Synthesis of 6, 4''-Di-O-methylerythromycin A and its derivatives

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Abstract. 6,4''-Di-O-methylerythromycin A is a relative substance of clarithromycin, which is due to incomplete protection of 4''-OH during the methylation process. In this study, 6,4''-Di-O-methylerythromycin A(5) and its derivatives including 2'-O-TMS-6-O-methylerythromycin A 9-[(1-ethoxy-1-methylethyl)oxime](2), 2'-O-TMS-6,4''-Di-O-methylerythromycin A 9-[(1-ethoxy-1-methylethyl)oxime](3) and 6,4''-Di-O-methylerythromycin A 9-oxime(4) was synthesized from 2',4''-O-bis(TMS)-6-O-methylerythromycin A 9-[(1-ethoxy-1-methylethyl)oxime](1). The structure of each compound was identified by ^{1}H-NMR and ^{13}C-NMR..

Introduction

Clarithromycin (6-O-methylerythromycin A), one of the best known macrolide antibiotics, has strong antibacterial activity and good pharmacokinetic properties [1]. Clarithromycin can inhibit *H.pylori*, which makes it widely used in clinic [2]. Clarithromycin was synthesized after oximation, etherification, silylation, methylation and de-protection with erythromycin A as the starting material in industrial production [3] [4] [5] [6]. There are various relative substances in clarithromycin products [7], whose type and content can be the direct indicator of the product's quality.

Group protection and region-selective methylation are the most important issues in industrial production of clarithromycin [8]. TMS is used as protecting group of 2'-OH and 4''-OH, derivatives of 6,4''-Di-O-methylerythromycin A will form if 4''-OH isn't protected completely. Furthermore, methylation reaction is accompanied by de-protection reaction. 4''-OTMS is easy to remove under alkaline conditions to form derivatives of 4''-O-methylerythromycin A in methylation procedure [9]. In this article, we synthesized the clarithromycin relative substance 5 and its derivatives 2, 3 and 4 with 1 as the starting material, the reaction pathway can be seen in Fig.1. Purification of each compound was performed by column chromatography and their structures were determined by ^{1}H-NMR and ^{13}C-NMR.

Reagents and Instruments

2',4''-O-bis(TMS)-6-O-methylerythromycin A 9-[(1-ethoxy-1-methylethyl)oxime] was provided by Zhejiang Guobang Pharmaceutical Company. All Reagents and solvents were purchased from Beijing Chemical Reagents Company.^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ on ARX500 spectrometer. HPLC was carried out on a 4.6×250mm column of Purospher STAR LP RP-18e (5 um) with 0.067M KH_2PO_4 (pH4.0) buffer/acetonitrile =55/45.

Synthesis of 2'-O-TMS-6-O-methylerythromycin A 9-[(1-ethoxy-1-methylethyl) oxime](2)

A solution of 1(5.0g, 5.03mmol) in 50ml of THF was treated with 4ml of water and 0.2g 85% KOH powder at 25℃. The solution was monitored by TLC (petroleum ether/ethyl acetate/ diethylamide, 10/1/1). The resultant reaction was added in 20 ml water and then basified (pH=9) using NH_4Cl solid. The mixture was extracted with petroleum ether and the organic layer was washed successively with saturated brine and water and dried over $MgSO_4$. The solvent was

evaporated in vacuo and the residue was crystallized from petroleum ether to afford 3.5g (75.5%) of 2 as colorless crystals:

^{1}H-NMR (500MHz, $CDCl_3$) δ(ppm) : 0.12 (s, 9H, 2'-OTMS), 0.85 (t, 3H, H-15), 0.96 (d, 3H, 8-CH3), 1.03 (d, 3H, 4-CH3), 1.24 (s, 3H, 3''-CH3), 1.29 (d, 3H, 5''-CH3), 1.41 [s, 6H, -O-C(CH3)2-O-], 1.46 (m, 1H, H-14), 1.48 (m, 1H, H-4'), 1.50 (s, 3H, 6-CH3), 1.52 (m, 1H, H-2''), 1.57 (m, 1H, H-7), 1.86 (m, 1H, H-14), 1.94 (m, 1H, H-4), 2.19 (m, 1H, 4''-OH), 2.25 [s, 6H, 3'-N $(CH_3)_2$], 2.33 (d, 1H, H-2''), 2.59 (m, 1H, H-10), 2.84 (m, 1H, H-2), 3.00 (t, 1H, H-4''), 3.07 (s, 3H, 6-OCH_3), 3.18 (m, 1H, H-2'), 3.29 (s, 1H, 12-OH), 3.33 (s, 3H, 3''-OCH_3), 3.47 (m, 2H, -O$\underline{C}H_2CH_3$), 3.51 (s, 1H, H-5'), 3.64 (d, 1H, H-5), 3.70 (m, 1H, H-8), 3.75 (s, 1H, H-11), 3.80 (d, 1H, H-3), 3.99 (m, 1H, H-5''), 4.33 (d, 1H, H-1'), 4.56 (s, 1H, 11-OH), 4.92 (d, 1H, H-1'') , 5.10 (dd, 1H, H-13).

^{13}C-NMR (500MHz, $CDCl_3$)δ(ppm): 1.06 (2'-OTMS) , 9.65 (4-CH_3), 10.64 (C-15), 15.11 (12-CH_3), 15.62 (8-CH_3), 16.14 (2-CH_3), 18.87 (10-CH_3), 18.89 (5''-CH_3), 20.12 (6- CH_3), 21.29 (C-14), 21.57 (5'-CH_3), 21.64 (3''-CH_3), 24.89 (C-8), 24.12, 24.67 [-O-C ($\underline{C}H_3$)2-O-], 29.45 (C-4'), 33.13 (C-10), 34.96 (C-2''), 37.70 (C-7), 39.71 (C-4), 41.00 [3' -N$(CH_3)_2$], 45.45 (3''-OCH3), 50.99 (6-OCH3), 56.68 (-O$\underline{C}H_2CH_3$), 65.68 (C-3'), 65.78 (C- 5''), 67.92 (C-5'), 70.10 (C-11), 72.65 (C-2'), 73.24 (C-3''), 73.95 (C-12), 76.86 (C-13), 77.93 (C-4''), 78.07 (C-3), 78.83 (C-6), 78.96 (C-5), 95.78 (C-1''), 102.85 (C-1'),102.87 [-O-$\underline{C}$(CH3)2-O-], 170.25 (C-9), 175.83 (C-1).

