 1 H), 2.70 (m, 1 H), 2.87 (dd, *J* = 13.8, 4.0 Hz, 1 H), 3.83 (m, 1 H), 5.14 (dd, *J* = 10.2, 1.6 Hz, 1 H), 5.19 (dd, *J* = 17.1, 1.6 Hz, 1 H), 5.92 (m, 1 H), 7.19 (d, *J* = 7.4 Hz, 2 H), 7.23 (t, *J* = 7.4 Hz, 1 H), 7.31 (dd, *J* = 7.4, 7.4 Hz, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 34.1 34.4, 36.9, 64.1, 68.4, 117.2, 126.5, 128.7, 129.1, 135.2, 138.8; HRCI calcd for $C_{13}H_{18}NO$ [$(M - H)^+$] 204.1388, found 204.1382.

(3*R*,4*R*)-3-Methylundec-1-en-4-ol (10). To a mixture of sublimed ᵗBuOK (11.05 g, 98.5 mmol) in THF (50 mL) at −78 °C were added *cis*-2-butene (about 25 mL) *via* cannula and then ⁿBuLi (47.9 mL in pentane, 95.8 mmol). The bright yellow mixture was stirred at −78 °C for 30 min, and a solution of (−)-*B*-methoxydiisopinocampheylborane (35.8 g, 113 mmol) in THF (110 mL) was cannulated into it, resulting in the disappearance of much of the yellow color. The solution was stirred at −78 °C for 30 min, and then $BF_3{\cdot}OEt_2$ (14.5 mL, 118 mmol) was added, giving a white mixture. The mixture thickened upon cannulation of a solution of octanal (15.4 mL, 98.5 mmol) in THF (110 mL) and was stirred for 2 h at −78 °C and 1.5 h without the dry ice/acetone bath. The mixture was cooled to 0 °C, and aqueous 30% H_2O_2 (33.6 mL) and aqueous 3 M NaOH (67 mL) were added slowly. After oxidation occurred overnight at 25 °C, most THF was removed *in vacuo* and extraction in EtOAc (3 × 400 mL) was conducted successively with brine (600 mL) and H_2O (600 mL). Flash chromatography with silica gel (gradient to 10% EtOAc/hexanes) and concentration *in vacuo* at 30 °C afforded **10** (15.84 g, 90% yield) as a colorless liquid in 90% de: $[\alpha]^{22}_D$ +28° (*c* 0.022, $CHCl_3$); IR (neat) 912, 1456, 2926, 3362 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J* = 6.9 Hz, 3 H), 1.01 (d, *J* = 6.9 Hz, 3 H), 1.20−1.38 (broad m, 10 H), 1.48 (broad m, 2 H), 2.26 (m, 1 H), 3.48 (m, 1 H), 5.05−5.12 (m, 2 H), 5.79 (m, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 13.9, 14.1, 22.6, 26.1, 29.3, 29.6, 31.8, 34.0, 43.4, 74.7, 115.1, 141.1; HREI calcd for $C_{12}H_{24}O$ (M^+) 184.1827, found 184.1828.

(3*R*,4*R*)-4-*p*-[(Methoxybenzyl)oxy]-3-methylundec-1-ene (11). To a solution of alcohol **10** (1.46 g, 7.92 mmol) in THF (30 mL) were added PMBTCAI (4.5 mL, 22 mmol) and then TfOH (2.1 μL, 0.024 mmol) as 350 μL of a stock solution of 6 μL of TfOH in 1 mL of THF. The solution was stirred for 5 h at 25 °C. TfOH was quenched with triethylamine (about 25 μL), and the solution was concentrated *in vacuo*. Flash chromatography with silica gel (gradient to 5% EtOAc/hexanes) led to PMB ether **11** (1.93 g, 80% yield) as a colorless oil: $[\alpha]^{22}_D$ +22° (*c* 0.018, $CHCl_3$); IR (neat) 1248, 1514, 2930 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.9 Hz, 3 H), 1.03 (d, *J* = 6.9 Hz, 3 H), 1.17−1.35 (broad m, 10 H), 1.45 (broad m, 2 H), 2.47 (m, 1 H), 3.23 (m, 1 H), 3.80 (s, 3 H), 4.43 (d, *J* = 11.0 Hz, 1 H), 4.49 (d, *J* = 11.0 Hz, 1 H), 5.02 (m, 2 H), 5.85 (m, 1 H), 6.87 (d, *J* = 8.7 Hz, 2 H), 7.27 (d, *J* = 8.7 Hz, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.1, 15.6, 22.7, 25.6, 29.3, 29.8, 31.1, 31.9, 40.8, 55.3, 71.4, 82.6, 113.7, 114.1, 129.3, 131.2, 141.3, 159.0; HREI calcd for $C_{20}H_{32}O_2$ (M^+) 304.2402, found 304.2396.

(2*S*,3*R*)-3-*p*-[(Methoxybenzyl)oxy]-2-methyldecanal (12). A solution of olefin **11** (1.78 g, 5.85 mmol) in CH_2Cl_2 (75 mL) was cooled to −78 °C, and ozone was bubbled in for 8 min with stirring. PPh_3 (2.7 g, 10 mmol) was added to the moderate gray solution, and the solution was stirred overnight at 25 °C. Concentration *in vacuo* and flash chromatography with silica gel (gradient to 10% EtOAc/hexanes) provided aldehyde **12** (1.20 g, 67% yield) as a colorless oil: $[\alpha]^{22}_D$ +26° (*c* 0.019, $CHCl_3$); IR (neat) 1248, 1514, 1725, 2928 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, *J* = 6.9 Hz, 3 H), 1.11 (d, *J* = 7.0 Hz, 3 H), 1.17−1.34 (broad m, 10 H), 1.39 (m, 1 H), 1.50 (m, 1 H), 2.55 (m, 1 H), 3.79 (m, 1 H), 3.80 (s, 3 H), 4.43 (d, *J* = 11.1 Hz, 1 H), 4.47 (d, *J* = 11.1 Hz, 1 H), 6.87 (d, *J* = 8.7 Hz, 2 H), 7.22 (d, *J* = 8.7 Hz, 2 H), 9.74 (d, *J* = 1.0 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 8.1, 14.1, 22.6, 25.8, 29.2, 29.5, 31.6, 31.8, 49.6, 55.2, 71.3, 78.1, 113.7, 129.3, 130.3, 159.2, 204.9; HREI calcd for $C_{19}H_{30}O_3$ (M^+) 306.2195, found 306.2192.

