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Structural Studies of an Impurity Obtained During the Synthesis of Telithromycin Derivatives

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Abstract

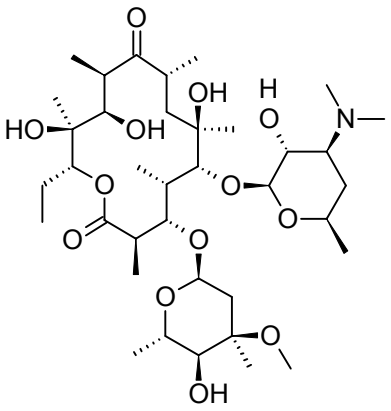
In an effort to synthesize a key intermediate, for synthesis of a variety of telithromycin derivatives a new by-product has been formed at the third stage of the synthetic scheme. The starting material, Clarithromycin, on treatment with hydrochloric acid and on benzoylation resulted in the formation of (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-14-ethyl-4,12,13-trihydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-2,10-dioxooxacyclotetradecan-6-yl 3,4,6-trideoxy-3-(dimethylamino)-2-*O*-(phenylcarbonyl)-β-D-*xylo*-hexopyranoside (**2**). Oxidation of this gave (3*R*,5*R*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-14-ethyl-12,13-dihydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-2,4,10-trioxooxacyclotetradecan-6-yl 3,4,6-trideoxy-3-(dimethylamino)-2-*O*-(phenylcarbonyl)-β-D-*xylo*-hexopyranoside (**3**), and also an unexpected by-product **4** in equivalent amounts. The O_{21}–H hydroxyl group in **3** was mesylated with dimethyl sulphoxide (DMSO) in pyridine leading to the precursor (3*R*,5*R*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-14-ethyl-12,13-dihydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-12-(methylsulfinyl)-2,4,10-trioxooxacyclotetradecan-6-yl 3,4,6-trideoxy-3-(dimethylamino)-2-*O*-(phenylcarbonyl)-β-D-*xylo*-hexopyranoside (**5**), which on further treatment with

1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in acetone and methylene chloride resulted in the formation of intermediate **6**. The by-product **4** and the intermediate **6** were isolated and characterized as (1S,2R,5R,7R,8R,9R)-2-ethyl-9-methoxy-1,5,7,9,11,13-hexamethyl-4,6-dioxo-3,15-dioxabicyclo[10.2.1]-pentadeca-11,13-dien-8-yl 3,4,6-trideoxy-3-(dimethylamino)-2-O-(phenylcarb-onyl)-β-D-xylo-hexopyranoside (**4**) and (3*R*,5*R*,6*R*,7*R*,9*R*,11*E*,13*S*,14*R*)-14-ethyl-13-hydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-2,4,10-trioxooxacyclo-tetradec-11-en-6-yl 3,4,6-trideoxy-3-(dimethylamino)-2-*O*-(phenylcarbonyl)-β-D-*xylo*-hexopyranoside (**6**) respectively by 2D NMR and single crystal X-ray diffraction.

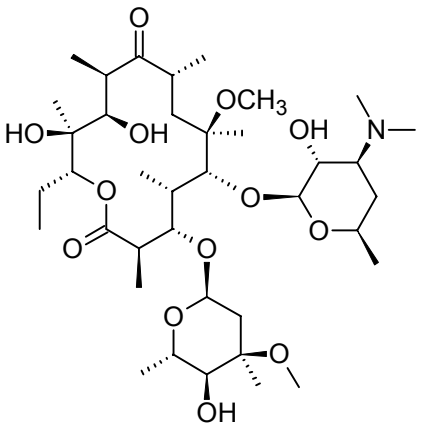
Keywords

Ketolide synthesis • By-product isolation • Characterization • 2D NMR • Single crystal X-ray structures