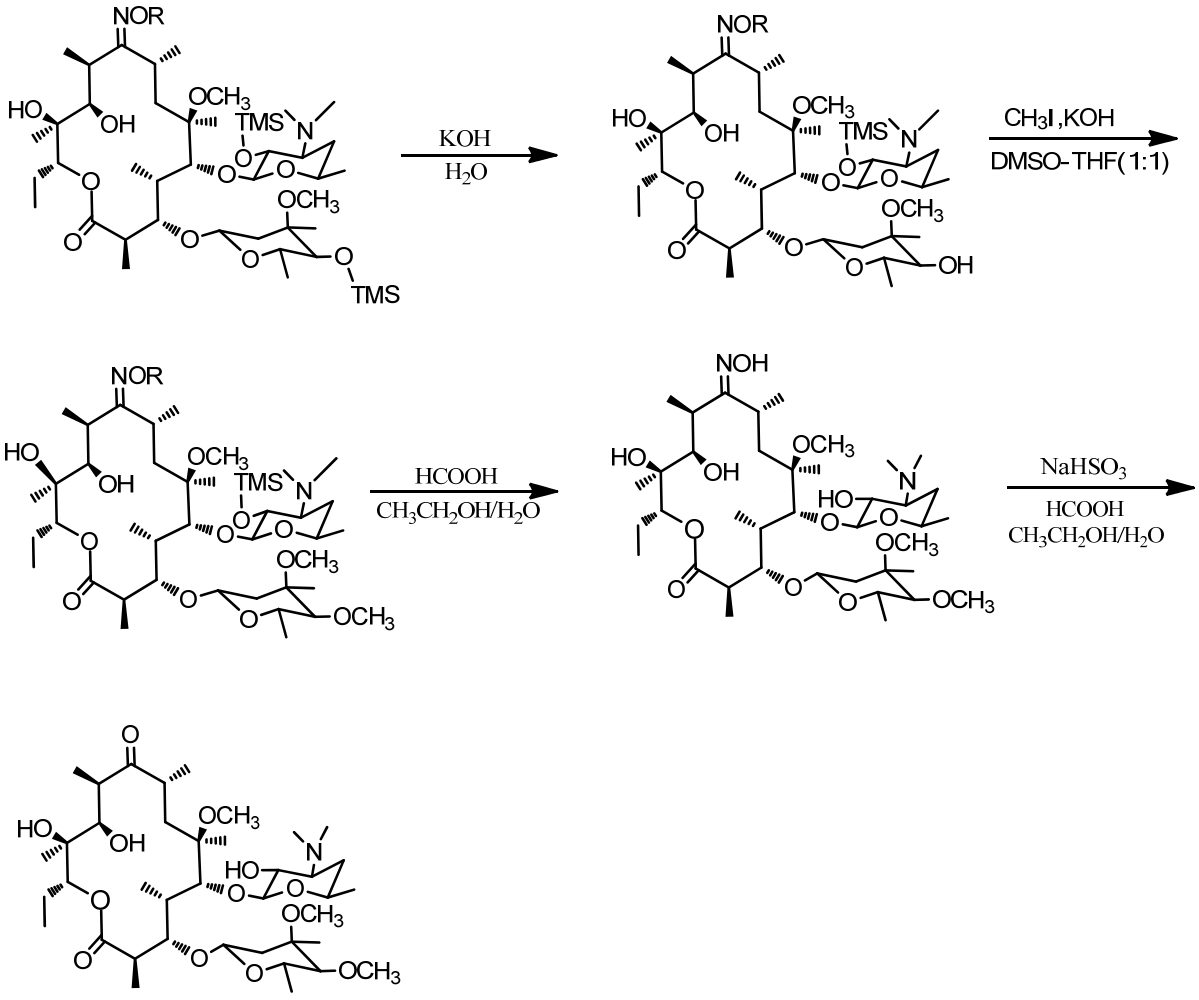

Fig.1 Synthesis pathway of 6, 4''-Di-O-methylerythromycin A

Synthesis of 2'-O-TMS-6, 4''-Di-O-methylerythromycin A 9-[(1-ethoxy-1-methylethyl)-oxime] (3)

To a solution of 2 (1.0g, 1.08mmol) in 20ml of a mixture of DMSO/THF (1/1) was added CH_3I (0.5ml, 7.92mmol) and then 85% KOH powder (0.3g, 4.58mmol), and the resulting mixture was stirred at room temperature for 3 hours. The solution was added 20ml of water; stirring was continued for 5 minutes and then extracted with petroleum ether (40ml + 20ml). The organic layer was combined and successively washed with water and saturated brine and then dried over anhydrous $MgSO_4$. The solvent was evaporated in vacuo to give 0.7g (69%) of 3 as colorless foam:

^{1}H-NMR (500MHz, $CDCl_3$) δ(ppm) : 0.09 (s, 9H, 2'-OTMS), 0.83 (t, 3H, H-15), 1.06 (d, 3H, 8-CH3), 1.11 (d, 3H, 4-CH3), 1.27 (s, 3H, 3''-CH3), 1.29 (d, 3H, 5''-CH3), 1.42 [s, 6H, -O-C(CH3)2-O-], 1.45 (m, 1H, H-14), 1.50 (m, 1H, H-4'), 1.50 (s, 3H, 6-CH3), 1.51 (m, 1H, H-2''), 1.53 (m, 1H, H-7), 1.92 (m, 1H, H-14), 1.97 (m, 1H, H-4), 2.20 [s, 6H, 3'-N $(CH_3)_2$], 2.36 (d, 1H, H-2''), 2.50 (m, 1H, H-3'), 2.63 (m, 1H, H-10), 2.91 (m, 1H, H-2), 3.10 (s, 3H, 6-OCH_3), 3.14 (m, 1H, H-2'), 3.14 (s, 1H, 12-OH), 3.32 (s, 3H, 3''-OCH_3), 3.48 (m, 2H, -O$\underline{CH_2}CH_3$), 3.53 (s, 3H, 4''-OCH_3), 3.56 (s, 1H, 11-OH), 3.56 (s, 1H, H-5'), 3.68 (s, 1H, H-11), 3.69 (d, 1H, H-5), 3.70 (m, 1H, H-8), 3.80 (d, 1H, H-3), 4.22 (m, 1H, H-5''), 4.36 (d, 1H, H-1'), 4.90 (d, 1H, H-1'') , 5.01 (dd, 1H, H-13).

Synthesis of 6,4''-Di-O-methylerythromycin A 9-oxime(4)

To a solution of 3 (0.7g, 0.75mmol) in a mixture of 20ml of ethanol and water (1/1) was added to formic acid (pH4.0) and then stirred under reflux. The reaction was monitored by TLC (petroleum ether/ethyl acetate/diethylamine, 10/4/1). The resulting solution was basified (pH9-10) using 4N NaOH aqueous and the precipitation was washed with water. The crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate/diethylamine, 10/1/0.5) to afford 0.3g (51.5%) of 4 as a colorless form:

^{1}H-NMR (500MHz, $CDCl_3$) δ(ppm) : 0.83 (t, 3H, H-15), 0.99 (d, 3H, 8-CH3), 1.10 (d, 3H, 4-CH3), 1.26 (s, 3H, 3''-CH3), 1.30 (d, 3H, 5''-CH3), 1.45 (m, 1H, H-14), 1.48 (s, 3H, 6-CH3), 1.54 (m, 1H, H-4'), 1.59 (m, 1H, H-2''), 1.62 (m, 1H, H-7), 1.94 (m, 1H, H-14), 2.05 (m, 1H, H-4), 2.31 [s, 6H, 3'-N $(CH_3)_2$], 2.35 (d, 1H, H-2''), 2.45 (m, 1H, H-3'), 2.58 (m, 1H, H-10), 2.95 (m, 1H, H-2), 3.03 (t, 1H, H-4''), 3.07 (s, 3H, 6-OCH_3), 3.10 (s, 1H, 12-OH), 3.22 (m, 1H, H-2'), 3.33 (s, 3H, 3''-OCH_3), 3.51 (s, 1H, 2'-OH), 3.51 (s, 1H, H-5'), 3.65 (s, 3H, 4''-OCH_3), 3.69 (d, 1H, H-5), 3.75 (d, 1H, H-3) 3.86 (m, 1H, H-8), , 4.04 (m, 1H, H-5''), 4.48 (d, 1H, H-1'), 4.92 (d, 1H, H-1'') , 4.98 (dd, 1H, H-13).

^{13}C-NMR (500MHz, $CDCl_3$) δ(ppm): 9.30 (4-CH_3), 10.62 (C-15), 15.51 (12-CH_3), 16.02 (8-CH_3), 17.20 (2-CH_3), 18.71 (10-CH_3), 18.71 (5''-CH_3), 19.88 (6-CH_3), 20.65 (C-14), 21.50 (5'-CH_3), 21.54 (3''-CH_3), 25.54 (C-8), 29.36 (C-4'), 33.39 (C-10), 35.07 (C-2''), 36.34 (C-7), 38.44 (C-4), 40.37 [3' –N $(CH_3)_2$], 45.04 (C-2), 49.45 (3''-OCH3), 50.44 (6-OCH3), 62.18 (4''-OCH_3), 65.61 (C-3'), 65.98 (C- 5''), 68.55 (C-5'), 71.16 (C-11), 71.16 (C-2'), 72.80 (C-3''), 75.59 (C-12), 77.79 (C-13), 77.91 (C-4''), 78.70 (C-3), 79.35 (C-6), 79.50 (C-5), 96.28 (C-1''), 102.56 (C-1'), 166.60 (C-9), 175.83 (C-1).