(2*S*,3*R*)-3-*p*-[(methoxybenzyl)oxy]-2-methyldecanoic Acid (13). To a solution of aldehyde **12** (1.15 g, 3.75 mmol) and 2-methyl-2-butene (0.7 mL, 6.4 mmol) in ᵗBuOH (25 mL) was added slowly a solution of 80% $NaClO_2$ (508 mg, 4.5 mmol) and NaH_2PO_4 (540 mg, 4.5 mmol) in H_2O (6 mL). Once the reaction solution was not too warm, the round-bottom flask was glass-stoppered and parafilmed, and stirring was continued overnight at 25 °C. After most of ᵗBuOH was removed *in vacuo* and extractions in CH_2Cl_2 (2 × 150 mL) with aqueous HCl (pH 1, 100 mL) and then H_2O (100 mL) were done, pure acid **13** (1.21 g, 100% yield) was obtained as a colorless oil: $[\alpha]^{22}_D$ +20° (*c* 0.011, $CHCl_3$); IR (neat) 1248, 1514, 1707, 2930 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.9 Hz, 3 H), 1.19 (d, *J* = 7.0 Hz, 3 H), 1.21−1.32 (broad m, 10 H), 1.53 (m, 2 H), 2.73 (m, 1 H), 3.68 (m, 1 H), 3.80 (s, 3 H), 4.49 (d, *J* = 11.0 Hz, 1 H), 4.54 (d, *J* = 11.0 Hz, 1 H), 6.87 (d, *J* = 8.7 Hz, 2 H), 7.25 (d, *J* = 8.7 Hz, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 11.7, 14.1, 22.6, 25.6, 29.2, 29.5, 31.4, 31.8, 42.4, 55.3, 71.9,

79.8, 113.8, 129.6, 129.8, 159.4, 178.5; HRFAB calcd for $C_{19}H_{30}O_4$ (M^+) 322.2144, found 322.2144.

(*S*)-1-Carboxy-2-methylpropyl (2*S*,3*R*)-3-*p*-[(methoxybenzyl)oxy]-2-methyldecanoate (5). To a solution of acid **13** (1.18 g, 3.66 mmol) in CH_2Cl_2 (40 mL) were added DIPEA (637 μL, 3.66 mmol) and $SOCl_2$ (400 μL, 5.49 mmol). The solution was stirred for 2 h at 0 °C, and then excess $SOCl_2$ was removed *in vacuo* carefully to obviate bumping. The crude acid chloride was dissolved in CH_2Cl_2 (40 mL) and cooled to 0 °C. DIPEA (1.40 mL, 8.05 mmol) was added slowly followed by (*S*)-α-hydroxyisovaleric acid (519 mg, 4.39 mmol). The solution was stirred overnight at 25 °C and extracted in CH_2Cl_2 (2 × 100 mL) with aqueous HCl (pH 1, 100 mL). Flash chromatography with silica gel (gradient to 60% EtOAc/hexanes) furnished **5** (811 mg, 52% yield) as a brown oil and no other diastereomer of **5**: $[\alpha]^{22}_D$ +19° (*c* 0.012, $CHCl_3$); IR (neat) 1248, 1514, 1736, 2932 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3 H), 1.00 (d, J = 7.0 Hz, 3 H), 1.02 (d, J = 7.0 Hz, 3 H), 1.22 (d, J = 7.1 Hz, 3 H), 1.14−1.34 (broad m, 10 H), 1.52 (m, 2 H), 2.30 (m, 1 H), 2.82 (m, 1 H), 3.72 (m, 1 H), 3.79 (s, 3 H), 4.47 (d, J = 10.9 Hz, 1 H), 4.53 (d, J = 10.9 Hz, 1 H), 4.96 (d, J = 4.0 Hz, 1 H), 6.86 (d, J = 8.7 Hz, 2 H), 7.25 (d, J = 8.7 Hz, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 12.0, 14.1, 17.0, 18.8, 22.6. 25.5, 29.2, 29.5, 30.1, 31.8, 42.8, 55.2, 71.8, 76.1, 80.0, 113.7, 129.4, 130.2, 159.2, 174.2, 174.3; HRFAB calcd for $C_{24}H_{38}O_6$ (M^+) 422.2668, found 422.2663.

[(*p*-Methoxybenzyl)oxy]alkenol 14. To a solution of ester acid **5** (699 mg, 1.65 mmol) in CH_2Cl_2 (20 mL) were added DIPEA (288 μL, 1.65 mmol) and $SOCl_2$ (190 μL, 2.60 mmol). The solution was stirred for 2 h at 0 °C, and then excess $SOCl_2$ was removed *in vacuo* carefully to obviate bumping. The crude acid chloride was dissolved in CH_2Cl_2 (13 mL) and cooled to 0 °C. DIPEA (570 μL, 3.30 mmol) was added slowly followed by a solution of amino alcohol **4** (373 mg, 1.82 mmol) in CH_2Cl_2 (7 mL). The ice bath was removed, and the solution was stirred for 1.5 h. After extraction in CH_2Cl_2 (2 × 100 mL) with saturated aqueous NH_4Cl (70 mL), flash chromatography with silica gel (gradient to 35% EtOAc/hexanes) provided **14** (707 mg, 70% yield) as a brown oil: $[\alpha]^{22}_D$ −36° (*c* 0.010, $CHCl_3$); IR (neat) 1248, 1514, 1640, 1732, 2930, 3424 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) both rotamers δ 0.88 (m, 3 H), 0.91 (d, J = 6.9 Hz, 3 H), 1.03 (d, J = 6.7 Hz, 3 H), 1.23 (d, J = 7.1 Hz, 3 H), 1.19−1.34 (broad m, 10H), 1.45 (m, 2 H), 1.99 (m, 1 H), 2.20−2.35 (m, 2 H), 2.67 (m, 1 H), 2.73 (s, 3 H), 3.01 (dd, J = 14.3, 3.8 Hz, 1 H), 3.17 (m, 1 H), 3.59 (broad m, 1 H), 3.76 (m, 1 H), 3.79 (s, 3 H), 3.93 (m, 1 H), 4.45 (m, 2 H), 4.84 (d, J = 5.3 Hz, 1 H), 5.10−5.22 (m, 2 H), 5.80 (m, 1 H), 6.85 (d, J = 8.6 Hz, 2 H), 7.14−7.29 (m, 7 H); ^{13}C NMR (101 MHz, $CDCl_3$) major conformer δ 11.7, 14.1, 17.2, 19.3, 22.6, 25.5, 29.2, 29.3, 29.6, 31.75, 31.83, 32.7, 39.6, 43.4, 55.2, 60.3, 71.9, 72.8, 75.3, 79.7, 113.6, 118.1, 126.3, 128.4, 129.0, 129.2, 131.0, 134.8, 139.1, 159.0, 170.8, 174.9; HRFAB calcd for $C_{37}H_{56}NO_6$ [(M + H)$^+$] 610.4108, found 610.4099.

