Hapalosin, a Cyanobacterial Cyclic Depsipeptide with Multidrug-Resistance Reversing Activity

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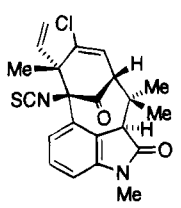
Hapalosin (**2**), a novel cyclic depsipeptide from the blue-green alga (cyanobacterium) *Hapalosiphon welwitschii* W. & G. S. West (Stigonemataceae), shows MDR-reversing activity. Its structure has been determined to be (3*S*,4*R*,8*R*,9*S*,12*S*)-9-benzyl-4-heptyl-8-hydroxy-12-isopropyl-3,10-dimethyl-1,5-dioxa-10-azacyclododecane-2,6,11-trione by a combination of spectroscopic and chemical methods. The carbon skeleton of **2** was determined by two-dimensional $^1H-^1H$ and $^1H-^{13}C$ NMR correlation experiments and confirmed by mass spectral analysis. The relative and absolute stereochemistry was determined by coupling constant data and Mosher analysis of **2** and three degradation products obtained from acid hydrolysis of **2**.

Agents that are capable of overcoming P-glycoprotein-mediated multidrug resistance (MDR)[1] have potential use in the treatment of cancer patients undergoing chemotherapy. Two types of anti-MDR drugs are desirable, viz. (1) cytotoxic drugs which are equally efficacious toward drug-sensitive and drug-resistant tumor cells,[2,3] and (2) MDR-reversing agents that are able to potentiate the cytotoxicity of common antitumor drugs like vinblastine and adriamycin toward drug-resistant cells.[4] In screening extracts of blue-green algae (cyanobacteria) for anti-MDR activity, about 1% of the extracts exhibited MDR-reversing activity. The lipophilic extracts of two strains of *Hapalosiphon welwitschii* W. & G. S. West (UH IC-52-3 and UTEX B1830) reversed MDR in a P-glycoprotein-overexpressing, vinblastine-resistant subline (SKV-LB1) of a human ovarian adenocarcinoma line (SKOV3). Some of the MDR-reversing activity was attributed to an unusual indole alkaloid, *N*-methylwelwitindolinone C isothiocyanate (**1**),[5] but much of the activity was due to a completely different compound. We report here the isolation and structure determination of a new cyclic depsipeptide,[6] hapalosin (**2**), which accounts for this remaining MDR-reversing activity.



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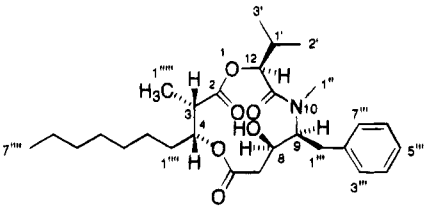

2

UH strain IC-52-3 was isolated from an Australian soil sample and mass cultured as previously described.[5] The lipophilic extract (1:1 CH_2Cl_2/2-propanol) of UH IC-52-3 was fractionated by successive size exclusion chromatography on Sephadex LH20 (MeOH elution), reversed-phase column chromatography on C18 (9:1 MeOH/H_2O elution), and RP-HPLC on C8 (23:17 CH_3CN/H_2O) to give **2** in 0.12% yield based on dry weight of alga. The lipophilic extract of UTEX B1830 was fractionated in a similar manner, except that the two RP-chromatography steps were carried out by HPLC, using 0.1% TFA in 22:3 MeOH/H_2O in the first step and 7:3 CH_3CN/H_2O in the second step, to give **2** in 0.28% yield.

Gross Structure Determination. The EIMS of **2** displayed a molecular ion peak at m/z 489 and a high

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(1) Endicott, J. A.; Ling V. *Annu. Rev. Biochem.* **1989,** *58,* 137–171.

(2) The cryptophycins are novel microtubule-depolymerizing agents which circumvent P-glycoprotein-mediated multidrug resistance. (a) Trimurtulu, G.; Golakoti, T.; Ohtani, I.; Patterson, G. M. L.; Moore, R. E.; Corbett, T. H.; Valeriote, F. A.; Demchik, L. *J. Am. Chem. Soc.* **1994,** *116,* 4729–37. (b) Smith, C. D.; Zhang, X.; Mooberry, S. L.; Patterson, G. M. L.; Moore, R. E. *Cancer Res.* **1994,** *54,* 3779–84.

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(4) Tolyporphin is an example of a MDR-reversing agent from a blue-green alga. Prinsep, M. R.; Caplan, F. R.; Moore, R. E.; Patterson, G. M. L.; Smith, C. D. *J. Am. Chem. Soc.* **1992,** *114,* 385–6.

(5) Stratmann, K.; Moore, R. E.; Bonjouklian, R.; Deeter, J. B.; Patterson, G. M. L.; Smith, C. D.; Smitka, T. A.; Staffer, S. *J. Am. Chem. Soc.* **1994,** *116,* in press.

(6) Five classes of cyclic depsipeptides (peptolides) from blue-green algae have been described to date. The cryptophycin class is the largest (21 members) [Trimurtulu, G.; Ogino, J.; Heltzel, C. E.; Moore, R. E.; Patterson, G. M. L. Manuscript in preparation]. The 11 members of the second largest class have structures related to a sea hare dietary constituent, dolastatin-13 [Pettit, G. R.; Kamano, Y.; Herald, C. L.; Dufresne, C.; Cerny, R. L.; Herald, D. L.; Schmidt, J. M.; Kizu, H. *J. Am. Chem. Soc.* **1989,** *111,* 5015–7], and all have been isolated from *Microcystis* spp., viz. aeruginopeptins [Harada, K-i.; Mayumi, T.; Shimada, T.; Suzuki, M.; Kondo, F.; Watanabe, M. F. *Tetrahedron Lett.* **1993,** *34,* 6091–4], cyanopeptolins [Martin, C.; Oberer, L.; Ino, T.; König, W. A.; Busch, M.; Weckesser, J. *J. Antibiotics* **1993,** *46,* 1550–6], micropeptins [Okino, T.; Murakami, M.; Haraguchi, R.; Munekata, H.; Matsuda, H.; Yamaguchi, K. *Tetrahedron Lett.* **1993,** 34, 8131–4], and microcystilide A [Tsukamoto, S.; Painuly, P.; Young, K. A.; Yang, X.; Shimizu, Y.; Cornell, L. *J. Am. Chem. Soc.* **1993,** *115,* 11046–7]. The other three classes are represented by majusculamide C (two members) [Carter, D. C.; Moore, R. E.; Mynderse, J. S.; Niemczura, W. P.; Todd, J. S. *J. Org. Chem.* **1984,** *49,* 236–41], microviridin [Ishitsuka, M. O.; Kusumi, T.; Kakisawa, H.; Kaya, K.; Watanabe, M. M. *J. Am. Chem. Soc.* **1990,** *112,* 8180–2], and aeruginosin 298-A [Murakami, M.; Okita, Y.; Matsuda, H.; Okino, T.; Yamaguchi, K. *Tetrahedron Lett.* **1994,** *35,* 3129–32].

