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SYNTHESIS OF THE MULTIDRUG RESISTANCE-REVERSING AGENT HAPALOSIN

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Abstract:

Cytotoxic drug often fail to kill tumour cells long because of over expression or activation of transmembrane p-glycoprotein in efflux of drugs.

Keywords: Hapalosin,2-hydroxy-3-methylbutyricacid,β-hydroxy-α-methylacid.

Introduction:

Hapalosin a novel cyclodepsipeptide isolated recently from the blue-green alga “Hapalosiphon Welwitschii” has shown important multidrug-resistance reversing activity. The term multidrug-resistance (MDR) is defined as the ability of tumor cells exposed to one drug to develop cross resistance to seemingly unrelated drugs. This phenomenon is apparently caused by an over expression of p-glycoprotein, a 170-200 K Da transmembrane protein that acts as an ATP-dependent drug efflux pump. As a consequence, chemotherapy very often becomes ineffective. Thus, the agents that are capable of reversing this p-glycoprotein induced MDR effect may have therapeutic potential for patients undergoing cancer chemotherapy.

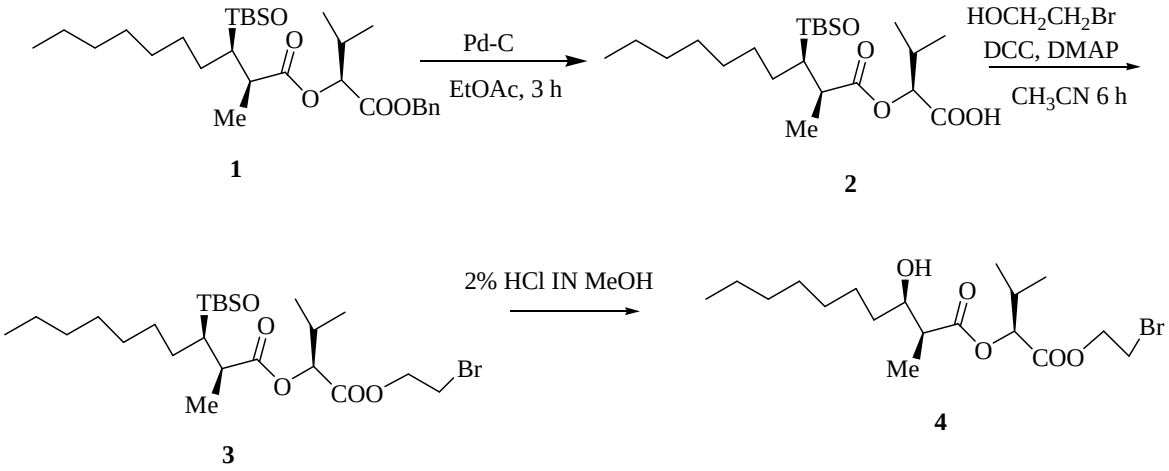
The structure of Hapalosin was assigned by Moore et al through spectroscopic and degradation studies. Besides this important MDR – reversing activity, one of the intriguing features of Hapalosin is that the molecule exists as a mixture of two conformers17, which has been proved by ^{1}H and ^{13}C NMR spectroscopy. Perhaps, only one of these conformers is responsible for the potent MDR-reversing activity. The interesting biological profile of this molecule and the level of interest in its chemistry prompted us to take up the total synthesis.