[(ᵗButyldimethylsilyl)oxy][(*p*-methoxybenzyl)oxy]-alkene 15. To a solution of alkenol **14** (360 mg, 0.59 mmol) in CH_2Cl_2 (6 mL) at 0 °C were added 2,6-lutidine (90 μL, 0.77 mmol) and TBSOTf (176 μL, 0.77 mmol). The solution was stirred at 0 °C for 1.5 h and concentrated *in vacuo*. Flash chromatography with silica gel (gradient to 16% EtOAc/hexanes) afforded olefin **15** (379 mg, 89% yield) as an oil: $[\alpha]^{22}_D$ −47° (*c* 0.010, $CHCl_3$); IR (neat) 835, 1061, 1250, 1514, 1663, 1732, 2930 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) both rotamers δ 0.04−0.14 (m, 6 H), 0.86−0.95 (m, 15 H), 1.03 (d, J = 6.5 Hz, 3 H), 1.23 (d, J = 7.1 Hz, 3 H), 1.16−1.34 (broad m, 10 H), 1.44 (broad m, 2 H), 1.94 (broad m, 1 H), 2.15−2.40 (m, 2 H), 2.64 (m, 4 H), 2.86−3.14 (m, 2 H), 3.71−3.83 (m, 5 H), 4.12 (m, 1 H), 4.41−4.49 (m, 2 H), 4.84 (d, J = 2.2 Hz, 1 H), 5.03−5.20 (m, 2 H), 5.92 (m, 1 H), 6.84 (d, J = 8.6 Hz, 2 H), 7.10−7.27 (m, 7 H); ^{13}C NMR (101 MHz, $CDCl_3$) both rotamers δ −4.72, −4.68, −4.0, −3.6, 11.4, 11.6, 14.1, 15.8, 16.9, 17.98, 18.01, 19.5, 22.6, 25.5, 25.6, 25.7 25.9, 28.7, 29.2, 29.3, 29.55, 29.61, 29.8, 31.8, 32.1, 32.8, 33.1, 40.4, 43.6, 43.7, 55.2, 61.7, 71.9, 72.0, 74.4, 74.9, 75.0, 79.56, 79.62, 113.5, 113.6, 117.7, 118.3, 126.0, 126.5, 128.2, 128.3, 128.6, 129.0, 129.1, 129.28, 129.34, 131.0, 131.1, 134.2, 134.5, 138.9, 158.9, 159.0, 169.1, 169.3, 174.8; HRFAB calcd for $C_{43}H_{70}O_6NSi$ [(M + H)$^+$] 724.4972, found 724.4964.

[(ᵗButyldimethylsilyl)oxy]alkenol 16. DDQ (231 mg, 1.02 mmol) was added to a mixture of PMB ether **15** (368 mg, 0.51 mmol) in 18:1 (v/v) CH_2Cl_2/H_2O (11.6 mL), and the mixture was stirred vigorously for 2 h at 25 °C. After extraction in CH_2Cl_2 (2 × 70 mL) with saturated aqueous $NaHCO_3$ (100 mL) and flash chromatography with silica gel (gradient to 10% EtOAc/hexanes), **16** (262 mg, 85% yield) was obtained as a colorless oil: $[\alpha]^{22}_D$ −43° (*c* 0.0095, $CHCl_3$); IR (neat) 1645, 1738, 2930, 3432 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) both rotamers δ 0.05−0.16 (m, 6 H), 0.84−1.00 (m, 15 H), 1.00−1.12 (m, 6 H), 1.23−1.37 (broad m, 10 H), 1.37−1.56 (broad m, 2 H), 1.56−1.91 (broad m, 1 H), 2.17−2.94 (m, 6 H), 3.00−3.19 (m, 2 H), 3.67−4.20 (broad m, 3 H), 4.88 (d, J = 2.8 Hz, 1 H), 5.00−5.25 (m, 2 H), 5.91 (m, 1 H), 7.08−7.29 (m, 5 H); ^{13}C NMR (101 MHz, $CDCl_3$) both rotamers δ −4.7, −3.8, 9.0, 14.1, 15.5, 16.3, 18.0, 19.5, 20.0, 22.63, 22.65, 25.9, 26.4, 28.5, 28.8, 29.3, 29.7, 30.2, 31.8, 32.9, 39.3, 40.4, 44.4, 61.6, 72.16, 72.21, 74.7, 74.9, 75.0, 117.8, 126.2, 126.7, 128.3, 128.9, 129.0, 133.9, 134.3, 138.7, 170.2, 170.5, 174.9, 175.1; HRFAB calcd for $C_{35}H_{62}NO_5Si$ [(M + H)$^+$] 604.4397, found 604.4404.