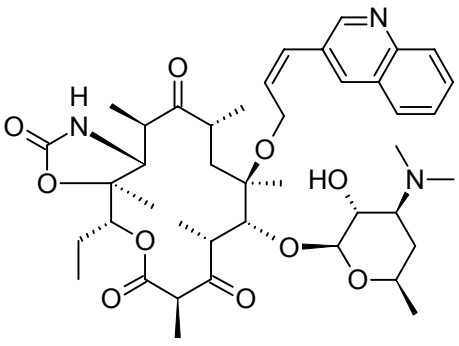
Introduction



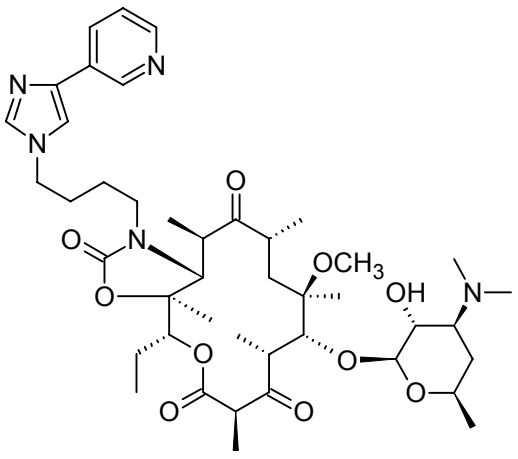
Erythromycin



Clarithromycin



Cethromycin



Telithromycin

Fig. 1. Macrolides discussed in this paper.

The macrolides including erythromycin are an old and well-known family of oral antibiotics. Their spectrum of activity covers most relevant bacterial species responsible for respiratory tract infections, especially for those caused by gram-positive pathogens [1]. Since erythromycin is quickly degraded into inactive products in the acidic medium of the stomach [2], second generation macrolides (derivatives of erythromycin) such as clarithromycin and azithromycin have been developed. Clarithromycin has favorable pharmacokinetic properties and has been used against the key respiratory tract pathogens [3]. However, second-generation derivatives of erythromycin have not shown significant efficacy against erythromycin resistant strains, this resistance has increased significantly in recent years. Hence new type of compounds called as ketolides have been synthesized as third generation macrolides. The third-generation derivatives of erythromycin, known as ketolides, Telithromycin [4, 5] and Cethromycin have potent activity against key respiratory pathogens including haemophilus influenzae and erythromycin-resistant streptococcus pneumonia (Fig.1). Our research work is focused on synthesis of various derivatives of Telithromycin. In an effort to synthesize a key intermediate **6**, for synthesis of variety of Telithromycin derivatives, a new by-product, **4** has been formed at the third stage of the synthetic scheme. Structure elucidation of both the by-product **4** and intermediate **6** are discussed in detail. The macrolides discussed in this paper are shown in Figure 1.

Results and Discussion

Hydrolysis of the clarithromycin followed by benzoylation gave compound **2** (Scheme 1). The oxidation of **2** was carried out with a modified Pfitzner–Moffat procedure [6]. The oxidation resulted in **3**, and also a by-product **4** in an equivalent amount. Similar 1,4 cyclized products have been reported by [7]. The by-product **4** was characterized by 2D-NMR and single crystal X-ray diffraction. The O_{21}–H hydroxyl group in **3** (Table 1) was mesylated with methane sulfonic anhydride $(MeSO_2)_2O$ in pyridine leading to **5** (not isolated), which on further treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in acetone and methylene chloride afforded **6**.