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Table 1. NMR Data for Hapalosin in $CDCl_3$

C no.–H_n	^1H NMR (500 MHz)	^{13}C NMR	COSY	HMBC
2	–	172.7		12-H,3-H,1''''-H_3,4-H
3-H	3.21 (m)	40.7	1''''-H_3,4-H	1''''-H_3
1''''-H_3	1.17 (d J = 7 Hz)	12.0	3-H	3-H
4-H	5.12 (m)	76.5	3-H,1'''-$H_{A,B}$	3-H,1''''-H_3
1''''-H_A	1.69	28.1	4-H,1''''-H,2''''-$H_{A,B}$	21-H
1''''-H_B	1.92		4-H,1''''-H_A,2''''-H_B	
2''''-H_A	1.35	26.0	1''''-H_A	4-H
2''''-H_B	1.26–1.30		1''''-$H_{A,B}$	
3''''-H_2	1.26–1.30	29.1		
4''''-H_2	1.26–1.30	29.2		
5''''-H_2	1.26–1.30	31.7		7''''-H_3
6''''-H_2	1.26–1.30	22.5	7''''-H_3	
7''''-H_3	0.89	14.0	6''''-H_2	
6-H	–	168.5		7-$H_{A,B}$
7-H_A	2.91 (dd, J = –18, 5 Hz)	37.1	7-H_B,8-H	
7-H_B	2.63 (m)		7-H_A,8-H	
8-H	3.85 (m)	70.2	7-$H_{A,B}$,9-H	7-$H_{A,B}$,9-H
9-H	3.70 (dt, J = 10, 2.5 Hz)	61.4	8-H,1'''-$H_{A,B}$	7-$H_{A,B}$,8-H,1'''-H_A,1''-H_3
1'''-H_A	3.40 (dd, J = –14, 2.5 Hz)	36.4	9-H,1'''-H_B	3'''-H,7'''-H
1'''-H_B	2.65 (m)		9-H,1'''-H_A	
2'''		137.4		1'''-$H_{A,B}$,4'''-H,6'''-H
3'''-H/7'''-H	7.18 (d, J = 7.5 Hz)	129.7		1'''-$H_{A,B}$,3'''-H,7'''-H
4'''-H/6'''-H	7.33 (t, J = 7.5 Hz)	128.9		4'''-H,6'''-H
5'''-H	7.25 (t, J = 7.5 Hz)	127.0		3'''-H,7'''-H
1''-H_3	2.85 (s)	28.8		
11	–	168.7		1''-H_3,12-H
12-H	4.31 (d, J = 8.5 Hz)	73.9	1'-H	1'-H,2'-H_3,3'-H_3
1'-H	2.01 (m)	29.1	12-H,2'-H_3,3'-H_3	
2'-H_3	0.23 (d, J = 7 Hz)	18.3	1'-H	12-H,1'-H,3'-H_3
3'-H_3	0.55 (d, J = 7 Hz)	17.5	1'-H	12-H,1'-H,2'-H_3

resolution measurement established its molecular composition as $C_{28}H_{43}NO_6$. Although the ^1H- and ^{13}C-NMR spectra were complicated by the presence of two conformers in approximately a 3:1 ratio, the formula was consistent with the NMR data (Table 1). A refocused INEPT experiment established that the ^{13}C spectrum was comprised of 5 methyl, 8 methylene, 11 methine, and 4 non-protonated carbon signals for the major conformer, indicating that **2** contained 28 carbons and 42 carbon-bonded hydrogens. Since **2** had an odd molecular weight, at least one nitrogen and one exchangeable hydrogen had to be present. The presence of exchangeable hydrogen was supported by a strong absorption at 3420 cm^{-1} in the IR spectrum. The ^1H NMR spectrum in $CDCl_3$, however, did not reveal a signal which lacked a ^1J-correlation with a ^{13}C signal in a HMQC experiment. When the ^{13}C NMR spectra of **2** in CD_3OH and CD_3OD were compared, however, one of the methine signals (70.3 ppm in CD_3OD; ^1J correlated with ^1H signal at 4.23 ppm) revealed a $\Delta\delta_C$ of 0.1 ppm in the two solvents, strongly suggesting that a hydroxyl group was attached to this methine.[7] One exchangeable hydrogen, therefore, appeared to be present in **2**. The remaining mass units (489 – 379 for $C_{28}H_{43}$ = 110) could only be accommodated by one nitrogen and six oxygens. Since one of the oxygens was in the proposed hydroxyl group, the other five oxygens and the single nitrogen in the molecule had to be located in two ester groups and one amide group (δ_C 168.5, 168.7, 172.7).

Three of the eight units of unsaturation required by the molecular formula could be assigned to the three carbonyl groups. Another four units were accounted for by a phenyl group, as shown by the three ^1H signals in the 7.15–7.35 ppm region and the four ^{13}C signals in the 127–137 ppm region (Table 1). Analysis of the HMQC and HMBC spectra confirmed the existence of this monosubstituted benzene ring. The remaining unit had to be due to another ring.

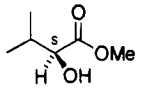
The gross structure of **2** was determined by a detailed analysis of one and two dimensional NMR spectra (Table 1). The structures of the two hydroxy acid units and the amino acid unit were determined by COSY experiments and the three units were connected together in the proper sequence by HMBC experiments. Confirmation of the structures of the three units as well as the sequencing was provided by EIMS data.

One of the hydroxy acid units was a 2-hydroxy-3-methylbutanoic acid residue (**2a**). The COSY and HMQC spectra revealed that an oxygen-bearing methine [δ 4.31 (12-H), 73.9 (C-12)] was connected to another methine [δ 2.01 (1'-H), 29.1 (C-1')] possessing two methyl groups [δ 0.23 (2'-H_3), 18.3 (C-2'); 0.55 (3'-H_3), 17.5 (C-3')]. A carbonyl group was also attached to the oxygen-bearing methine, since HMBC correlations could be seen from the δ 2.01 and 4.31 signals to the carbon signal at 168.7 ppm (C-11).

The second hydroxy acid unit was deduced to be a 3-hydroxy-2-methyldecanoic acid unit (**2b**). COSY and HMQC analysis indicated that this unit possessed a methine group [δ 3.21 (3-H), 40.7 (C-3)] that was connected to a methyl group [δ 1.17 (1''''-H_3), 12.0 (C-1'''')] as well as to an oxygen-bearing methine [δ 5.12 (4-H), 76.5 (C-4)]. The latter methine was in turn joined to the first of a chain of methylenes terminated by a methyl [δ 0.89 (7''''-H_3), 14.0 (C-7'''')]. The molecular formula of **2** required that the chain be comprised of six methylenes. The methyl-bearing methine was also connected to a

(7) Signals for OH- or NH-bearing carbons generally show isotope shifts of 0.1–0.2 ppm compared with the corresponding OD- or ND-bearing carbon signals. Isotope shifts have been used to distinguish OH- and NH-bearing carbon signals from other types of O- and N-bearing carbon signals in the structure determination of natural products such as gambieric acids [Nagai, H.; Torigoe, K.; Satake, M.; Murata, M.; Yasumoto, T.; Hirota, H. *J. Am. Chem. Soc.* **1992**, *114*, 1102–3], cylindrospermopsin [Ohtani, I.; Moore, R. E.; Runnegar, M. T. C. *J. Am. Chem. Soc.* **1992**, *114*, 7941–2], and zooxanthellatoxin-A [Nakamura, H.; Asari, T.; Murai, A.; Kondo, T.; Yoshida, K.; Ohizumi, Y. *J. Org. Chem.* **1993**, *58*, 313–4].

carbonyl, as shown by HMBC correlations from the proton signals at 1.17, 3.21, and 5.12 ppm to the carbonyl signal at 172.7 ppm (C-2).