Hydroxyaldehyde 17. Ozone was bubbled into a solution of olefin **16** (252 mg, 0.42 mmol) in CH_2Cl_2 (22 mL) at −78 °C until it turned blue. PPh_3 (164 mg, 0.63 mmol) was added, and the solution was stirred overnight at 25 °C. Concentration *in vacuo* and flash chromatography with silica gel (gradient to 20% EtOAc/hexanes) furnished aldehyde **17** (214 mg, 85% yield) as a colorless oil: $[\alpha]^{22}_D$ −60° (*c* 0.010, $CHCl_3$); IR (neat) 1644, 1732, 2930, 3430 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), both rotamers δ 0.05−0.18 (m, 6 H), 0.80−0.98 (m, 15 H), 1.05 (d, J = 7.0 Hz, 3 H), 1.07 (d, J = 7.0 Hz, 3 H), 1.20−1.36 (broad m, 10 H), 1.36−1.58 (broad m, 2 H), 1.83 (broad m, 1 H), 2.50−2.90 (m, 6 H), 3.04−3.30 (m, 2 H), 3.74−4.41 (m, 3 H), 4.82 (d, J = 2.6 Hz, 1 H), 7.09−7.31 (m, 5 H), 9.79 (m, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$), both rotamers δ −4.8, −4.7, −4.2, −4.1, 8.6, 8.7, 14.1, 15.6, 16.1, 17.9, 18.0, 19.7, 20.0, 22.6, 25.7, 25.8, 26.3, 26.4, 28.4, 29.2, 29.3, 29.55, 29.64, 31.8, 32.9, 33.0, 34.0, 34.5, 44.3, 44.5, 48.2, 64.0, 68.6, 69.1, 72.1, 72.2, 74.9, 75.1, 126.4, 126.9, 128.4, 128.9, 129.1, 138.1, 170.7, 171.1, 175.1, 175.3, 200.5; HREI calcd for $C_{30}H_{50}O_6NSi$ [(M − ᵗBu)$^+$] 548.3407, found 548.3412.

Hydroxy Acid 3. To a solution of aldehyde **17** (200 mg, 0.33 mmol) and 2-methyl-2-butene (70 μL, 0.66 mmol) in ᵗBuOH (4.5 mL) was added slowly a solution of 80% $NaClO_2$ (49 mg, 0.43 mmol) and NaH_2PO_4 (52 mg, 0.43 mmol) in H_2O (1.1 mL). The round-bottom flask was glass-stoppered and parafilmed and the solution was stirred fast overnight at 25 °C. After removal of most of the ᵗBuOH *in vacuo* and successive extractions in CH_2Cl_2 (2 × 50 mL) with aqueous HCl (pH 2, 40 mL) and H_2O (40 mL), a colorless oil was obtained that was used without further purification: $[\alpha]^{22}_D$ −51° (*c* 0.014, $CHCl_3$); IR (neat) 1186, 1636, 1734, 2930, 3422 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) both rotamers δ 0.07−0.17 (m, 6 H), 0.77−0.98 (m, 15 H), 1.05 (d, J = 7.2 Hz, 3 H), 1.08 (d, J = 7.0 Hz, 3 H), 1.17−1.37 (broad m, 10 H), 1.37−1.57 (broad m, 2 H), 1.82 (broad m, 1 H), 2.43−2.95 (broad m, 6 H), 3.05−3.28 (broad m, 2 H), 3.92−4.34 (broad m, 3 H), 4.85 (d, J = 2.4 Hz, 1 H), 7.09−7.30 (m, 5 H); ^{13}C NMR (101 MHz, $CDCl_3$) both rotamers δ −4.99, −4.96, −4.3, −4.2, 8.6, 9.0, 14.0, 15.5, 16.1, 17.8, 17.9, 19.4, 20.0, 22.5, 22.6, 25.7, 25.8, 26.2, 26.3, 28.3, 28.5, 29.1, 29.2, 29.45, 29.54, 31.7, 32.7, 32.8, 33.3, 34.4, 38.9, 44.2, 62.6, 69.5, 70.4, 72.1, 72.3, 74.7, 75.1, 126.3, 126.8, 128.3, 128.9, 129.1, 138.1, 170.5, 171.2, 175.0, 175.4, 175.7, 176.1; HRFAB calcd for $C_{34}H_{60}NO_7Si$ [(M + H)$^+$] 622.4139, found 622.4133.

Hapalosin (1). Crude hydroxy acid **3** (30 mg, 0.048 mmol) was azeotroped with benzene once alone and once with DMAP (29 mg, 0.24 mmol). A solution of **3** and DMAP in MeCN (20 mL) was added to a refluxing solution of 2-chloro-*N*-methylpyridinium iodide (246 mg, 0.96 mmol) and DMAP (89 mg, 0.72 mmol) in MeCN (100 mL) by a syringe pump over 16 h. After 18 h of refluxing, most MeCN was removed *in vacuo* and extraction in CH_2Cl_2 (2 × 30 mL) with aqueous HCl (pH 2, 50 mL) was performed. Crude TBS ether of hapalosin was

collected in a broad area (R_f about 0.35) in preparative TLC (10% EtOAc/hexanes).

To a solution of the crude macrolide in THF (0.6 mL) was added TBAF (15 μL in THF, 0.015 mmol). The solution was stirred for 45 min at 0 °C and then extracted in CH_2Cl_2 (2 × 13 mL) with aqueous NH_4Cl (10 mL). Preparative TLC (50% EtOAc/hexanes) furnished hapalosin (3.0 mg, R_f 0.45) in 13% yield for the last three steps: $[\alpha]^{22}_D$ −48° (*c* 0.0029, CH_2Cl_2) [natural $[\alpha]_D$ −49.2° (*c* 0.35, CH_2Cl_2)]; IR (neat) 1632, 1734, 2926, 3420 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$, 2.5:1 conformers), major conformer δ 0.23 (d, J = 7.0 Hz, 3 H), 0.55 (d, J = 7.0 Hz, 3 H), 0.89 (3 H), 1.18 (d, J = 7.0 Hz, 3 H), 1.20−1.40 (broad m, 10 H), 1.68 (m, 1 H), 1.92 (m, 1 H), 2.01 (m, 1 H), 2.62 (m, 1 H), 2.65 (m, 1 H), 2.85 (s, 3 H), 2.92 (dd, J = −17.8, 5.1 Hz, 1 H), 3.22 (m, 1 H), 3.41 (dd, J = −14.0, 2.5 Hz, 1 H), 3.70 (dt, J = 10.1, 2.5 Hz, 1 H), 3.85 (m, 1 H), 4.31 (d, J = 8.4 Hz, 1 H), 5.12 (m, 1 H), 7.18 (d, J = 7.6 Hz, 2 H), 7.25 (t, J = 7.6 Hz, 1 H), 7.33 (dd, J = 7.6, 7.6 Hz, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) major conformer δ 12.1, 14.1, 17.6, 18.3, 22.6, 26.0, 28.1, 28.8, 29.08, 29.15, 29.2, 31.7, 36.4, 37.1, 40.7, 61.4, 70.2, 73.8, 76.5 127.1, 128.9, 129.7, 137.4, 168.5, 168.7, 172.7; HREI calcd for $C_{28}H_{43}NO_6$ (M^+) 489.3090, found 489.3094.