Synthesis of 6, 4''-Di-O-methylerythromycin A(5)

To a solution of 4 (0.3g, 0.39mmol) in a mixture of 20ml of ethanol and water (1/1) was added to $NaHSO_3$ and formic acid (pH4.0) and then stirred under reflux. The reaction was monitored by TLC (petroleum ether/ethyl acetate/diethylamine, 10/ 4/1). The resulting solution was basified (pH9-10) using 4N NaOH aqueous and the precipitation was washed with water and afford 0.2g of 5(68.0%) as colorless crystals (the purity was 77.4% determined by HPLC):

^{1}H-NMR (500MHz, $CDCl_3$) δ(ppm) : 0.85 (t, 3H, H-15), 1.11 (d, 3H, 8-CH3), 1.12 (d, 3H, 4-CH3), 1.28 (s, 3H, 3''-CH3), 1.30 (d, 3H, 5''-CH3), 1.41 (s, 3H, 6-CH3), 1.53 (m, 1H,

H-14), 1.57 (m, 1H, H-4'), 1.65 (m, 1H, H-2''), 1.78 (m, 1H, H-7), 1.86 (m, 1H, H-14), 2.07 (m, 1H, H-4), 2.27 [s, 6H, 3'-N(CH_3)$_2$], 2.38 (d, 1H, H-2''), 2.54 (m, 1H, H-3'), 2.64 (m, 1H, H-10), 2.98 (m, 1H, H-2), 3.04 (m, 1H, H-4''), 3.13 (s, 3H, 6-OCH_3), 3.15 (s, 1H, 12-OH), 3.17 (m, 1H, H-2'), 3.32 (s, 3H, 3''-OCH_3), 3.45 (s, 1H, H-5'), 3.45 (s, 1H, 2'-OH), 3.54 (s, 3H, 4''-OCH3), 3.67 (d, 1H, H-5), 3.69 (m, 1H, H-3), 3.77 (m, 1H, H-8) , 4.22 (m, 1H, H-5''), 4.51 (m, 1H, H-1'), 4.91 (d, 1H, H-1'') , 5.43 (d, 1H, H-13).

Results

Absence of 4''-OTMS in compound 2 was indicated by the absence of corresponding absorptions in the ^{1}H-NMR and ^{13}C-NMR spectra (15ppm and 0.86ppm, respectively). The ^{1}H-NMR spectrum also showed a δ value of 2.19ppm for 4''-OH, suggesting that 4''-OTMS has been taken off in compound 2, it can be determined that compound 2 was 2'-O-TMS-6-O-methylerythromycin A 9-[(1-ethoxy-1-methylethyl)oxime].

The ^{1}H-NMR spectrum showed that the peak at 2.19ppm (m, 1H, 4''-OH) had disappeared and a new peak at 3.53ppm (s, 3H, 4''-OCH_3) had appeared in compound 3 compared to compound 2, indicating that compound 3 is 2'-O-TMS-6,4''-Di-O-methylerythromycin A 9- [(1-ethoxy-1-methyl-ethyl)oxime].

In ^{1}H-NMR spectrum, chemical absorptions of 0.09, 1.42 and 3.48ppm were vanished, suggesting that 2'-OTMS and -O-C(CH_3)$_2$-O-CH_2CH_3 were taken off from compound 3. In ^{13}C-NMR spectrum, disappearance of corresponding chemical absorptions of 2'-OTMS, -O-<u>C</u>-(CH_3)$_2$-O-, -O-C(<u>C</u>H_3)$_2$ -O-, -O-<u>C</u>H_2CH_3 (δ value : 1.06, 102.87, 24.12 and 26.57, 56.68ppm, respectively) with other chemical shifts basically unchanged in compound 4 compared to compound 3, suggesting that compound 4 was 6,4''-Di-O-methylerythromycin A 9-oxime.