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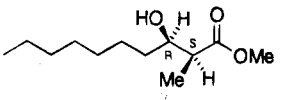

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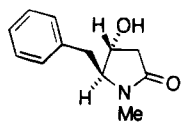

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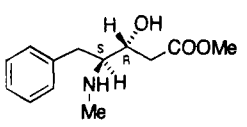


6

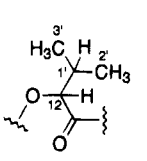
The amino acid unit in hapalosin was shown to be a 3-hydroxy-4-(methylamino)-5-phenylpentanoic acid unit (**2c**). HMBC correlations from the methylene proton signals at 2.65 (1‴-H_A) and 3.40 ppm (1‴-H_B) to the carbon signals at 137.4 (C-2‴) and 129.7 ppm (C-3‴) showed that a methylene was attached to the phenyl ring and this suggested that **2** might possess a phenylalanine-type residue. COSY correlations revealed that this methylene group was connected successively to two contiguous methines [3.70 (9-H) and 3.85 ppm (8-H)] and another methylene [2.91 (7-H_A) and 2.63 ppm (7-H_B)]. A carbonyl group was attached to the latter methylene as shown by a HMBC correlation from the ^{1}H signals at 2.63 and 2.91 ppm to a ^{13}C signal at 168.5 ppm (C-6). The nitrogen of an *N*-methyl amide group was connected to the methine resonating at 3.70 (9-H) and 61.4 ppm (C-9), as a HMBC correlation was observed from a methyl signal, which had to be assigned to an *N*-methyl group because of its chemical shift (δ_H 2.85), to the carbon signal at 61.4 ppm. The hydroxyl group was on the adjacent methine [δ 3.85 (8-H), 70.2 (C-8)].

The three units could be joined together from HMBC data. Firstly, the oxygen on the α-carbon of **2a** was connected to the carbonyl carbon of **2b** on the basis of the HMBC correlation from 4.31 ppm (12-H) to 172.7 ppm (C-2). Secondly, the nitrogen of **2c** had to be attached to the carbonyl of **2a**, since the signal for the protons of the *N*-methyl group showed a HMBC correlation to the carbonyl carbon signal at 168.7 ppm (C-11). Thirdly, the oxygen on the β-carbon of **2b** was connected to the carbonyl carbon of **2c**, as the proton signal at 5.12 ppm (4-H) displayed a HMBC correlation to the carbon signal at 168.5 ppm (C-6). The sequencing established that hapalosin was a cyclic depsipeptide.

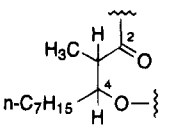
Fragment ion peaks in the EI mass spectrum of **2** supported the structural conclusions from NMR analysis. Intense fragment ions at *m*/*z* 267 ($C_{16}H_{27}O_3$) and 167 ($C_{11}H_{19}O$) were consistent with acyl cations resulting from successive cleavages of the amide and ester bonds flanking **2a** in an acyclic molecular ion of **2**. Initial β-elimination of the 3-acyloxy group in the **2b** unit via a McLafferty rearrangement would be expected to give the prerequisite acyclic molecular ion (Scheme 1). Another intense fragment ion at *m*/*z* 398 was appropriate for the loss of a benzyl radical from the molecular ion. Cleavage of the ester bond in the *m*/*z* 398 ion followed by loss of valic acid then accounted for the fragment ion at *m*/*z* 298. The presence of a hydroxy group in **2** was supported by the loss of water from the molecular ion and the fragment ions at *m*/*z* 398 and 298 to form the ions at *m*/*z* 471, 380, and 280, respectively.

The ^{1}H and ^{13}C NMR spectra of **2** clearly showed the presence of two components. We concluded that these two components were conformational isomers for two reasons. Separation could not be achieved by chromatography and chemical degradation (*vide infra*) led to products that were consistent with the presence of only one component. A variable temperature experiment, however, which would have further established that the two components were conformers, was not carried out.

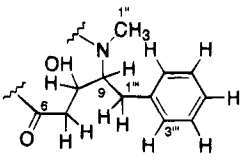
Absolute Stereochemistry. Hydrolysis of **2** with refluxing 2.25 N HCl in 1:3 MeOH/H_2O followed by treatment of the acid hydrolyzate with diazomethane produced a mixture of methyl 2-hydroxy-3-methylbutanoate (**3**, methyl 2-hydroxyisovalerate), methyl 2-methyl-3-hydroxydecanoate (**4**) and a lactam (**5**). The three hydrolysis products were separated by normal phase chromatography on silica gel and their absolute stereochemistries elucidated as follows.



2a



2b



2c

Methyl 2-hydroxy-3-methylbutanoate (**3**) was shown to have the *S*(L)-configuration by GC/MS and modified Mosher analysis.[8] Commercially available L- and D-2-hydroxy-3-methylbutanoic acid were esterified with CH_2N_2 and the methyl esters separated by GC on a chiral (Chirasil-Val) column. The retention time and the mass spectrum of the authentic methyl L-2-hydroxy-3-methylbutanoate were found to be identical with those for the degradation product. Furthermore, modified Mosher analysis of the (*S*)- and (*R*)-MTPA (2-methoxy-2-(trifluoromethyl)-2-phenylacetyl) esters of **3** led to the same result. A positive $\Delta\delta$ ($\delta_S - \delta_R$) value for the ester methoxy group and negative $\Delta\delta$ values for all protons on C-3 and C-4/4′ were found (Figure 1). Therefore, C-12 in **2** had to have the *S*(L)-configuration.

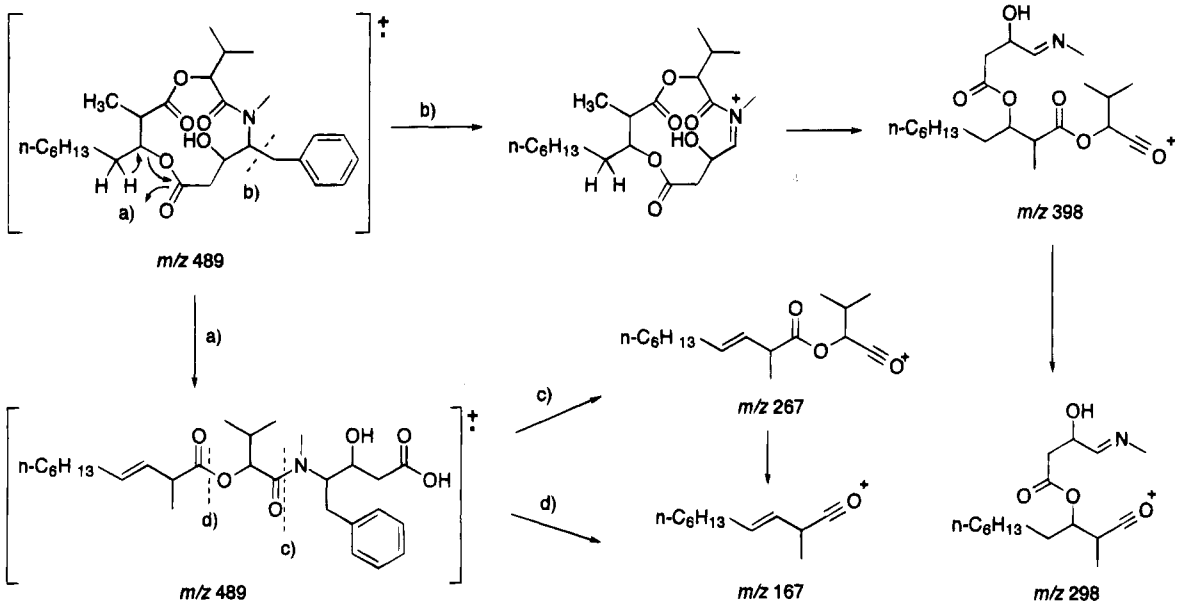
The absolute stereochemistry of C-2 and C-3 in **4** was determined to be 2*S*,3*R* by the modified Mosher method[8] in combination with analysis of the coupling constant between 2-H and 3-H. The (*S*)- and (*R*)-MTPA esters of **4** were prepared. Negative $\Delta\delta$ values for the C-1/C-2 side and positive $\Delta\delta$ values for the C-4 to C-10 side of the MTPA plane were found (Figure 1). This meant that C-3 had to be 3*R*. Since the coupling constant between 2-H and 3-H for **4** was 4.0 Hz, the methyl and hydroxy substituents had to be *syn* to each other, which meant that C-2 was 2*S*. In general, methyl *anti*- and *syn*-3-hydroxy-2-methylalkanoates show coupling constants of 7 and 4 Hz, respectively, for $J_{2,3}$.[9] The absolute configurations of C-3 and C-4 in **2** were therefore 3*S* and 4*R*.