^{t}Butyldimethylsilyl Ether 18: $[\alpha]^{22}_D$ −31° (*c* 0.036, $CHCl_3$); IR (neat) 1192, 1701, 1742, 2930 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) both rotamers δ 0.06−0.15 (four singlets, 6 H), 0.85−0.94 (m, 6 H), 0.92 (s, 9 H), 0.96 (d, J = 6.8 Hz, 3 H), 1.15 (d, J = 7.1 Hz, 3 H), 1.20−1.30 (broad m, 10 H), 1.53−1.65 (broad m, 2 H), 2.23 (m, 1 H), 2.44−2.54 (m, 1 H), 2.57 (s, 3 H), 2.61−2.77 (m, 3 H), 2.86 (m, 1 H), 3.13−3.21 (m, 1 H), 4.08−4.51 (broad multiplets, 2 H), 4.84 (d, J = 4.2 Hz, 1 H), 4.97−5.19 (m, 4 H), 7.06 (d, J = 7.1 Hz, 1 H), 7.10−7.36 (m, 14 H); ^{13}C NMR (126 MHz, $CDCl_3$) both conformers δ −4.84, −4.77, −4.12, −4.09, 12.1, 12.6, 13.0, 14.1, 17.1, 17.97, 18.00, 18.8, 22.6, 25.4, 25.86, 25.89, 25.93, 29.1, 29.2, 29.4, 30.0, 31.7, 32.0, 34.4, 40.0, 42.8, 43.1, 66.5, 66.77, 66.80, 67.2, 69.8, 70.1, 74.4, 74.5, 126.1, 126.2, 127.3, 127.7, 128.2, 128.27, 128.28, 128.33, 128.4, 128.5, 128.8, 128.9, 135.3, 135.4, 136.4, 136.9, 138.9, 156.3, 169.11, 169.14, 170.8, 173.27, 173.33; HRFAB calcd for $C_{49}H_{72}O_9NSi$ [$(M + H)^+$] 846.4976, found 846.4971.

(1*R*,2*R*)-1-^{n}Heptyl-2-methyl-3-butenyl Acetate (24). To a solution of alcohol **10** (1.750 g, 9.49 mmol) in CH_2Cl_2 (17 mL) at 0 °C were added successively pyridine (920 μL, 11.4 mmol), DMAP (116 mg, 0.95 mmol), and acetyl chloride (740 μL, 10.4 mmol) slowly. Stirring transpired for 10 min at 0 °C and for 1 h without the ice bath. The mixture was extracted in CH_2Cl_2 (2 × 150 mL) successively with 0.1 N $NaHSO_4$ (100 mL) and H_2O (100 mL). Concentration at water aspirator pressure at 35 °C provided clean acetate **24** (2.149 g, 100% yield) as a colorless liquid: $[\alpha]^{22}_D$ +24° (*c* 0.014, $CHCl_3$); IR (neat) 1240, 1742, 2928, 2957 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3 H), 1.00 (d, J = 6.9 Hz, 3 H), 1.18−1.34 (broad m, 10 H), 1.43−1.57 (broad m, 2 H), 2.05 (s, 3 H), 2.39 (m, 1 H), 4.82 (m, 1 H), 5.01−5.06 (m, 2 H), 5.73 (m, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.0, 15.2, 21.0, 22.6, 25.4, 29.1, 29.4, 31.3, 31.7, 41.4, 76.8, 115.0, 140.0, 170.8; HREI calcd for $C_{14}H_{27}O_2$ [$(M + H)^+$] 227.2011, found 227.2008.

(1*R*,2*R*)-1-^{n}Heptyl-2-methyl-3-butenyl (4*S*)-4-[(Benzyloxycarbonyl)amino]-3-oxo-5-phenylpentanoate (23). A solution of L-*N*-Cbz-Phe (425 mg, 1.42 mmol) and 1,1′-carbonyldiimidazole (CDI, 253 mg, 1.56 mmol) in THF (13.5 mL) was stirred at 25 °C for 2 h and then cooled to −78 °C. In the meantime, a solution of acetate **24** (1.286 g, 5.68 mmol) and LDA (4.73 mL, 5.96 mmol, 1.26 M in cyclohexane) in THF (22 mL) was stirred at −78 °C for 1 h. The enolate solution at −78 °C was added via syringe with a big-bore needle to the imidazolide solution at −78 °C at a moderate rate with *fast* stirring. The resulting mixture was stirred at −78 °C for 15 min, the −78 °C bath was removed, and the mixture was quenched with saturated aqueous NH_4Cl and extracted in CH_2Cl_2 (2 × 150 mL) with saturated aqueous NH_4Cl (100 mL). Flash chromatography with silica gel (gradient to 20% EtOAc/hexanes) furnished acetate **24** (0.939 g) and γ-amino-β-keto ester **23** (519 mg, 72% yield) as a colorless oil: $[\alpha]^{22}_D$ +22° (*c* 0.019, $CHCl_3$); IR (neat) 698, 1046, 1250, 1499, 1712, 2928, 3333 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, J = 6.8 Hz, 3H), 0.98 (d, J = 6.9 Hz, 3 H), 1.15−1.34 (broad m, 10 H), 1.43−1.58 (broad m, 2 H), 2.39 (m, 1 H), 3.01 (dd, J = 14.1, 7.0 Hz, 1 H), 3.17 (dd, J = 14.1, 6.2 Hz, 1 H), 3.44 (d, J = 16.0 Hz, 1 H), 3.50 (d, J = 16.0 Hz, 1 H), 4.67 (m, 1 H), 4.85 (m, 1 H), 5.00−5.10 (m, 4 H), 5.36 (d, J = 7.8 Hz, 1 H), 5.71 (m, 1 H), 7.14 (d, J = 6.5 Hz, 2 H), 7.20−7.37 (m, 8 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.0, 15.1, 22.6, 25.3, 29.1, 29.3, 31.1, 31.7, 37.0, 41.1, 46.9, 60.9, 67.0, 78.5, 115.3, 127.1, 128.0, 128.2, 128.5, 128.7, 129.2, 135.7, 136.1, 139.6, 155.7, 166.5, 201.3; HREI calcd for $C_{31}H_{41}NO_5$ (M^+) 507.2985, found 507.2996.