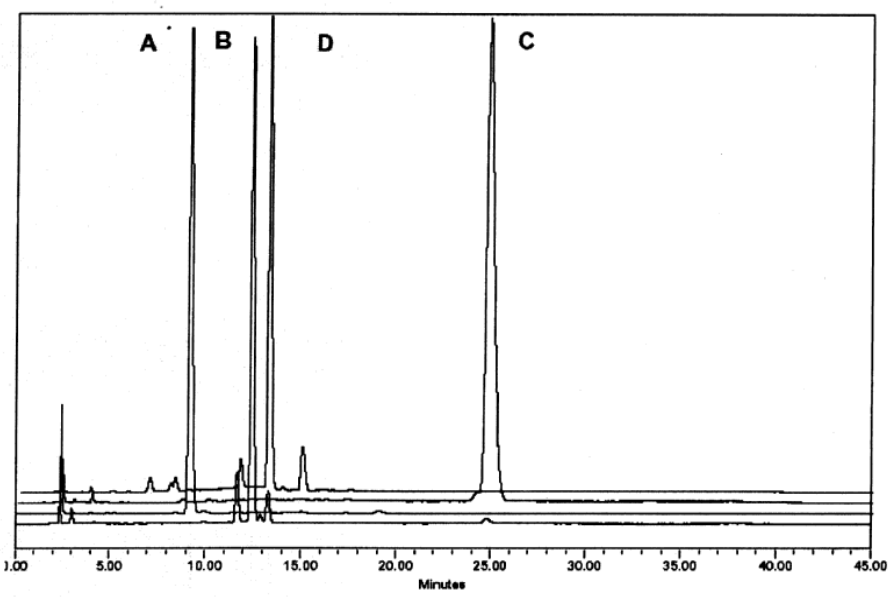
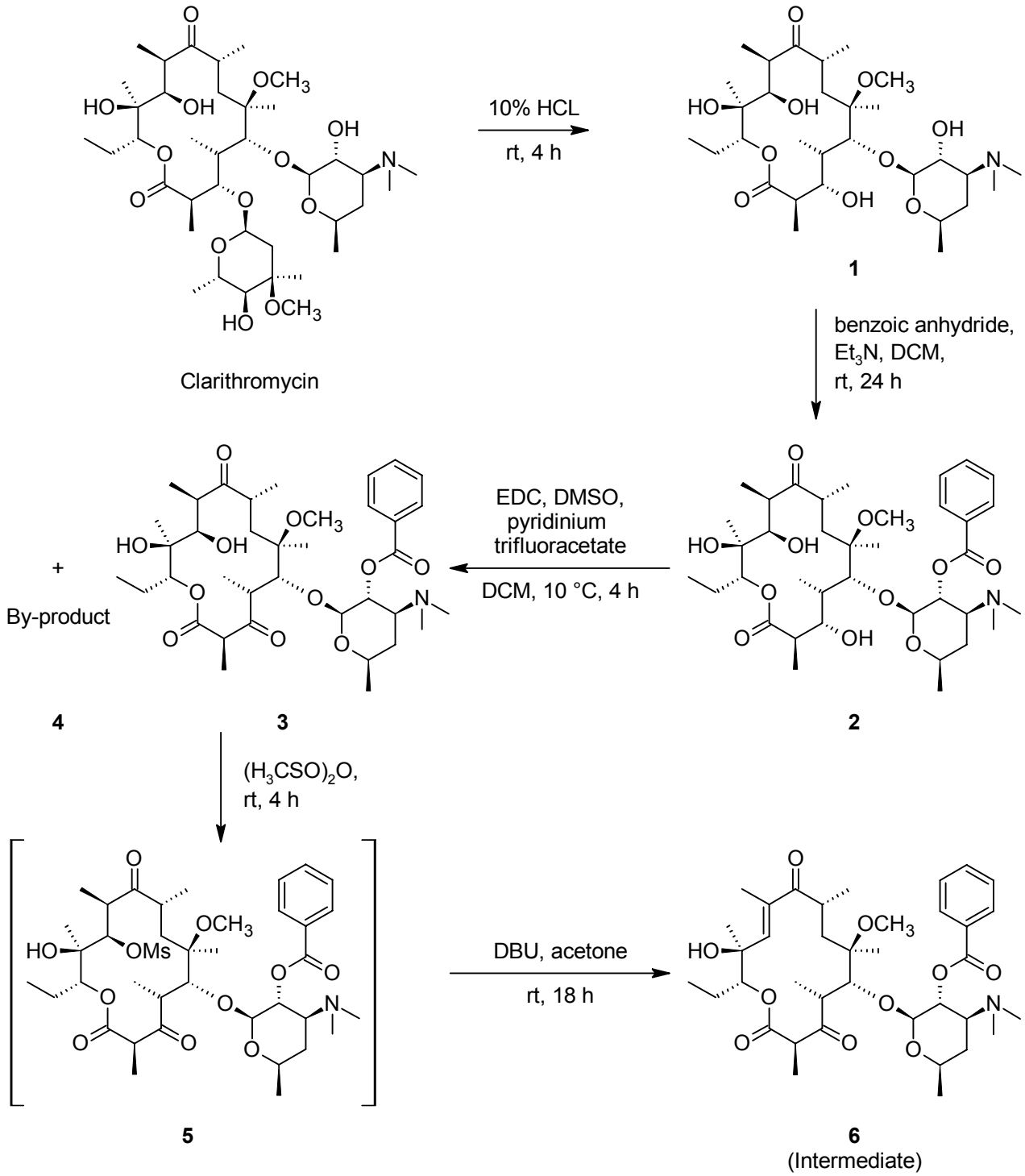