Determination of the total stereochemistry of γ-lactam **5**, however, was more complicated. Conformational analysis was required to solve the relative stereochemistry with a high degree of confidence. In $CDCl_3$ the ^{1}H and ^{13}C NMR spectra indicated that **5** was a 6:1 mixture of two conformational isomers. In CD_3OD, however,

(8) (a) Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1973**, *95*, 512–9. (b) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–6. (c) Kusumi, T.; Fukushima, T.; Ohtani, I.; Kakisawa, H. *Tetrahedron Lett.* **1991**, *32*, 2939–42.

(9) Meyers, A. I.; Yamamoto, Y. *Tetrahedron* **1984**, *40*, 2309–15.

Scheme 1. Electron Impact Mass Spectral Fragmentation of Hapalosin



signals were observed for only one conformer. The coupling between 4-H and 5-H for the major conformer in $CDCl_3$ was found to be 1.6 Hz (0 Hz for the single conformer detected in CD_3OD) and this suggested that the dihedral angle was near 90°. By comparison, $J_{4,5}$ for the minor conformer in $CDCl_3$ was considerably larger (6.0 Hz). Inspection of Dreiding models allowed us to conclude that the OH and benzyl substituents were *trans* to each other. Only in the *trans* isomer was it possible to have a conformation (**5a**) for the major conformer where a dihedral angle of 90° separated 4-H and 5-H. In **5a** a dihedral angle of 150° separated the *anti* OH and benzyl groups. Interestingly, the Dreiding model also showed that another conformation (**5b**) was possible, one in which the OH and benzyl groups were *gauche* (dihedral angle near 90°) and 4-H and 5-H were *anti* (dihedral

5a **5b**

angle near 150°). The $J_{4,5}$ value found for the minor conformer was consistent with this latter conformation (coupling near 7 Hz predicted for a dihedral angle of 150°). Molecular models generated from CSC Chem3D Plus corroborated these findings, showing that (1) 4-H and 5-H are separated by dihedral angles of 93° and 151° and (2) the OH and benzyl groups are separated by dihedral angles of 149° and 92° in **5a** (8.3 kcal/mol) and **5b** (9.6 kcal/mol), respectively. Modeling studies further showed that the lactam was not the *cis* isomer. For the two conformations that one could generate for the *cis* lactam, the dihedral angle between 4-H and 5-H was approximately 30° in both cases. If the lactam had been the *cis* isomer, a 7 Hz coupling would have been expected.

Although (*S*)- and (*R*)-MTPA esters of **5** could be readily prepared, Mosher analysis[8] did not give completely unambiguous results. Large negative $\Delta\delta$ values were found for all but one of the protons on the left side of the MTPA plane (1′-$H_{S\ or\ R}$) (Figure 1). On the right side of the MTPA plane, however, one of the two diastereotopic protons on C-3 displayed a positive $\Delta\delta$, whereas

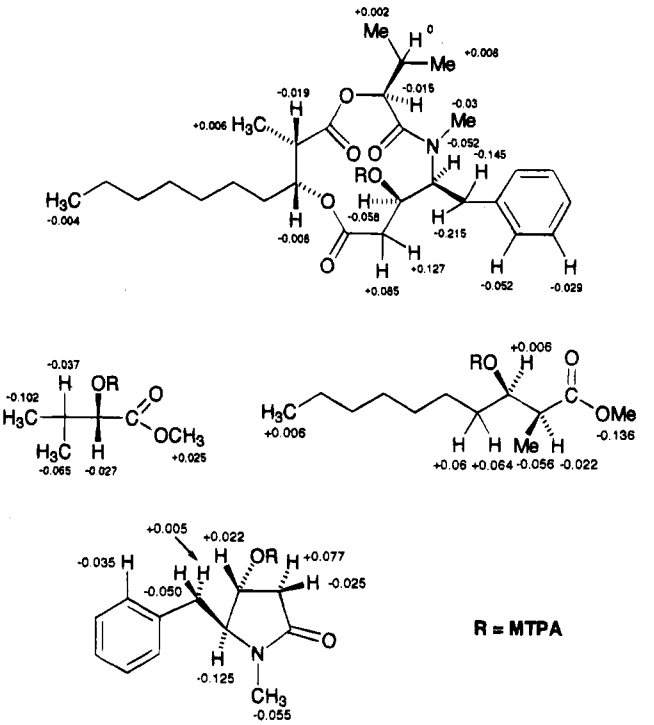


Figure 1. $\Delta\delta$ ($\delta S - \delta R$) values for MTPA esters of **2**–**5**.

the other one gave a negative $\Delta\delta$ (Figure 1). In most cases, Mosher analysis can be used with a high degree of confidence to determine the absolute configuration of a chiral secondary alcohol, but caution must be exercised if the OH group is located in a crowded environment.[10] In the case of **5**, however, steric hindrance is not obvious. Modeling studies of the two MTPA esters indicate that 4-H and the ester carbonyl atoms are not coplanar in the energy-minimized structures, but it is not known whether the deviations (30°) are large enough to account for the discrepancies.

We consequently subjected the intact peptide **2** to Mosher analysis to solve the absolute configuration at

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C-8 for hapalosin. Large positive $\Delta\delta$ values were found for the two protons on C-7, whereas negative $\Delta\delta$ values were observed for H-9, the benzyl and *N*-methyl protons, and H-12 (Figure 1). Therefore the configuration of C-8 in **2** is 8*R*. Since the configuration of C-4 in **5** is *R* based on Mosher analysis of **2**, C-5 in **5**, and by extension C-9 in **2**, is *S*.

Lactam **5** could be opened up to (3*R*,4*S*)-*N*-methyl-4-amino-3-hydroxy-5-phenylpentanoic acid (**6**) by acid hydrolysis. The amino acid, as the hydrochloride, exhibited a positive optical rotation, $[\alpha]_D$ +35°, in contrast to (3*R*,4*S*)-4-amino-3-hydroxy-5-phenylpentanoic acid (AHPA)[11] which has been reported to show a negative $[\alpha]_D$ as the free amino acid. The sign of the optical rotation for AHPA has been found to depend on the configuration of C-4, not C-3.[12] All 4*S*-AHPA's display a negative $[\alpha]_D$. The positive $[\alpha]_D$ for **6** may be due to the presence of a *N*-methyl group and/or the protonation of the methylamino group.