(1*R*,2*R*)-1-^{n}Heptyl-2-methyl-3-butenyl (3*R*,4*S*)-4-[(Benzyloxycarbonyl)amino]-3-[(*tert*-butyldimethylsilyl)oxy]-5-phenylpentanoate (25). To a solution of γ-amino-β-keto ester **23** (298 mg, 0.587 mmol) in EtOH (6.0 mL) at 0 °C was added KBH_4 (127 mg, 2.35 mmol). The mixture was stirred at 0 °C for 6.5 h, carefully quenched with saturated aqueous NH_4Cl at 0 °C, and extracted in CH_2Cl_2 (2 × 40 mL) successively with saturated aqueous NH_4Cl (40 mL) and H_2O (40 mL). The crude product was a mixture of inseparable diastereomers.

The crude alcohol (284 mg, 0.557 mmol) was dissolved in CH_2Cl_2 (5.3 mL) and cooled to 0 °C. 2,6-Lutidine (130 μL, 1.11 mmol) and TBSOTf (154 μL, 0.668 mmol) were added, and the solution was stirred at 0 °C for 30 min. After successive extractions in CH_2Cl_2 (2 × 40 mL) with saturated aqueous $NaHCO_3$ (30 mL) and aqueous 0.1 N $NaHSO_4$ (30 mL), flash chromatography with silica gel (gradient to 10% EtOAc/hexanes) afforded the undesired TBS ether diastereomer (62 mg, 17% yield for two steps) and the desired diastereomer **25** (275 mg, 75% yield for two steps) as a colorless oil: $[\alpha]^{22}_D$ −5.0° (*c* 0.020, $CHCl_3$); IR (neat) 837, 1051, 1252, 1732, 2928, 3349 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.05 (s, 3 H), 0.08 (s, 3 H), 0.87 (t, J = 7.0 Hz, 3 H), 0.90 (s, 9 H), 0.98 (d, J = 6.8 Hz, 3 H), 1.17−1.35 (broad m, 10H), 1.51 (broad m, 2 H), 2.40 (m, 1 H), 2.56 (m, 2 H), 2.69 (m, 1 H), 2.98 (m, 1 H), 3.99 (broad m, 1 H), 4.33 (broad m, 1 H), 4.77 (d, J = 7.8 Hz, 1 H), 4.84 (m, 1 H), 4.97−5.05 (m, 2 H), 5.73 (m, 1 H), 7.15−7.32 (m, 10 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ −5.0, −4.5, 14.1, 15.2, 18.0, 22.6, 25.5, 25.9, 29.1, 29.5, 31.1, 31.8, 35.5, 39.7, 41.1, 56.6, 66.3, 70.5, 77.4, 115.1, 126.3, 27.76, 127.84, 128.3, 128.4, 129.1, 136.6, 138.0, 139.8, 155.7, 170.7; HRFAB calcd for $C_{37}H_{58}NO_5Si$ [$(M + H)^+$] 624.4084, found 624.4091.

(2*S*,3*R*)-2-Formyl-3-decyl (3*R*,4*S*)-4-[(Benzyloxycarbonyl)amino]-3-[(*tert*-butyldimethylsilyl)oxy]-5-phenylpentanoate (26). Into a solution of olefin **25** (275 mg, 0.441 mmol) in CH_2Cl_2 (14 mL) and MeOH (1 mL) at −78 °C was bubbled ozone for 3 min, at which time TLC showed no more olefin. PPh_3 (180 mg, 0.685 mmol) was added to the clear, colorless solution, the −78 °C bath was removed, and stirring transpired at 25 °C for 15 h. Flash chromatography with silica gel (gradient to 20% EtOAc/hexanes) furnished aldehyde **26** (234 mg, 85% yield) as a colorless oil: $[\alpha]^{22}_D$ +7.1° (*c* 0.024, $CHCl_3$); IR (neat) 837, 1252, 1728, 2928, 3357 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.04 (s, 3 H), 0.07 (s, 3 H), 0.88 (t, J = 7.0 Hz, 3 H), 0.89 (s, 9 H), 1.08 (d, J = 7.1 Hz, 3 H), 1.20−1.39 (broad m, 10 H), 1.56 (broad m, 1 H), 1.65 (broad m, 1 H), 2.49−2.60 (m, 3 H), 2.67 (m, 1 H), 2.97 (m, 1 H), 3.96 (broad m, 1 H), 4.30 (broad m, 1 H), 4.72 (d, J = 7.8 Hz, 1 H), 4.97 (s, 2 H), 5.27 (m, 1 H), 7.16 (d, J = 7.3 Hz, 2 H), 7.18−7.32 (m, 8 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ −4.9, −4.6, 8.1, 14.0, 18.0, 22.6, 25.7, 25.8, 29.0, 29.2, 31.6, 31.7, 35.6, 39.6, 49.7, 56.7, 66.4, 70.6, 73.2, 126.4, 127.8, 127.9, 128.36, 128.44, 129.1, 136.6, 137.9, 155.7, 170.5, 202.2; HRFAB calcd for $C_{36}H_{56}NO_6Si$ [$(M + H)^+$] 626.3877, found 626.3877.