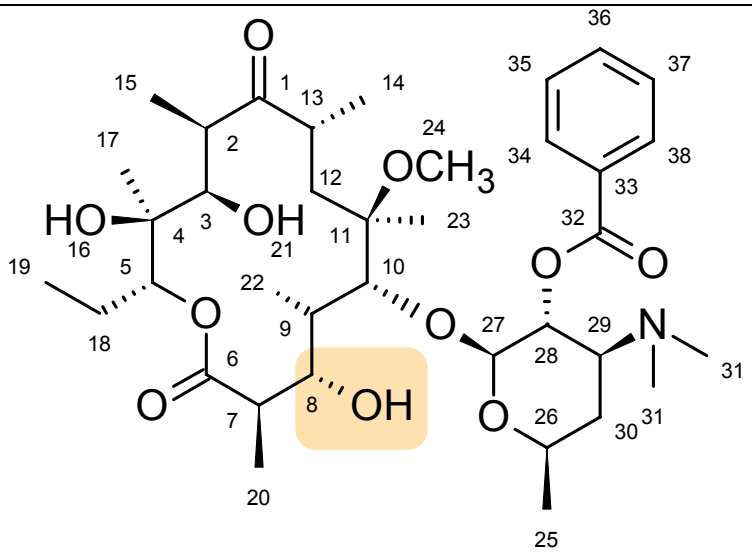
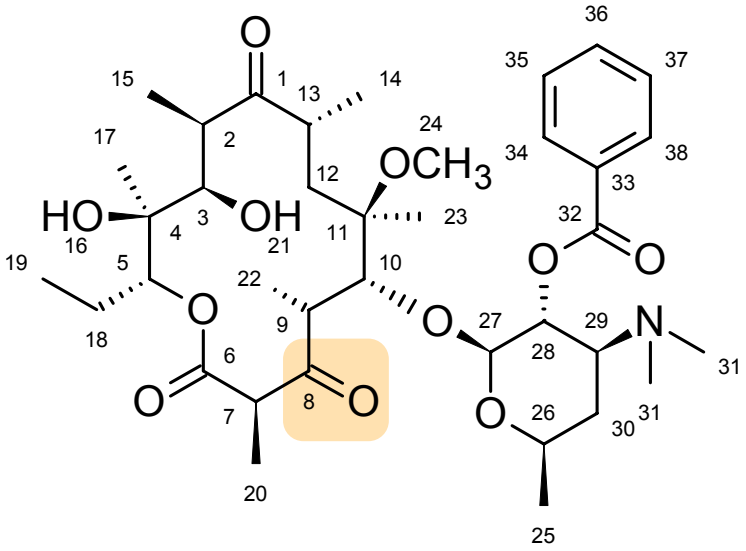
Fig. 2. Overlay of HPLC chromatograms of **2** (A), **3** (D), **4** (C) and **6** (B).



Sch. 1. Synthetic scheme for the intermediate (**6**) of Telithromycin.