In summary, hapalosin has been found to be (3*S*,4*R*,-8*R*,9*S*,12*S*)-9-benzyl-4-heptyl-8-hydroxy-12-isopropyl-3,-10-dimethyl-1,5-dioxa-10-azacyclododecane-2,6,11-trione (**2**).

Biological Activity. Chemotherapy often provides only temporary clinical improvement. Tumor cells that survive initial chemotherapy often recover with increased resistance to both the original therapeutic agent and other seemingly unrelated drugs, resulting in the ultimate failure of chemotherapy. The phenomenon, termed multiple-drug resistance (MDR), is frequently caused by an overexpression or activation of P-glycoprotein, a 170–200 kDa transmembrane protein (homologous with certain bacterial transport ATPases) which acts as an ATP-dependent drug efflux pump. Enhanced efflux results in a reduction of intracellular drug accumulation with a concomitant decrease in cytotoxicity. Over the past decade, several drugs have been discovered that reverse P-glycoprotein mediated MDR. Verapamil is one of the first MDR reversing agents to be discovered and studied in the clinic.[13]

Hapalosin was found to have better MDR reversing activity than verapamil. As shown in Figure 2, SKVLB1 cells accumulated only 2 pmol of [^{3}H]vinblastine/10^6 cells and 0.7 pmol of [^{3}H]taxol/10^6 cells in a control experiment. Verapamil caused dose-dependent increases in the accumulation of [^{3}H]vinblastine and [^{3}H]taxol in the SKVLB1 cells, reaching 190 and 270%, respectively, of the control at 20 μM. In the case of **2**, similar increases in the accumulation of [^{3}H]vinblastine in SKVLB1 cells were noted; however, **2** was clearly more efficacious than verapamil in promoting [^{3}H]taxol accumulation, reaching 440% of control at 20 μM. Since **2** did not increase the accumulation of either [^{3}H]vinblastine or [^{3}H]taxol in SKOV3 cells (data not shown), we concluded that **2** probably induced drug accumulation in SKVLB1 cells by inhibiting the P-glycoprotein efflux pump.[14]

P-Glycoprotein-overexpressing breast carcinoma (MCF-7/ADR) cells were used to characterize the efficacy of **2**

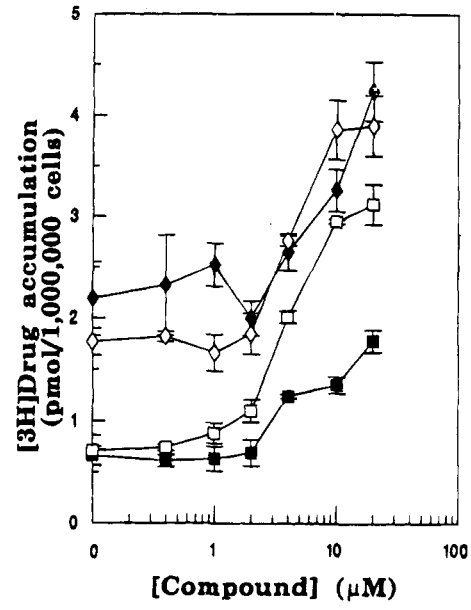

Figure 2. Effects of hapalosin (**2**) and verapamil on drug accumulation in SKVLB1 cells. SKVLB1 cells were incubated with the indicated concentrations of **2** (□,◇) or verapamil (■,♦) for 30 min as indicated in the Experimental Section. [^{3}H]-Vinblastine (■,□) or [^{3}H]taxol (♦,◇) was then added and its intracellular accumulation after 60 min was determined. Values represent the mean ±SD accumulation of [^{3}H]drug in one of three similar experiments.

in modulating drug resistance. MCF-7/ADR cells were found to be more susceptible to combinations of vinblastine and **2** than to vinblastine alone (Figure 3a). Moreover, vinblastine showed as much cytotoxicity toward MCF-7/ADR cells in the presence of **2** as it did to MCF-7 cells in the absence of **2**, suggesting that **2** was able to completely overcome P-glycoprotein-mediated MDR in this model system. As indicated in Figure 3b, **2** increased the cytotoxicities of daunomycin, vinblastine, actinomycin D, taxol, and colchicine (drugs known to be transported by P-glycoprotein) toward MCF-7/ADR cells, without potentiating the cytotoxicities of 5-fluorouracil or cisplatin (nontransported drugs). Chemosensitization of MCF-7/ADR cells was apparent with doses of **2** as low as 2.5 μM, approximately the same doses required to observe increases in intracellular drug accumulation. Compound **2** was mildly cytotoxic, showing IC_{50}s in the 5–15 μM range. The therapeutic index (TI), i.e. the ratio of the concentration of **2** required for reversal of drug resistance to the IC_{50} for MCF-7/ADR, however, was >5, similar to the TI's exhibited by several other MDR reversing agents, e.g. verapamil and trifluorperazine.[15]

The efficacy of **2** to (1) selectively potentiate the cytotoxicity of P-glycoprotein-transported drugs and (2) increase intracellular accumulation of vinblastine and taxol are properties characteristic of MDR-reversing agents that act as antagonists for drug transport by P-glycoprotein. While numerous compounds with this activity have been identified in the past 10 years,[15] none to date show satisfactory clinical activity.[16] Hapalosin (**2**) represents a lead compound for a new class of potential anti-MDR agents and studies are in progress to evaluate its activity *in vivo*.

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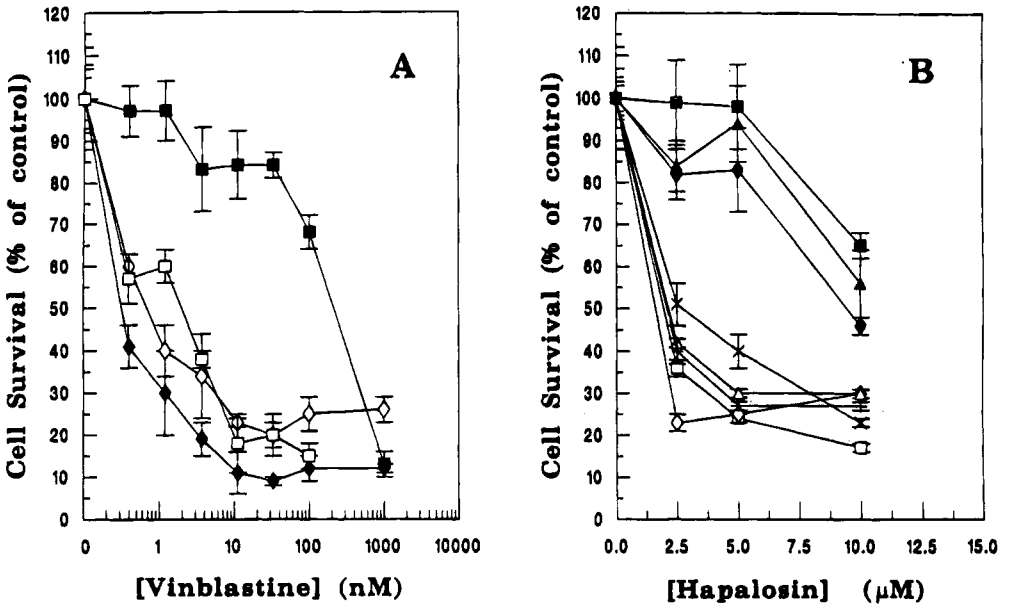

Figure 3. Chemosensitization of MCF-7/ADR cells by hapalosin (**2**). (A) MCF-7 cells (□) or MCF-7/ADR cells treated with 1% DMSO (■), 10 mM verapamil (◇), or 5 μM hapalosin (♦) were incubated with the indicated concentrations of vinblastine. After 48 h, cell survival was assayed as indicated in the Experimental Section. (B) MCF-7/ADR cells were treated with the indicated concentrations of hapalosin in the presence of PBS (■), 15 μM daunomycin (□), 250 nM vinblastine (◇), 200 nM actinomycin D (△), 750 nM colchicine (+), 3 μM taxol (×), 4 μM cisplatin (♦), or 40 μM 5-fluorouracil (▲). After 48 h, cell survival was assayed as indicated in the Experimental Section. In the absence of hapalosin, these drug concentrations inhibited cell growth by 20–30%.