Results and Discussion:

Synthesis of 3R-[4'S-(Benzy1oxycarbony1-methy1-amino)-3'R-(tert.buty1dimethylsilanyloxy)-5'-pheny1-pentanoyloxy]-2S-methy1-decanoic acid-1"S-(2-bromo-ethoxycarbonyl)-2"-methy1-propy1 ester (6):

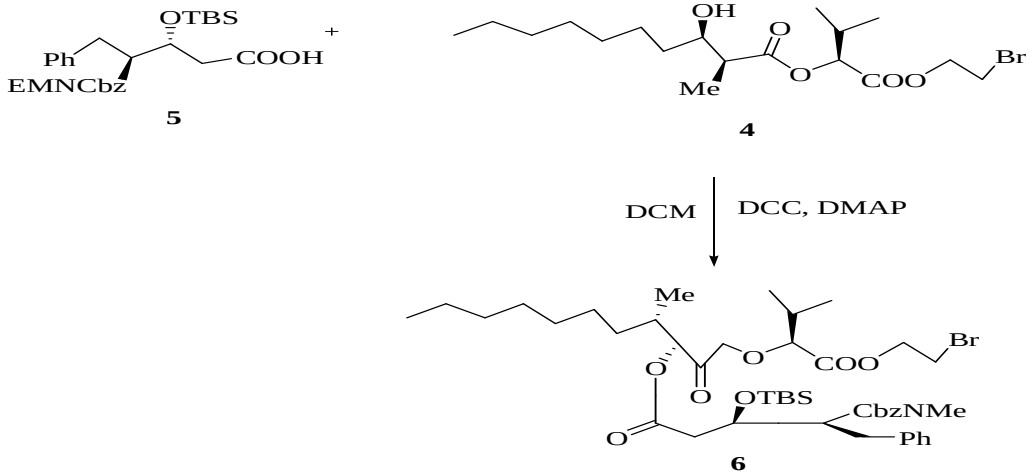
To a stirred solution of 3R-Hydroxy-2S-methy1-decanoic acid - 1' S-(2-bromethoxycarbony1)-2-methy1 - propy1 ester (4)in THF and of water were added freshly activate zinc dust and sodium iodide The mixture was refluxed for 8 h to give 3R-[4S-(Benzy1oxyearbony1-methy1-amino)-3R-(tert.buty1dimethylsilanyloxy)-5'-pheny1-pentanoyloxy]-2S-methy1-decanoic acid-1" S-carboxy-2-methy1-propy1 ester.