The structures of macrolide (**2**) and product ketolide (**3**) with atom numbering and retention times of **2–4** and **6** by HPLC are tabulated in Table 1. The analytical HPLC chromatograms of **2**–**4** and **6** are shown in Fig. 2. The HPLC shows that the compound **2** (labeled as A in Fig. 2) eluted at retention time 9.11 min. While **3**, **4** and **6** eluted around 13.30 min, 24.73 min and 12.43 min, respectively (labeled as D, C and B in Fig. 2).

Tab. 1. HPLC retention times and structures of macrolide **2** and **3**.

S. No.	HPLC RT (min)	Structure	Remarks
1	9.11 min		**2** (ketolide)
1	13.30 min		**3** (product Ketolide)

Structure elucidation of 2 and 3

The HR-MS spectrum of **2** (Fig.S1) displayed exact mass of the protonated molecular ion at *m/z* 694.4137 (Calc. 694.4166 for $C_{37}H_{60}NO_{11}$) which corresponds to the molecular formula $C_{37}H_{59}NO_{11}$. The HR-MS spectrum of **3** displayed exact mass of the protonated molecular ion *m/z* 692.4012 (Calc 692.4010 for $C_{37}H_{58}NO_{11}$) (Fig. S2) which corresponds to the molecular formula $C_{37}H_{57}NO_{11}$. This indicates that **2** displayed protonated molecular ion at *m/z* 694 which is two atomic units (a.m.u's) more than **3** *m/z* 692 this could be due to the loss of hydrogen atoms.

Tab. 2. ^{1}H and ^{13}C NMR assignments of macrolide (**2**) and product (**3**).

Atom No.	Macrolide (2)				Product (3)			
	^{1}H	Ppm/J*	^{13}C	DEPT	^{1}H	Ppm/J*	^{13}C	DEPT
1	–	–	221.04	-	–	–	221.18	–
2	1H	2.87/m	37.60	CH	1H	2.99	46.45	CH
3	1H	3.74/d 10	69.80	CH	1H	4.34	77.33	CH
4	–	–	74.51	–	–	–	78.11	–
5	1H	5.12/m	77.01	CH	1H	5.14	79.56	CH
6	–	–	174.97	–	–	–	169.70	–
7	1H	2.56/m	44.25	CH	1H	3.72	51.18	CH
8	1H	3.44/dd 2.5	78.07	CH	–	–	205.83	–
9	1H	1.97/m	35.87	CH	1H	2.99	46.39	CH
10	1H	3.76/d 1.8	80.85	–	1H	4.33	83.29	CH
11	–	–	78.26	CH	–	–	80.14	–
12	2H	1.39/m 1.61/m	38.62	CH_2	2H	1.41/m 1.59/m	39.33	CH_2
13	1H	2.52/m	45.64	CH	–	2.60	45.16	–
14	3H	1.06/dd 7.0	17.88	CH_3	3H	1.14	18.78	CH_3
15	3H	1.07/dd 5.6	12.72	CH_3	3H	0.97/d1.5	14.72	CH_3
16	1H	3.90/br	–	–	–	3.90	–	–
17	3H	1.02/s	16.46	CH_3	3H	1.02	25.51	CH_3
18	2H	1.91/m 1.47/m	32.09	CH_2	2H	1.91m 1.69/m	24.94	CH_2
19	3H	0.80/t 7.5	10.64	CH_3	3H	0.87/t8.0	11.00	CH_3
20	3H	1.22/d 7.5	15.60	CH_3	3H	1.30/d 6.5	21.37	CH_3
21	OH	3.90br	–	–	–	3.90/br	–	–
22	3H	0.66/d 7.0	7.76	CH_3	3H	0.97	14.70	CH_3
23	3H	1.29/s	21.17	CH_3	3H	1.77/s	23.84	CH_3
24	3H	2.92/s	49.91	CH_3	3H	3.63/s	47.60	CH_3
25	3H	1.29/s	19.56	CH_3	3H	1.26/d6	22.14	CH_3
26	1H	3.61/m	68.94	CH	1H	3.60/m	69.44	CH
27	1H	4.80/d7.4	99.77	CH	1H	4.57/d 8.0	102.38	CH
28	1H	5.12/d8.8	77.00	CH	1H	5.11/dd 8.0,10.5	72.49	CH
29	1H	3.21/m	62.92	CH	1H	2.84/m	64.35	CH
30	2H	1.40/m, 1.88m	21.68	CH_2	2H	1.45/m, 1.80m	31.19	CH_2
31	6H	2.41/s	40.39	$2CH_3$'s	6H	2.81/s	64.30	$2CH_3$s
32	–	–	165.76	–	–	–	165.33	–
33	–	–	130.17	–	–	–	130.17	–
34	1H	8.05/dd 1.5	130.03	CH	1H	7.99/dd 1.5,7.5	129.91	CH
35	1H	7.36/dd 7.5	128.37	CH	1H	7.41/d7.5	128.26	CH
36	1H	7.48/t 7.5	132.85	CH	1H	7.58/t 7.5	132.74	CH
37	1H	7.34/dd 7.5	128.28	CH	1H	7.41/d7.5	128.26	CH
38	1H	7.86/dd 7.5	129.99	CH	1H	7.94/dd 1.5,7.5	129.91	CH