Experimental Section

Spectral Analysis. NMR spectra were determined on a 11.75-T instrument operating at 500 MHz for ^1H and 125 MHz for ^{13}C. ^1H chemical shifts are referenced in $CDCl_3$ to residual $CHCl_3$ (7.26 ppm); ^{13}C chemical shifts are referenced to the solvent ($CDCl_3$, 77.0 ppm). One-bond heteronuclear $^1H-^{13}C$ connectivities were determined by HMQC; two- and three-bond $^1H-^{13}C$ connectivities were determined by HMBC. All ^1H and ^{13}C assignments are based on detailed COSY, HMQC, and HMBC analyses. Homonuclear ^{13}C connectivities were determined by 1D decoupling experiments. Mass spectra, including high resolution mass measurements, were determined in the EI mode. UV spectra were measured in MeOH at 20 °C.

Isolation and Cultivation of Alga. *Hapalosiphon welwitschii* W. & G. S. West, designated UH strain IC-52-3, was grown in mass culture as previously described.[5] The yield of lyophilized cells was 0.3–0.4 g/L.

H. welwitschii UTEX B1830 was purchased from the University of Texas Culture Collection and grown in a similar manner. Incubation time ranged from 25 to 28 d. The yield of lyophilized cells was 0.32–0.39 g/L.

Isolation of Hapalosin. Freeze-dried alga (34.2 g, *H. welwitschii* IC-52-3) was extracted twice with 2 L portions of 1:1 CH_2Cl_2/2-propanol overnight with stirring. The extracts were combined and evaporated under reduced pressure to a dry green solid (1.5 g). The extract was dissolved in 20 mL of MeOH and the filtered solution was applied to a column of Sephadex LH20-120 (Fluka, 70 cm × 4.5 cm diameter, flowrate 50 mL/h) equilibrated in MeOH. After approximately 400 mL of MeOH had passed, green pigments began to be eluted. The fractions from 600 to 1000 mL were analyzed by ^1H NMR for hapalosin. The fractions which showed characteristic doublets at 0.55 and 0.23 ppm (800–900 mL) were combined and the solvent was evaporated. The residual solid (200 mg) was further purified by RP-chromatography on ODS (YMC-gel, 120A; 2.5 × 33 cm), using successively 300 mL amounts of 4:1 MeOH/H_2O, 9:1 MeOH/H_2O, and MeOH. The material eluted in the fraction from 450 to 600 mL (60 mg) contained **2** as shown by RP-TLC on C18 (R_f 0.3 with 9:1 MeOH/H_2O). Final purification was achieved by RP-HPLC on C8 (Econosil C8, 10 μm, 250 × 10 mm, UV-detection at 230 nm, flow rate 3.0 mL/min) using 23:17 CH_3CN/H_2O as the eluant to give 42 mg (0.12% yield) of **2** (t_R 62 min) as a white solid.

Freeze dried alga (10.4 g, *H. welwitschii* UTEX B1830) was extracted with two 400 mL portions of 3:7 EtOH/H_2O, each one for 24 h at room temperature. The cells were then extracted with 400 mL of 1:1 iPrOH/CH_2Cl_2 for 24 h at room temperature to give 1.68 g of a dark brown oil. The lipophilic extract was passed through a 130 × 2 cm column of Sephadex LH20 with 4:1 MeOH/CH_2Cl_2 in two portions and 5 mL fractions were collected. The fraction eluting from 110 to 120 mL was estimated by NMR to be 50% **2**. Final purification was accomplished by two RP-HPLC steps, first on Econosil C18 with 0.1% TFA in 22:3 MeOH/H_2O and then on Econosil C18 with 7:3 CH_3CN/H_2O. The yield of **2** was 29 mg (0.28% yield).

Hapalosin (2). $[\alpha]_D$ −49.2° (*c* 0.35, CH_2Cl_2); EIMS *m/z* (rel intensity) 489 (M^+, 1.8), 398 (54), 380 (41), 298 (15), 280 (18), 267 (72), 217 (26), 167 (100), 134 (39); HREIMS *m/z* 489.3146 ($C_{28}H_{43}NO_6$, Δ −5.5 mmu), 398.2569 ($C_{21}H_{36}NO_6$, Δ −2.6 mmu), 380.2459 ($C_{21}H_{34}NO_5$, Δ −2.2 mmu), 298.2034 ($C_{16}H_{28}NO_4$, Δ −1.6 mmu), 280.1891 ($C_{16}H_{26}NO_3$, Δ +2.2 mmu), 267.1957 ($C_{16}H_{27}O_3$, Δ + 0.3 mmu), 167.1436 ($C_{11}H_{19}O$, Δ 0.0 mmu); UV (MeOH) λ_{max} nm (ε) 208 (10 600); IR (neat) ν_{max} 3420, 1730, 1645 cm^{-1}. ^1H and ^{13}C NMR of major conformer ($CDCl_3$): see Table 1. ^1H NMR of major conformer (MeOH-d_4): *amino or hydroxy acid unit* δ (proton position) **2a** 5.02 (12-H), 1.85 (1′-H), 0.10 (2′-H_3), 0.78 (3′-H_3); **2b** 3.17 (3-H), 1.15 (1‴″-H_3), 4.95 (4-H), 1.55/1.81 (1‴″-H_2), 1.27 (2‴″-H_2/3‴″-H_2/4‴″-H_2/5‴″-H_2), 1.30 (6‴″-H_2), 0.90 (7‴″-H_3); **2c** 2.45/2.55 (7-H_2), 4.23 (8-H), 4.99 (9-H), 2.74/3.44 (1‴-H_2), 7.21 (3‴-H/7‴-H), 7.33 (4‴-H/6‴-H), 7.25 (5‴-H), 2.83 (1″-H_3). ^{13}C NMR of major conformer (MeOH-d_4): *unit* δ (carbon position) **2a** 172.2 (11), 84.1 (12), 29.1 (1′), 17.3 (2′), 19.9 (3′); **2b** 173.8 (2), 41.7 (3), 11.0 (1‴″), 77.0 (4), 26.3 (1‴″), 30.1/30.3/31.6 (2‴″/3‴″/4‴″), 33.0 (5‴″), 23.7 (6‴″), 14.5 (7‴″); **2c** 171.5 (6), 41.9 (7), 70.3 (8), 61.0 (9), 35.8 (1‴), 139.2 (2‴), 130.2 (3‴/7‴), 129.4 (4‴/6‴), 127.5 (5‴), 30.5 (1″).