Scheme-1



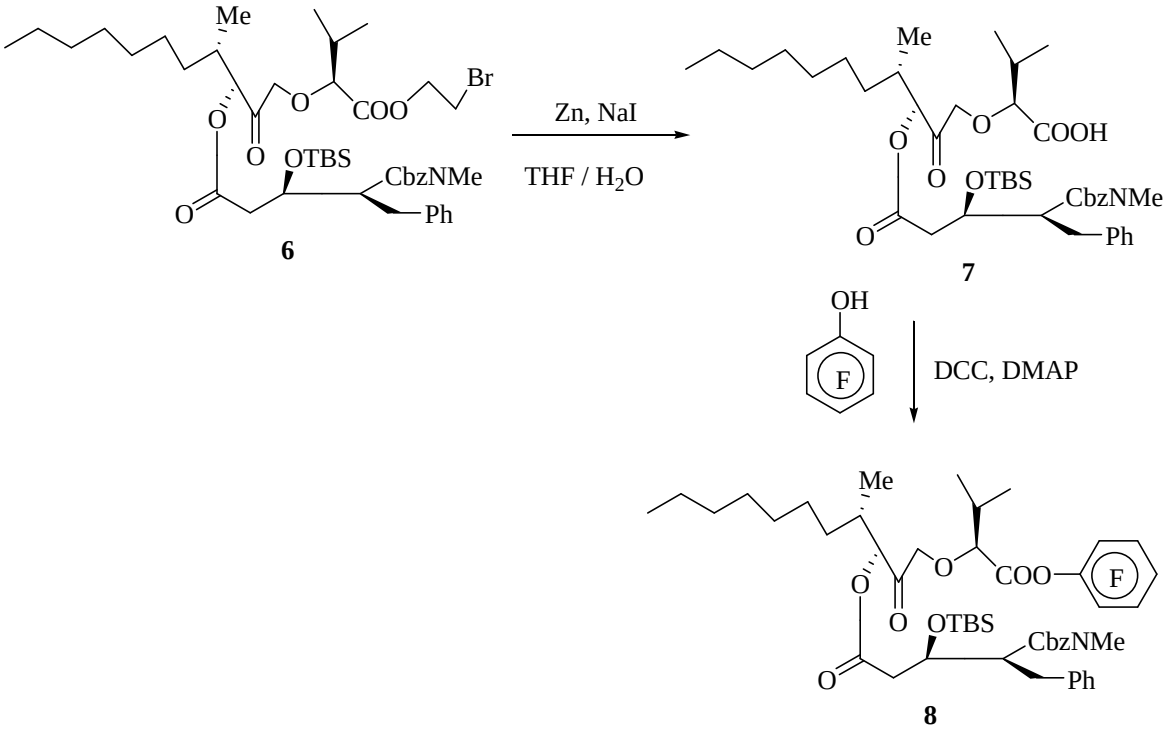
1 **2** **3** **4**

Scheme-2



5 **4** **6**

Scheme-3



Experimental Section:

General: - Melting points were determined in a sulfuric acid-bath and are uncorrected. IR spectra were recorded in KBr on a Shimadzu 435 spectrometer, ^{1}H NMR spectra on a Varian Gemini 200 MHz spectrometer with TMS as an internal standard and mass spectra on a Perkin Elmer Hitachi RDO-62 and MS-30 instrument.

i. General procedure for the synthesis 3R-(tert.Buty1dimethylsilanyloxy)-2S-methy1-decanoic acid-1'-S-benzyloxycarbony1-2-methy1-propy1ester (1):

To a mixture of compound (2S, 3R)-3-(tert.buty1dimethy1silanyloxy)-2-methy1decanoic acid (2.1 g, 6.6 mmol) and compound Benzy1 (2S)-2-hydroxy-3-methy1butanoate (1.38 g, 6.6 mmol) in dry DCM, at 0^{0}C, N,N-deimethylamino pyridine (0.081 g, 0.6 mmol) was added. To this mixture N,N-dicyclohexy1carbodiimide (1.65 g, 7.9mmol) dissolved in DCM was also added slowly over a period of 1h. After stirring the reaction mixture at the room temperature for 12h, DCM was removed. Diethy1 ether was then added to it and the solid (urea) separated

was filtered off. Evaporation of diethy1 ether gave the crude product. This residue was purified by column chromatography using 5% ethy1 acetate/hexane as eluent to give compound **1** as syrup (2.3 g, 68.5%).

$[\alpha]_D$ -16.50 (c=1.15 in $CHCl_3$).

^{1}H NMR ($CDC1_3$, 200 MHz): 7.32 (s, 5H, aromatic), 5.15 (dd, J=9.36 and 14.89 Hz, 2H, -CH_2Ph), 4.80 (d, J=4.26 Hz, 1H, -$CHCO_2$Bn), 4.00 (q, J=5.10 and 9.36 Hz, 1H, -CHOSi), 2.70-2.55 (m, 1H, CH_3-CH-CO-), 2.30-2.15 (m, 1H, -CH(CH_3)2) 1.60 - 1.45 (m, 2H-CH_2CH(OSi), 1.30 (s, 10H, -(CH_2)5CH_3), 1.20 (d, J=6.38 Hz, 3H, CH_3-CH-CO-), 1.02-0.85 (m, 18H, tBuSi, (CH_3)2 CH- and CH_3 (CH_2)6-), 0.05 (2s, 6H, (CH_3)2Si).

FAB MS: 507 (MH^+).

HRMS: Calculated for $C_{29}H_{51}O_5Si$ (MH^+) 507.3505, found 507.3505.

ii. (S)-1'-Carboxy-2'-methy1propy1 (2S, 3R)-3(tert.butyidimethylsilanyloxy)-2-me-thyldecanoate (2):

Debenzylation of compound **1** (0.75 g, 1.5 mmol) was done, with 10% palladium on charcoal (100 mg) in methanol (5 ml) under hydrogen at ambient temperature for 3h to give compound **2** (0.58 g, 94%) as a syrup.

