Pergamon

Tetrahedron Letters, Vol. 37, No. 20, pp. 3467-3470, 1996

0040-4039/96 $15.00 + 0.00

S0040-4039(96)00592-8

Synthetic Study on Hapalosin, a Cyclic Depsipeptide Possessing Multidrug Resistance Reversing Activities

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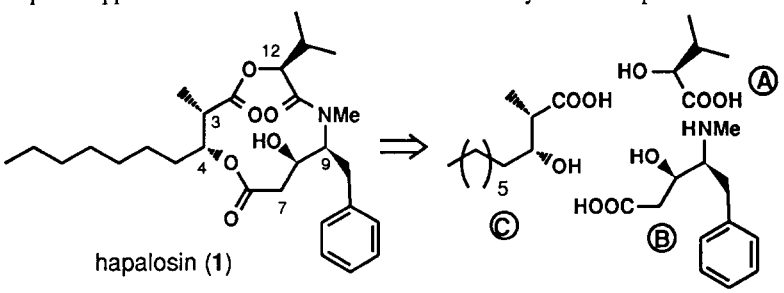
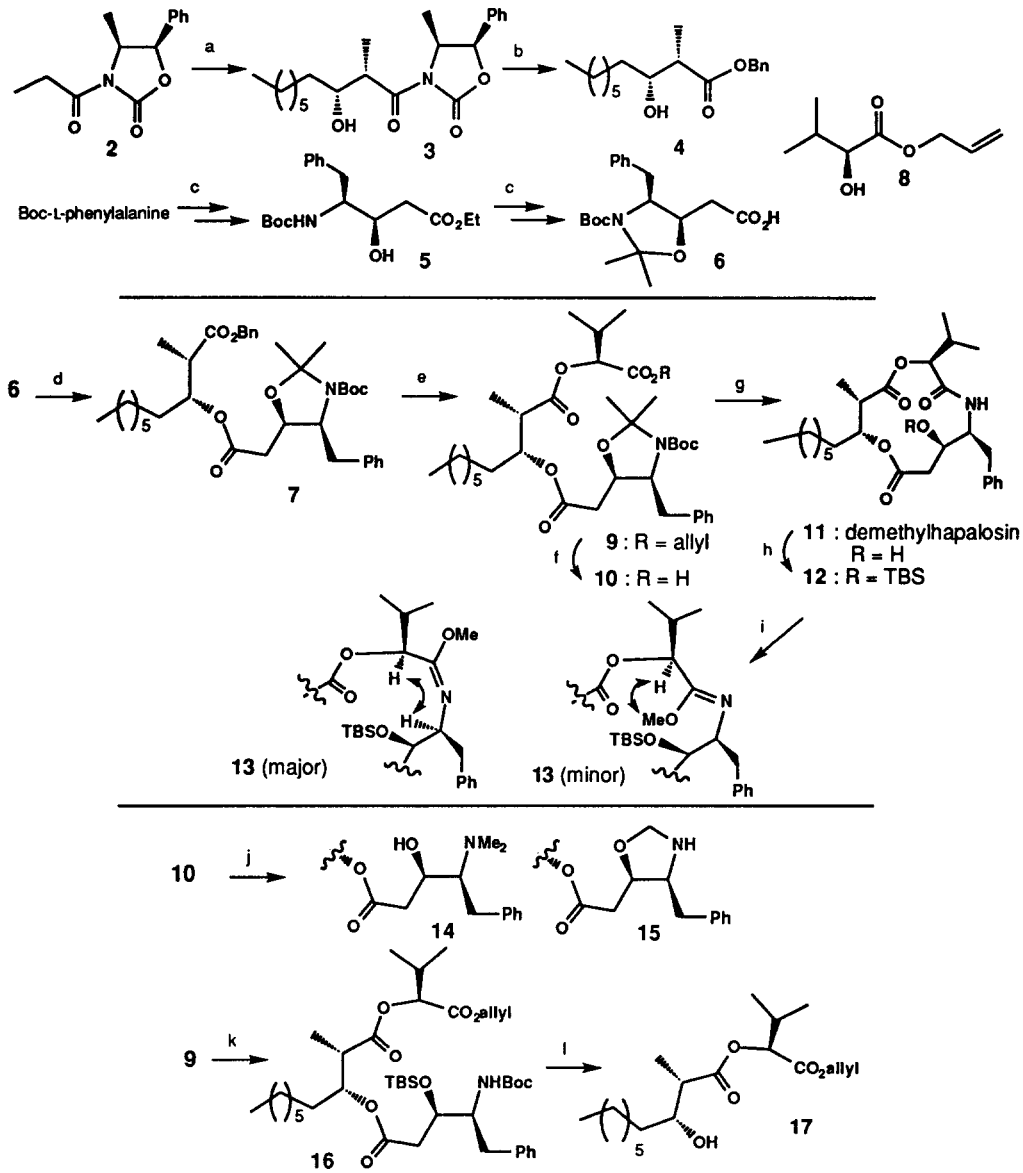
Abstract: Hapalosin possessing a multidrug resistance reversing activity, has been synthesized from the corresponding hydroxy acids and γ-amino acids. The stereochemistry of the natural product and related derivatives is discussed. Copyright © 1996 Elsevier Science Ltd