Discussion

In the synthesis of 6, 4''-Di-O-methylerythromycin A and its derivatives, temperature is a crucial issue. The reaction proceeds very slowly and is incomplete when the temperature is too low, while the temperature is too high, much more side effects will occur. For instance, 1 was no longer reduced after one week at 10℃, suggesting that the reaction was terminated but the raw material was not completely reacted. However, it would generate a lot of 6-O-methylerythromycin A 9-[(1-ethoxy-1-methylethyl) oxime] instead of 2 when the temperature was 40℃; 2 generated a variety of byproducts rather than 3 at room temperature in the methylation reaction.

Reaction time is very important on synthesis of 6, 4''-Di-O-methylerythromycin A and its derivatives. 1 generated a large number of 2 and a small amount of 6-O-methylerythromycin A 9-[(1-ethoxy-1-methylethyl)oxime] after 3 days at 25℃ but a lot of 2 would be converted into 6-O-methylerythromycin A 9-[(1-ethoxy-1-methylethyl)oxime] after 5 days. It would generate a lot of unknown byproducts when the reaction time was too long in the reaction from 4 to 5, which resulting in the amount of target product reduced. Therefore, it is extremely important to control reaction time.

Conclusion

6,4''-Di-O-methylerythromycin A and its derivatives were synthesized in 18.2% overall yield from 2',4''-O-bis(TMS)-6-O-methylerythromycin A 9-[(1-ethoxy-1-methylethyl)oxime] in this study and their structures were confirmed by ^{1}H-NMR and ^{13}C-NMR. The control of temperature and reaction time is significantly important in order to get a high yield of the target products. Further studies on optimization of the reaction conditions are needed to obtain a higher yield of 6,4''-Di-O-methylerythromycin A and its derivatives.

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References:

[1] Morimoto S, Takahashi Y, Watanabe Y, et al. Chemical modification of erythromycin Ⅱ.Synthesis and antibacterial activity of O-alkyl derivatives of erythromycin A [J]. The Journal of Antibiotics, 1990, 43: 286-294.

[2] Yilmaz O, Demiray E, Clinical role and importance of fluorescence in situ hybridization method in diagnosis of H pylori infection and determination of clarithromycin resistance in H pylori eradication therapy [J]. World Journal of Gastroenterology, 2007, 13(5): 671-675.

[3] Sasaki J, Mizoue K, Moromoto S, et al. Microbial transformation of 6-O-methyl erythromycin derivatives [J]. The Journal of Antibiotics, 1988, 41: 908-915.

[4] Adachi T, Morimoto S, Kondoh H, et al. 14-hydroxy-6-O-methylerythromycins A, active metabolites of 6-O-methylerythromycin A in human[J]. The Journal of Antibiotics, 1989 ,41: 966–975.

[5] Watanabe Y, Morimoto S, Goi M, et al. Chemical modification of erythromycin Ⅻ. A facile synthesis of clarithromycin (6-O-methylerythromycin A) via 2'-silyethers of erythromycin A derivatives [J]. The Journal of Antibiotics, 1993, 36(2): 243.

[6] Watanabe Y, Masato K, Toshifumi A, et al. Chemical modification of erythromycin Ⅺ. Synthesis of clarithromycin (6-O-methylerythromycin A) via erythromycin A quaternary ammonium salt derivatives [J]. Heterocycles, 1993, 36:243-247.

[7] Sun Li-quan, Zhang Yu-wen, Fan Xin-yuan, et al. Research progress of related substances of clarithromycin [J]. Huaxue Shiji, 2012, 34(12): 1083-1088.

[8] Sun Jing-guo, Liang Jian-hua, Yao-Guowei, et al. Progress in synthesis of clarithromycin [J]. Chinese Journal of Organic Chemistry, 2002, 22(12): 951-963.

[9] Zheng Shao-jun, Bai Jing-rui, Yao Guo-wei, et al. Deprotective reaction in the synthesis of clarithromycin [J]. Fine Chemicals, 2004, 21(3): 185-187.