^{1}H NMR ($CDC1_3$, 200 MHz): 4.82 (bs, 1H, -$CHCO_2$H), 4.00-3.88 (m, 1H, -CH-OSi), 2.70-2.52 (m, 1H, CH_3-CH-CO), 2.38-2.15 (m, 1H, -CH(CH_3)$_2$), 1.50-1.40 (m, 2H, -CH_2-CH(OSi)), 1.23 (s, 10H, -(CH_2)5CH_3), 1.14 (d, J=6.74, 3H, CH_3-CH-CO), 1.00 (bs, 6H, (CH_3)2-CH-), 0.85 (s, 12H, tBuSi and CH_3(CH_2)6-), 0.02 (2xs, 6H (CH_3)2Si).

iii. 3R-(tert. Buty1dimethy1silanyloxy)-2S-methy1decanoic acid -1' S-(2-bromoethoxy-carbony1)-2-methy1propy1 ester (3):

To a stirred solution of acid **2** (0.52 g, 1.3 mmol) and 2-bromeothanol (0.1 ml, 1.5 mmol) in acetonitrile (6 ml) at 0^0C, pyridine (0.2 ml, 0.25 mmol) and N, N-dicyclohexy1 carbodiimide (0.31 g, 1.5 mmol) were added. The mixture was stirred at 0^0C, for 6h. the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography using 5% ethy1 acetate/hexane mixture as eluent to give bromo ester **3** (0.41 g, 63%).

^{1}H NMR ($CDC1_3$, 200 MHz): 4.73 (d, J=4.21 Hz, 1H, -$CHCO_2$(CH_2)$_2$Br), 4.50-4.34 (m, 1H, -CO_2CH_2-), 3.98 (q, J=4.63 and 9.26 Hz, -CHOSi), 3.48 (t, J=6.32 Hz, 2H, -CH_2Br), 2.70-2.52 (m, 1H, CH_3-CH-CO), 2.32-2.14 (m,

1H, $CH(CH_3)_2$, 1.56-1.40 (m, 2H, $-CH_2CH(OSi)$), 1.26 (bs, 10H,$-(CH_2)_5CH_3$). 1.18 (d, J=6.32 Hz, 3H, CH_3-CH-CO), 1.00 (2xd, J=6.32Hz, 6H, $(CH_3)_2CH$-), 0.86 (bs, 12H, tBuSi and $CH_3(CH_2)_6$), 0.04 (2xs, 6H, $(CH_3)_2Si$).

iv. 3R-Hydroxy-2S-methy1-decanoic acid - 1' S-(2-bromethoxycarbony1)-2'-methy1 - propy1 ester (4):

Desilylation of compound **3** (0.37 g, 0.7 mmol) was carried out by the procedure described earlier using 2% hcl in methanol. The free hydroxyl compound **4** was eluted, from a silica gel column using ethy1 acetate: light petroleum (1:9) as syrup (0.267 g, 92%).

^{1}H NMR ($CDC1_3$, 200 MHz): 4.82 (d, J=4.04 Hz, 1H, $-CHCO_2(CH_2)_2Br$), 4.50- 4.26 (m, 2H, $-CO_2CH_2$-), 4.00 - 3.90 (m, 1H, -CH-OH), 3.42 (t, J=5.25 Hz, 2H , ICH_2Br), 2.63-2.50 (m 1H, CH_3-CH-CO), 2.30 - 2.12 (m, 1H, -$CH(CH_3)2$), 1.50-1.10 (m, 12H, $-(CH_2)6CH_3$), 1.06 (d, J=6.06 HZ, 3H, CH_3-CH-CO-), 0.94 (t, J=6.06 Hz, 6H, $(CH_3)2CH$-), 0.80 (bt, J=6.0 Hz, 3H, CH_3 $(CH_2)6$-).