Acid Hydrolysis. A solution of 10.5 mg of **2** in 2.5 mL of MeOH and 7.5 mL of 3 N HCl was refluxed under N_2 for 20 h and then evaporated. The acid hydrolyzate was treated with an ethereal solution of CH_2N_2 and the solvent was carefully (methyl ester **3** is volatile) removed under reduced pressure. The residue was chromatographed on a 4 × 0.6 cm of silica gel with successive 10 mL amounts of 1:9 Et_2O/hexane, 3:17 Et_2O/hexane, 1:4 Et_2O/hexane, 7:3 Et_2O/hexane, and 9:1

EtOAc/2-propanol. Fractions (3.3 mL) were collected and analyzed by ^{1}H-NMR. Fractions 2–3 contained **3** (yield undetermined) and fractions 3–5 contained 4.3 mg (93%) of **4**. Lactam **5** (3.3 mg, 71%) was present in the fractions eluted with 9:1 EtOAc/2-propanol.

Chiral GC-Analysis. The fractions containing **3** were dissolved in 1 mL of CH_2Cl_2 for gas chromatographic analysis on a 25 m × 0.25 mm Chirasil-Val column (Alltech). Standards of 1 mg of commercial D- and L-2-hydroxy-3-methylbutanoic acid were treated with excess CH_2N_2 in Et_2O and the volume of the solution adjusted to 1 mL. The following conditions were used for GC: a 12 psi head pressure (flow rate estimated to be about 0.75 mL/s) and a column temperature held at 30 °C for 6 min after injection of the sample and then increased from 30° to 180 °C at 7°/min and finally held at 180° for 2 min. The retention times were found to be 8 min 5 s and 8 min 42 s for the authentic L- and D-2-hydroxy-3-methylbutanoic acid methyl esters, respectively. The retention time for **3** from hydrolysis of **2** was 8 min 5 s.

Methyl (2*S*,3*R*)-3-hydroxy-2-methyldecanoate (4): HREIMS m/z 201.1489 ($[M-CH_3]^+$; $C_{11}H_{21}O_3$, Δ +0.2 mmu); ^{1}H-NMR ($CDCl_3$) δ 3.88 (td; J = 8.2 and $J_{2,3}$ = 4.0 Hz; 3-H), 3.71 (s, OMe), 2.55 (qd; J = 7.2 and $J_{2,3}$ = 4.0 Hz; 2-H), 2.35 (OH), 1.45 (m; 4-H_2), 1.28 (br s; 5,6,7,8,9-H_2), 1.17 (d; J = 7.2 Hz; Me on C-2), 0.88 (t; J = 6.4 Hz; 10-H_3); ^{13}C-NMR ($CDCl_3$) δ 173.6 (C-1), 71.8 (C-3), 51.8 (OCH_3), 44.1 (C-2), 33.8 (C-8), 31.8/29.5/29.2 (C-5,6,7), 26.0 (C-4), 22.6 (C-9), 14.1 (C-10), 10.6 (Me on C-2).

Lactam 5. HREIMS m/z 205.1080 ($C_{12}H_{15}NO_2$, Δ +2.3 mmu); ^{1}H-NMR of major conformer ($CDCl_3$) δ 7.25–7.35 (m; 4′-H/5′-H/6′-H); 7.17 (br dd; J = 8 and 1.5 Hz; 3′-H/7′-H), 4.19 (dt; J = 6.3 and 1.6 Hz; 4-H), 3.66 (ddd; $J_{5,1'S}$ = 7.9, $J_{5,1'R}$ = 4.8 and $J_{4,5}$ = 1.6 Hz; 5-H), 3.00 (dd; J_{gem} = −14.0 and $J_{5,1'R}$ = 4.8 Hz, 1′-H_R), 2.89 (s; N-CH_3), 2.68 (dd; J_{gem} = −14.0 and $J_{5,1'S}$ = 7.9 Hz; 1′-H_S), 2.37 (dd; J_{gem} = −17.8 and $J_{3R,4}$ = 6.3 Hz; *pro-R* 3-H), 2.18 (dd; J_{gem} = −17.8 and $J_{3S,4}$ = 1.6 Hz; *pro-S* 3-H); ^{1}H-NMR of minor conformer ($CDCl_3$) δ 7.25–7.35 (m; 4′-H/5′-H/6′-H); 7.16 (d, 3′-H/7′-H), 4.19 (td; 4-H), 3.66 (dt; $J_{5,1'S}$ = 8.5, $J_{5,1'R}$ and $J_{4,5}$ = 6 Hz; 5-H), 3.00 (dd; J_{gem} = −14.0 and $J_{5,1'R}$ = 8.5 Hz, 1′-H_R), 2.89 (s; NCH_3), 2.68 (dd; J_{gem} = −14.0 and $J_{5,1'S}$ = 6.0 Hz; 1′-H_S), 2.37 (dd; J_{gem} = −17.0 and $J_{3R,4}$ = 6.5 Hz; *pro-R* 3-H), 2.18 (dd; J_{gem} = −17.0 and $J_{3S,4}$ = 3.2 Hz; *pro-S* 3-H); ^{13}C-NMR of major/minor (5:1) conformers ($CDCl_3$) δ 172.6/nd (C-2), 136.2/nd (C-2′), 129.1/129.2 (C-3′/C-7′), 128.9/128.8 (C-4′/C-6′), 127.1/126.8 (C-5′), 70.2/65.6 (C-5), 68.9/66.4 (C-4), 39.6/40.2 (C-3), 37.1/33.3 (C-1′), 28.3/28.1 (NCH_3).

Conversion of 5 to 6. A solution of 3.8 mg of **5** in 2 mL of acetic acid and 6 mL of 3 N HCl was refluxed overnight. After evaporating the solvents and drying in vacuo, [3*R*,4*S*]-3-hydroxy-4-(methylamino)-5-phenylpentanoic acid (**6**), as the hydrochloride, was obtained as a water soluble oil in quantitative yield; $[\alpha]_D$ +35° (*c* 0.14, H_2O); ^{1}H-NMR (D_2O) δ 7.35–7.45 (m; phenyl 3-H/4-H/5-H); 7.27 (d; J = 6.8 Hz; phenyl 2-H/6-H), 4.29 (dd; $J_{2,3}$ = 5.4 and $J_{3,4}$ = 0.5 Hz; 3-H), 3.87 (ddd; $J_{4,5}$ = 6.6, $J_{4,5'}$ = 4.5 and $J_{3,4}$ = 0.5 Hz; 4-H), 2.90 (dd; J_{gem} = −14.2 and $J_{4,5'}$ = 4.5 Hz; 5-H′), 2.96 (s; N-CH_3), 2.93 (dd; J_{gem} = −14.2 and $J_{4,5}$ = 6.6 Hz; 5-H), 2.13 (dd; J_{gem} = −18.5 and $J_{2,3}$ = 5.4 Hz; 2-H), 2.07 (d; J_{gem} = −18.5 Hz; 2-H′).

Preparation of (*R*)- and (*S*)- MTPA-esters of 2–5. A solution of 1 mg of **2** (2 μmol), 2.4 mg of (*R*)- or (*S*)-MTPA acid (10 μmol; 1.74 μL), 2.76 mg of DCC (13 μmol) and 0.5 mg of DMAP in 500 μL of dry CH_2Cl_2 was stirred at room temperature for 3 h. After evaporating the solvent, the reaction product was passed through a 2 × 0.4 cm column of silica gel (230–425 mesh) with CH_2Cl_2. The fraction eluting from 2–4 mL contained a quantitative yield of 90% pure (*R*)- or (*S*)-MTPA ester, respectively. Each ester was further purified by preparative TLC (silica gel 60 Å, Whatman, 20 × 10 cm plate; CH_2Cl_2 as mobile phase).