Hapalosin (**1**), isolated from *Hapalosiphon welwitschii* W. & S. West, indicates better P-glycoprotein-mediated multidrug resistance reversing activity than verapamil to potentiate the cyctotoxicities of such antitumor drugs transported by P-glycoprotein as daunomycin, vinblastine, actinomycin D, colchicine and taxol against resistant cells.[1] Among the many chemotherapeutic approaches to cancer disease, drug resistance remains one of the most urgent problems to be solved. Along with chemical interest in the cyclic depsipeptide structure, this background prompted us to initiate syntheses of **1** and its congeners.[2] When cleaved at the two esters and the amide bonds, the molecule was found to consist of 3-hydroxy-2-methylbutyric acid (**A**), 3-hydroxy-γ-amino acid (**B**) and β-hydroxy acid (**C**). In addition to commercially available **A** and the known structure (**B**),[3] **C** might be prepared by the Evans aldol protocol.[4] To enable the easy cyclization to the target molecule, the amide linkage would be finally constructed, and the following N-methylation might promise a facile introduction of diverse alkyl groups to supplement information on the structure-activity relationship.

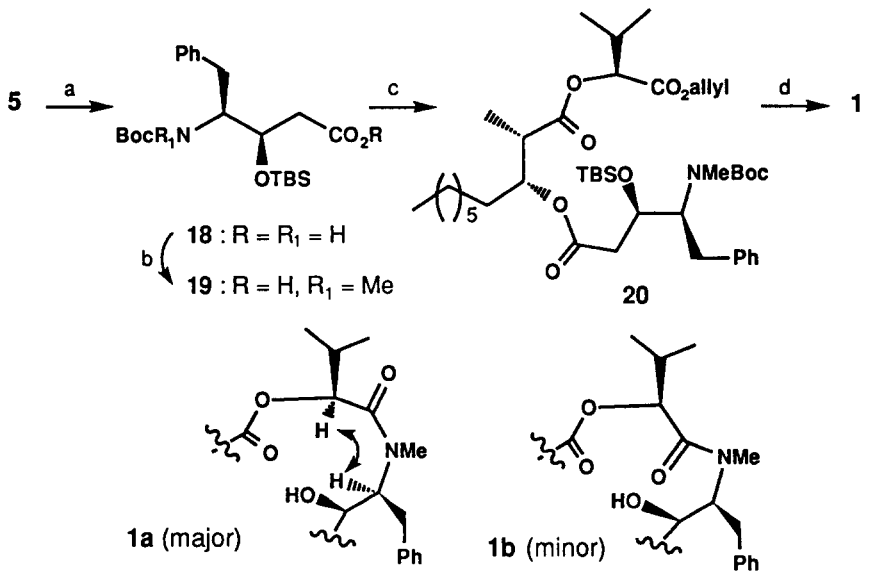


Oxazolidinone **2** was coupled with octanal in the presence of Bu_2BOTf and Et_3N to give the corresponding amide (**3**) in 90% yield, which on recrystallization contained no diastereomers detectable by the ^1H NMR spectrum. Treatment of **3** with LiOBn prepared from *n*BuLi - BnOH provided the corresponding benzyl ester (**4**, 71%), which was condensed with the γ-amino acid (**6**)[3] to give **7** in 86% yield based on **6**. Hydrogenolysis of **7** effected the abstraction of a benzyl group, leading to a carboxylic acid, which on coupling with **8** under the DCC - DMAP conditions furnished **9** in 81% yield in two steps. After removal of the protective groups of **9** by a 2-step procedure via **10**, the resulted acid underwent cyclization by a high dilution method (1 mmol/l) to produce demethylhapalosin (**11**).[5] Transformation into the natural product (**1**) by