(2*S*,3*R*)-2-Carboxy-3-decyl (3*R*,4*S*)-4-[(Benzyloxycarbonyl)amino]-3-[(*tert*-butyldimethylsilyl)oxy]-5-phenylpentanoate (27). To a solution of aldehyde **26** (234 mg, 0.374 mmol) and 2-methyl-2-butene (374 μL, 0.75 mmol, 2.0 M in THF) in ^{t}BuOH (3.2 mL) was added dropwise a solution of 80% sodium chlorite (55 mg, 0.49 mmol) and NaH_2PO_4 (58 mg, 0.49 mmol) in H_2O (0.8 mL). The round-bottom flask was glass-stoppered and parafilmed, and the solution was vigorously stirred at 25 °C for 16 h. Successive extractions in CH_2Cl_2 (2 × 40 mL) with 0.1 N $NaHSO_4$ (40 mL) and H_2O (40 mL) produced pure acid **27** (240 mg, 100% yield) as a colorless oil: $[\alpha]^{22}_D$ −8.8° (*c* 0.016, $CHCl_3$); IR (neat) 837, 1252, 1717, 1736, 2928, 3265, 3324 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.07 (s,

6 H), 0.87 (t, J = 7.0 Hz, 3 H), 0.90 (s, 9 H), 1.18 (d, J = 6.8 Hz, 3 H), 1.20−1.35 (broad m, 10 H), 1.54 (broad m, 1 H), 1.64 (broad m, 1 H), 2.50 (m, 1 H), 2.59 (dd, J = 15.8, 7.1 Hz, 1 H), 2.64−2.76 (m, 2 H), 3.00 (m, 1 H), 4.00 (broad m, 1 H), 4.30 (broad m, 1 H), 4.80 (d, J = 8.9 Hz, 1 H), 4.96 (s, 2 H), 5.25 (broad m, 1 H), 7.15−7.32 (m, 10H); ^{13}C NMR (126 MHz, $CDCl_3$) δ −4.9, −4.6, 11.2, 14.1, 18.0, 22.6, 25.6, 25.8, 29.1, 29.3, 31.4, 31.7, 34.9, 40.0, 42.2, 56.0, 66.6, 71.2, 74.6, 126.4, 127.87, 127.95, 128.4, 129.3, 136.4, 137.9, 155.9, 170.5, 177.1; HRFAB calcd for $C_{36}H_{56}NO_7Si$ [$(M + H)^+$] 642.3826, found 642.3827.

Benzyl (2*S*)-2-Hydroxy-3-methylbutanoate (28). To a solution of (*S*)-α-hydroxyisovaleric acid (272 mg, 2.30 mmol) in MeCN (3.5 mL) were added successively 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU, 344 μL, 2.30 mmol), which warmed up the solution, and BnBr (274 μL, 2.30 mmol). After being stirred for 17 h at 25 °C, the solution was extracted in CH_2Cl_2 (2 × 40 mL) with 0.1 N $NaHSO_4$ (40 mL). Flash chromatography with silica gel (gradient to 20% EtOAc/hexanes) provided hydroxy ester **28** (418 mg, 87% yield) as a colorless oil: $[\alpha]^{22}_D$ −11° (*c* 0.025, $CHCl_3$); IR (neat) 1138, 1734, 2963, 3505 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, J = 6.9 Hz, 3 H), 1.00 (d, J = 6.9 Hz, 3 H), 2.09 (m, 1 H), 2.79 (d, J = 6.2 Hz, 1 H), 4.08 (dd, J = 6.2, 3.5 Hz, 1 H), 5.19 (d, J = 12.1 Hz, 1 H), 5.24 (d, J = 12.1 Hz, 1 H), 7.31−7.39 (m, 5 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 15.8, 18.7, 32.1, 67.2, 75.0, 128.4, 128.5, 128.6, 135.1, 174.8; HREI calcd for $C_{12}H_{16}O_3$ (M^+) 208.1099, found 208.1105.

Triester 22. Acid **27** (80 mg, 0.12 mmol) and alcohol **28** (26 mg, 0.12 mmol) were dissolved in CH_2Cl_2 (2.0 mL), and DMAP (15 mg, 0.12 mmol) followed by 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC, 31 mg, 0.16 mmol) were added. The round-bottom flask was glass-stoppered and parafilmed, and stirring transpired at 25 °C for 17 h. After extraction in CH_2Cl_2 (2 × 25 mL) with 0.1 N $NaHSO_4$ (25 mL), flash chromatography with silica gel (gradient to 20% EtOAc/hexanes) afforded triester **22** (88 mg, 85% yield) as a colorless oil: $[\alpha]^{22}_D$ −21° (*c* 0.022, $CHCl_3$); IR (neat) 1252, 1740, 2930, 3386 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.05 (s, 3 H), 0.07 (s, 3 H), 0.87 (t, J = 7.0 Hz, 3 H), 0.89 (s, 9 H), 0.92 (d, J = 6.8 Hz, 3 H), 0.96 (d, J = 6.9 Hz, 3 H), 1.18 (d, J = 7.1 Hz, 3 H), 1.20−1.38 (broad m, 10 H), 1.57 (broad m, 1 H), 1.65 (broad m, 1 H), 2.22 (m, 1 H), 2.55 (dd, J = 16.3, 6.2 Hz, 1 H), 2.64 (dd, J = 16.3, 5.6 Hz, 1 H), 2.71 (m, 1 H), 2.79 (m, 1 H), 2.97 (m, 1 H), 3.98 (broad m, 1 H), 4.35 (broad m, 1 H), 4.84 (1 H), 4.85 (d, J = 4.4 Hz, 1 H), 4.97 (s, 2 H), 5.09 (d, J = 12.3 Hz, 1 H), 5.15 (d, J = 12.3 Hz, 1 H), 5.20 (m, 1 H), 7.15−7.36 (m, 10 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ −5.0, −4.6, 12.2, 14.0, 17.1, 18.0, 18.7, 22.6, 25.5, 25.8, 29.1, 29.3, 30.0, 31.5, 31.7, 35.4, 39.5, 42.6, 56.7, 66.3, 66.8, 70.3, 74.5, 76.8, 126.3, 127.7, 127.8, 128.2, 128.32, 128.34, 128.5, 129.1, 135.3, 136.6, 138.2, 155.6, 169.2, 170.5, 173.3; HRFAB calcd for $C_{48}H_{70}NO_9Si$ [$(M + H)^+$] 832.4820, found 832.4832.