v. 3R-[4'S-(Benzyloxycarbony1-methy1-amino)-3'R-(tert.buty1dimethyisilanyloxy)- 5'-pheny1-pentanoyloxy]-2S-methy1-decanoic acid - 1"S-(2-bromo-ethoxycarbony1)-2"- methy1-propy1 ester (6):

Compound **6** was prepared by esterification of 3R-(tert.Buty1dimethy1silanyloxy)-4S-(methy1benzyloxy carbon1 amino)-5-pheny1pentanoic acid (0.27 g, 0.6 mmol) with alcohol **4** (0.22 g, 0.5 mmol) by the procedure described earlier, using N, N-dimethylamino pyridine (0.007 g, 0.06 mmol) and N, N-dichlohexy1carbodiimide (0.154 g, 0.7 mmol). The ester **6** was purified by column chromatography using 10% ethy1 acetate/hexane as eluent (0.063 g, 13.6%).

^{1}H NMR ($CDC1_3$, 200 MHz): 7.40-7.10 (m, 10H, aromatic), 5.30-5.10 (m, 1H, C_3-H), 5.05 (s, 2H, $-CH_2$ of Cbz), 4.80 (d, J=4.20 Hz, 1H, C1"-H), 4.60-4.30 (m, 4H, C_3-H, C_4-H and $-CO_2CH_2$), 3.50 (t, J=5.89 Hz, 2H, $-CH_2Br$), 3.17-2.70 (2xm, 6H, C_2-H, -N-CH_3 and C5-2H), 2.65-2.55 (m, 2H, C_2-2H), 2.40-2.20 (m, 1H, C_2, -H), 1.75-1.45 (m, 2H, C_4-2H), 1.40-1.15(m, 13H, C_5-C_9-$(CH_2)5$ and C_2-CH_3), 1.05 (2xd, J=6.32 Hz, 6H, C_2-$(CH_3)2$), 0.90 (bs, 12H, tBuSi and C_{10}-3H), 0.10 (2xs, 6H, $(CH_3)2$ Si).

vi. 3R-[4'S-(Benzy1oxyearbony1-methy1-amino)-3'R-(tert.buty1dimethylsilanyloxy)-5'-pheny1-pentanoyloxy]-2S-methy1-decanoic acid-1"S-carboxy-2"-methy1-propy1 ester (7):

To a stirred solution of **6** (0.05g, 0.06 mmol) in 6 ml of THF and 5ml of water were added freshly activated zinc dust (0.038 g, 0.6 mmol) and sodium iodide (0.044 g, 0.3 mmol). The mixture was refluxed for 8 h. The white precipitate separated was filtered off and the cake was washed well with THF (4X5 ml). The filtrate was concentrated in vacuuo, the residue was taken up in DCM, dried over anhydrous Na_2SO_4 and purified by column chromatography using 1:1 mixture of ehy1 acetate / hexane as eluent (0.029 g, 66%).

^{1}H NMR ($CDC1_3$, 200 MHz): 7.40-7.10 (m, 10H, aromatic), 5.40-5.20 (m, 1H, C_3-H), 5.05 (s,2H,-CH_2 of Cbz), 4.60-4.40 (m, 2H, C1 -H and C_3-H), 4.05-3.80 (m, 1H, C_4-H), 3.10-2.50 (bm, 8H, C_2-H, C_2-2H-, C_5-2H- and N-CH), 2.40-2.18 (m, 1H, C2' -H), 1.75-1.50 (m, 2H, C_4-2H-), 1.30 (bs, 13H, -C2-CH_3 and C5-C9(CH_2), 0.90 (bs, 18H, tBuSi, C2' -(CH_3) and C10-3H), 0.10 (2xs, 6H, (CH_3)2Si).

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