Scheme 1. a. Bu_2OTf, Et_3N/CH_2Cl_2, then $Me(CH_2)_6CHO$ (90%). b. *n*BuLi, BnOH / THF (71%). c. ref. 3. d. i) 2M NaOH: ii) **4**, DCC, DMAP / CH_2Cl_2 (86% from **6**). e. i) H_2, $Pd(OH)_2$ / EtOH: ii) **8**, DCC, DMAP / CH_2Cl_2 (81% in two steps). f. $(Ph_3P)_4Pd$, morpholine / THF (80%). g. i) TFA / CH_2Cl_2: ii) DPPA, $EtNiPr_2$ / DMF (1 mmol/l) (83% in two steps). h. TBSCl, Imd / DMF (92%). i. Me_3OBF_4, Proton Sponge™ / CH_2Cl_2 (66%). j. i) TFA / CH_2Cl_2: ii) HCHO, $NaBH_3CN$ / MeOH (**14**, 72%): i) TFA / CH_2Cl_2: ii) HCHO, then $NaBH_3CN$ (**15**, 67%). k. i) *p*-TsOH / MeOH (67%): ii) TBSCl, Imd / DMF (82%). l. MeI, NaH / DMF (35%)

employing the protected derivative (**12**) was troublesome, probably owing to labile properties under the usual N-methylation conditions examined (MeI - NaH, MeI - Ag_2O and MeI - LDA). During such attempts, exposure to Me_3OBF_4 and Proton Sponge™,[6] produced **13**[7,8] as a 1.8:1 (cis / trans) mixture of geometrical isomers, whose structures were deduced by the NOESY experiments, as depicted in Scheme 1. Interestingly, the



Scheme 2. a. i) TBSCl, Imd / DMF (100%): ii) 2M NaOH (84%). b. MeI, NaH / THF (77%). c. i) **4**, DCC, DMAP / CH_2Cl_2 (77%): ii) H_2, $Pd(OH)_2$ / EtOH: iii) **8**, DCC, DMAP / CH_2Cl_2 (86% in two steps). d. i) $(Ph_3P)_4Pd$, morpholine / THF (97%): ii) TFA / CH_2Cl_2 (94%): iii) DPPA, $EtNiPr_2$ / DMF (1 mmol/l) (44%).

preferential formation of the cis-type product is closely similar to the ratio of the natural conformers.[1] Additionally, N-methylation by using the primary amine was also unsuccessful; HCHO - $NaBH_3CN$ gave a mixture of the dimethyl derivative (**14**), or acetal **15**. Upon employing **16** as a substrate, the MeI - NaH method gave rise to an ester cleavage to afford the fragment (**17**).

To circumvent the above-mentioned difficulties in the N-methylation, the N-methyl group was incorporated at an early stage of the synthesis. Thus, **5** was protected as a siloxy ether, followed by hydrolysis under basic conditions to give **18** in 84% yield in two steps (Scheme 2). Compound **18** was submitted to MeI - NaH in THF[9] to yield **19**. The following homologation reactions were performed by essentially the same procedure as mentioned above to give **20**. After removal of the protective groups, cyclization under high dilution conditions provided the target (**1**)[10] in 44% yield. Spectral data of synthetic **1** were superimposable to those reported for the natural sample.[1]

In particular, the ^1H NMR spectrum of synthetic **1** at room temperature showed a ~3:1 (**1a / 1b**) mixture of the conformers as reported by Moore.[1] By further measurement (DMSO-d_6) at the elevated temperature, it was observed that the ratio of **1a** and **1b** reversibly changed to 1.7:1 (100 °C). Since NOE information was not sufficiently obtained, the conformation of the whole molecule could not be discussed. However, the NOESY experiments indicated that the major isomer (**1a**) exhibited a correlation between H_9 and H_{12} ascribed to a cis-amide, while the minor (**1b**) adopted a trans-amide without a NOE effect at the same position.[11] Interestingly, contrary to the case of **1**, the ^1H NMR spectrum of demethylhapalosin (**11**) showed the presence of a trans-amide as a single isomer. Based on these observations, the N-methyl group may be a crucial factor to control the geometry of the amide bond.