To a solution of 10 μmol of **3**, **4**, or **5**, 15 mg of DMAP (4-(dimethylamino)pyridine, 123 μmol), and 6.2 mL of triethylamine (44 μmol) in 500 μL of CH_2Cl_2 (dried over 3 Å molecular sieves) was added 11.2 μL of (*R*)-(−) MTPA chloride (60 μmol), and the solution was allowed to stand overnight. The reaction product was subjected to preparative TLC [silica gel 60 Å, Whatman, 20 × 10 cm; hexane–Et_2O, 1:1 (v/v)] in the case of **3** or **4**, or to chromatography on a short column of silica (500 mg) in the case of **5**, to give the pure (*S*)-MTPA ester in 35–40% yield. The (*R*)-MTPA ester was made from the (*S*)-MTPA chloride in a similar manner. R_f values for TLC are shown below.

(*S*)-MTPA-Ester of 2: R_f 0.02–0.28; 300 MHz ^{1}H-NMR ($CDCl_3$) δ_H (carbon position) **2a** 5.110 (12), 1.76 (1′), 0.145 (2′), 0.775 (3′); **2b** 3.063 (3), 1.162 (1‴″), 4.909 (4), 1.52/1.82 (1‴″), 1.28 (2‴″/3‴″/4‴″/5‴″/6‴″), 0.882 (7‴″); **2c** 2.684/2.593 (7), 5.560 (8), 5.325 (9), 2.837/2.531 (1‴), 7.112 (3‴/7‴), 7.193 (4‴/6‴), 7.122 (5‴), 2.807 (1″).

(*R*)-MTPA-Ester of 2: R_f 0.02–0.25; 300 MHz ^{1}H-NMR ($CDCl_3$) δ_H (carbon position) **2a** 5.125 (12), 1.76 (1′), 0.137 (2′), 0.773 (3′); **2b** 3.082 (3), 1.156 (1‴″), 4.917 (4), 1.52/1.82 (1‴″), 1.28 (2‴″/3‴″/4‴″/5‴″/6‴″), 0.886 (7‴″); **2c** 2.599/2.466 (7), 5.619 (8), 5.377 (9), 3.052/2.676 (1‴), 7.164 (3‴/7‴), 7.222 (4‴/6‴), 2.837 (1″).

(*S*)-MTPA-Ester of 3: R_f 0.55; ^{1}H-NMR ($CDCl_3$) δ 5.000 (d, 2-H), 3.788 (s, CO_2CH_3), 2.295 (m, 3-H), 0.925 (d, 4-H_3), 0.901 (d, Me on C-3).

(*R*)-MTPA-Ester of 3: R_f 0.55; ^{1}H-NMR ($CDCl_3$) δ 5.027 (d; 2-H), 3.763 (s, CO_2CH_3), 2.332 (m; 3-H); 1.027 (d, 4-H_3), 0.966 (d, Me on C-3).

(*S*)-MTPA-Ester of 4: R_f 0.58; EIMS m/z (rel intensity) 199 (24), 189 (100), 167 (40), 149 (10), 105 (17), 97 (26), 88 (13), 83 (49), 77 (10), 69 (63); HREIMS m/z 199.1697 ($C_{12}H_{23}O_2$, Δ +0.1 mmu), 189.0518 ($C_9H_8OF_3$, Δ +0.9 mmu); ^{1}H-NMR ($CDCl_3$) δ 5.417 (dt, J = 7.5/4.9; 3-H), 3.532 (s, $COOCH_3$), 2.733 (qd, J = 7.1/4.9 Hz; 2-H), 1.72 (m, 4-H); 1.61 (m, 4-H′), 1.28/1.25 (m/m, 5,6,7,8,9-H_2), 1.119 (d, J = 7.1 Hz, Me on C-2), 0.878 (t, J = 7.1 Hz; 10-H_3).

(*R*)-MTPA-Ester of 4: R_f 0.59; EIMS m/z (rel intensity) 199 (25), 189 (100), 167 (37), 149 (9), 105 (19), 97 (23), 88 (10), 83 (53), 77 (10), 69 (51); HREIMS m/z 199.1688 ($C_{12}H_{23}O_2$, Δ +0.9 mmu), 189.0532 ($C_9H_8OF_3$, Δ −0.5 mmu); ^{1}H-NMR ($CDCl_3$) δ 5.411 (dt; 3-H), 3.668 (s, $COOCH_3$), 2.755 (qd, 2-H), 1.65 (m, 4-H), 1.55 (m, 4-H′), 1.25 / 1.20 (m, 5,6,7,8,9-H_2), 1.175 (d, Me on C-2), 0.871 (t; 10-H_3).

(*S*)-MTPA-Ester of 5: ^{1}H-NMR of major conformer ($CDCl_3$) δ 7.125 (br dd, 3′-H/7′-H), 5.262 (dd, 4-H), 3.689 (t, 5-H), 3.007 (dd, 1′-H_R), 2.844 (s, N-CH_3), 2.897 (dd, 1′-H_S), 2.205 (dd; J_{gem} = −17.0 and $J_{3R,4}$ = 6.5 Hz; *pro-R* 3-H), 2.235 (dd; J_{gem} = −17.0 and $J_{3S,4}$ = 3.2 Hz; *pro-S* 3-H). Ratio of major conformer to minor conformer is 14:1.

(*R*)-MTPA-Ester of 5: ^{1}H-NMR ($CDCl_3$) δ 7.16 (d, 3′-H/7′-H), 5.240 (dt, 4-H), 3.814 (t, 5-H), 3.057 (dd, 1′-H_R), 2.899 (s, N-CH_3), 2.892 (dd, 1′-H_S), 2.230 (dd; J_{gem} = −17.0 and $J_{3R,4}$ = 6.5 Hz; *pro-R* 3-H), 2.158 (dd; J_{gem} = −17.0 and $J_{3S,4}$ = 3.2 Hz; *pro-S* 3-H). Chemical shifts for only one conformer are observed.

Cytotoxicity of 2. IC_{50} values for KB (a human nasopharyngeal carcinoma cell line) and LoVo (a human colon adenocarcinoma cell line) were determined to be approximately 2.5 and 2 μg/mL, respectively.[17]

Evaluation of MDR Reversal. MCF-7 breast carcinoma cells and MCF-7/ADR cells, an MDR subline,[18] were obtained from the Division of Cancer Treatment of the National Caner Institute. SKVLB1 cells, which were selected for resistance to vinblastine and overexpressed P-glycoprotein at least 100-fold, were kindly provided by Dr. Victor Ling of the Ontario Cancer Institute.[19] The effects of drugs on cell growth were characterized using the sulforhodamine binding assay[20] as

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previously described.[21] The effects of **2** and verapamil on intracellular drug accumulation were determined as previously described[21] using 20 nM [^{3}H]vinblastine sulfate (10–15 Ci/mmol from Amersham) or [^{3}H]taxol (~19 Ci/mmol from Moravek Biochemicals).

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Supplementary Material Available: 500 MHz ^{1}H- and 125 MHz ^{13}C-NMR spectra of **2** and **5** in $CDCl_3$; 300 MHz ^{1}H-NMR spectrum of **5** in CD_3OD (5 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.