TBS Ether of the Non-*N*-Me Analog of Hapalosin (29). The round-bottom flask containing a mixture of triester **22** (83 mg, 0.10 mmol) and moist $Pd(OH)_2$/C (Pearlman's catalyst, 56 mg) in MeOH (4.0 mL) was purged with N_2, and H_2 was then bubbled into the mixture for 5 min. After the mixture was stirred for about 15 h at 25 °C under a balloon full of H_2, H_2 was again bubbled into the mixture for 5 min. After the mixture was stirred for an additional 15 h under the same conditions, the flask was purged with N_2 and the mixture was filtered through a short column of Celite and washed well with MeOH and EtOAc. ^{1}H NMR of the crude product showed that bis-deprotection was complete.

To a solution of the crude amino acid (34 mg, 0.056 mmol) in PhMe (60 mL) were added DIPEA (146 μL, 0.84 mmol) followed by BOP-Cl (143 mg, 0.56 mmol). The mixture was stirred at 85 °C for 15 h and then extracted with 0.1 N $NaHSO_4$ (30 mL), and the aqueous layer was back-extracted with EtOAc (30 mL). Preparative TLC with two silica gel-coated plates of 0.5 mm thickness (the eluent was 10% EtOAc/hexanes) resulted in a green band (*p*-anisaldehyde stain, R_f = 0.28) containing the silylated analog of hapalosin, **29** (20 mg colorless oil, 60% yield for two steps): $[\alpha]^{22}_D$ −45° (*c* 0.034, CH_2Cl_2); IR (neat) 839, 1088, 1175, 1198, 1541, 1663, 1732, 1744, 2928, 3318 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.13 (s, 3 H), 0.14 (s, 3 H), 0.56 (d, J = 6.7 Hz, 3 H), 0.76 (d, J = 6.6 Hz, 3 H), 0.88 (t, J = 7.0 Hz, 3 H), 0.91 (s, 9 H), 1.17 (d, J = 7.1 Hz, 3 H), 1.20−1.37 (broad m, 10H), 1.55 (m, 1 H), 1.69 (m, 2 H), 2.48 (d, J = 5.7 Hz, 2 H), 2.66 (dd, J = 14.1, 9.8 Hz, 1 H), 3.07 (m, 1 H), 3.2 (dd, J = 14.1, 4.5 Hz, 1 H), 4.22 (m, 1 H), 4.31 (m, 1 H), 4.80 (d, J = 8.3 Hz, 1 H), 5.16 (m, 1 H), 5.53 (d, J = 10.5 Hz, 1 H), 7.17 (t, J = 7.2 Hz, 1 H), 7.19 (d, J = 7.2 Hz, 2 H), 7.25 (t, J = 7.2 Hz, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ −4.7, −4.5, 10.7, 14.1, 17.8, 17.9, 18.2, 22.6, 25.68, 25.71, 29.1, 29.3, 29.5, 30.4, 31.7, 38.8, 40.5, 40.8, 55.6, 71.7, 75.7, 80.0, 126.4, 128.3, 129.2, 137.6, 168.9, 171.6, 173.0; HRFAB calcd for $C_{33}H_{56}NO_6Si$ [$(M + H)^+$] 590.3877, found 590.3878.

Non-*N*-Me Analog of Hapalosin (2). A stock solution of HF/pyridine was prepared from 0.25 mL of 70% HF/pyridine (Aldrich), 0.5 mL of pyridine, and 2.0 mL of THF in a polyethylene bottle. This stock solution of HF/pyridine (1.5 mL) was added to a solution of TBS ether **29** (13 mg, 0.022 mmol) in THF (0.8 mL) in a glass shell vial, and the solution was stirred at 25 °C for 18 h. After extraction in CH_2Cl_2 (2 × 20 mL) with 0.1 N $NaHSO_4$ (2 × 15 mL), preparative TLC with two silica gel-coated plates of 0.5 mm thickness (the eluent was 40% EtOAc/hexanes) was performed. A broad green band (*p*-anisaldehyde stain, R_f = 0.33) contained the non-*N*-Me analog of hapalosin, **2** (7.5 mg colorless solid, 71% yield): $[\alpha]^{22}_D$ −66° (*c* 0.0061, CH_2Cl_2); IR (neat) 1194, 1545, 1661, 1728, 1742, 2921, 3303, 3385 (shoulder) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.66 (d, J = 6.8 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 3 H), 0.89 (t, J = 6.8 Hz, 3 H), 1.21 (d, J = 7.0 Hz, 3 H), 1.23−1.39 (broad m, 10 H), 1.58 (broad m, 1 H), 1.71 (broad m, 1 H), 1.77 (m, 1 H), 2.34 (d, J = 11.2 Hz, 1 H), 2.57 (dd, J = 13.9, 5.8 Hz, 1 H), 2.65 (dd, J = 13.9, 3.6 Hz, 1 H), 2.92 (m, 1 H), 2.96 (m, 1 H), 3.01 (dd, J = 14.2, 6.1 Hz, 1 H), 4.07 (broad m, 1 H), 4.54 (d, J = 8.0 Hz, 1 H), 4.64 (m, 1 H), 5.46 (d, J = 10.4 Hz, 1 H), 5.52 (m, 1 H), 7.20 (t, J = 7.2 Hz, 1 H), 7.23 (d, J = 7.2 Hz, 2 H), 7.27 (t, J = 7.2 Hz, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 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, 41.3, 54.0, 70.6, 75.9, 81.8, 126.7, 128.6, 129.0, 137.0, 169.7, 173.7, 174.1; HREI calcd for $C_{27}H_{41}NO_6$ (M^+) 475.2934, found 475.2936.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of all new compounds in the synthesis of hapalosin and its non-*N*-Me analog (50 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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