Further investigation of the relationship between the N-alkyl group and the stereochemistry of the whole molecule, and concomitant biological activities are in progress.

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5. **11**: $[\alpha]_D^{22}$ -31.7° (*c* 0.50, $CHCl_3$). Found *m/z* 475.2945. Calcd for $C_{27}H_{41}NO_6$: 475.2822 (M+). IR (film) 3320, 2940, 1735, 1665, 1595 cm^{-1}; ^1H NMR (400MHz, $CDCl_3$) δ 0.66 (3H, d, J= 6.8 Hz), 0.85 - 0.92 (6H, complex), 1.20 - 1.81 (16H, complex), 2.57 (1H, dd, J= 5.6, 14.0 Hz), 2.65 (1H, dd, J= 3.6, 14.0 Hz), 2.87 - 3.04 (3H, complex), 4.08 (1H, brs), 4.53 (1H, d, J= 8.0 Hz), 4.63 (1H, m), 5.46 (1H, d, J= 10.8 Hz), 5.52 (1H, m), 7.18 - 7.30 (5H, complex); ^{13}C NMR (100.5 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, 41.2, 54.0, 70.6, 75.8, 81.8, 126.7, 128.6, 129.0, 136.9, 169.8, 173.4, 174.1.
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7. **12**: ^1H NMR (400MHz, $CDCl_3$) major confomer: δ 0.02 - 0.19 (6H, complex), 0.52 (3H, d, J= 6.8 Hz), 0.86 - 0.92 (6H, complex), 0.95 (9H, s), 1.17 (3H, d, J= 7.6 Hz), 1.20 - 1.49 (11H, complex), 1.95 - 2.05 (2H, complex), 2.30 (1H, dd, J= 10.0, 12.8 Hz), 2.54 (1H, dd, J= 5.6, 16.2 Hz), 2.76 (1H, dd, J= 2.4, 16.2 Hz), 2.87 (1H, m), 3.34 (1H, dd, J= 2.1, 12.8 Hz), 3.72 (1H, ddd, J= 2.1, 7.9, 10.0 Hz), 3.88 (1H, ddd, J= 2.4, 5.6, 7.9 Hz), 4.22 (1H, d, J= 8.8 Hz), 5.16 (1H, m), 7.08 - 7.31 (5H, complex).
8. Treatment with TBAF to remove a silyl protective group resulted in an inseparable mixture.
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10. **1**: $[\alpha]_D^{18}$ -41.0° (*c*1.00, CH_2Cl_2). Found *m/z* 489.3079. Calcd for $C_{28}H_{43}NO_6$: 489.3090 (M+). IR (film) 3430, 2940, 1735, 1635cm^{-1}; ^1H NMR (400MHz,$CDCl_3$) major confomer: δ 0.23 (3H, d, J= 6 Hz), 0.57 (3H, d, J= 6 Hz), 0.88 - 0.92 (3H, complex), 1.14 - 1.40 (13H, complex), 1.50 - 2.05 (3H, complex), 2.61 (1H, m), 2.65 (1H, m), 2.86 (3H, m), 2.92 (1H, dd, J= 18.5 Hz), 3.22 (1H, m), 3.41 (1H, dd, J= 2.6, 14 Hz), 3.69 (1H, dt, J= 2.6, 10 Hz), 3.85 (1H, m) 4.31 (1H, d, J= 8.4 Hz), 5.12 (1H, m), 7.17 - 7.35 (5H, complex); ^{13}C NMR (100.5 MHz, $CDCl_3$) major conformor: 12.1, 14.1, 17.5, 18.3, 22.6, 26.0, 28.0, 28.2, 28.8, 29.1, 29.2, 31.7, 36.4, 37.0, 40.7, 61.4, 70.2, 73.8, 76.5, 127.0, 128.9, 129.7, 137.4, 168.5, 168.7, 172.7.
11. The same observation was reported by Armstrong (see ref.2a).

(*Received in Japan* 19 *February* 1996; *revised* 18 *March* 1996; *accepted* 25 *March* 1996)