* Refer Tab. 1 for numbering; ** J Hz: This column gives the ^{1}H–^{1}H coupling constant in hertz, (br) broad, (dd) doublet of doublet, (s) Singlet, (t) triplet, (m) multiplet.

Tab. 3. ^{1}H and ^{13}C NMR assignments of by-product (**4**) and Intermediate (**6**).

Atom	By product (4)				Intermediate (6)			
No.	^{1}H	Ppm/J*	^{13}C	DEPT	^{1}H	Ppm/J*	^{13}C	DEPT
1	–	–	154.02	–	–	–	206.89	–
2	–	–	135.02	–	–	–	72.02	–
3	1H	5.64(s)	130.29	–	1H	6.52s	143.77	CH
4	–	–	88.05	–	–	–	72.02	–
5	1H	4.93/dd 3.0, 10.0	79.56	CH	1H	4.80m	71.27	CH
6	–	–	169.70	–	–	–	170.10	
7	1H	3.50m	51.28	CH	1H	3.80/d 7.0	49.68	CH
8	–	–	205.75	–	–	–	204.70	–
9	1H	3.69 (m)	47.13	CH	1H	1.14m	19.74	CH
10	1H	4.17/d 9.5	83.27	CH	1H	4.01d	80.40	CH
11	–	–	80.14	–	–	–	78.20	–
12	2H	1.51/d 14.0 1.48/m	36.85	CH_2	2H	1.36m 1.74m	39.13	CH_2
13	–	–	103.50	–	1H	3.18m	35.69	CH
14	3H	1.67/s	18.78	CH_3	3H	1.01/d 6.5	18.15	CH_3
15	3H	1.81/d 1.5	16.69	CH_3	3H	1.88/s	13.86	CH_3
16	–	–	–	–	1H	5.25/s	–	–
17	3H	1.27	25.51	CH_3	3H	1.22s	20.47	CH_3
18	2H	1.36/m, 1.69/m	24.94	CH_2	2H	1.22/m, 1.84/m	20.89	CH_2
19	3H	0.87/t 8.0	11.00	CH_3	3H	0.79/t 7.5	10.62	CH_3
20	3H	1.21/d 6.5	14.50	CH_3	3H	2.92/d 7.0	49.67	CH_3
21	–	–	–	–	–	–	–	–
22	3H	0.91	14.82	CH_3	3H	0.84/d 7.5	12.65	CH_3
23	3H	1.27/s	23.84	CH_3	3H	1.87s	12.30	CH_3
24	3H	3.03/s	47.60	CH_3	3H	2.79/m	49.74	CH_3
25	3H	1.26/d 6.0	22.14	CH_3	3H	1.01s	18.15	CH_3
26	1H	3.60/m	69.44	CH	1H	3.66m	68.06	CH
27	1H	4.58/d 8.0	102.38	CH	1H	4.53/d 7.5	100.44	CH
28	1H	5.11/dd 8.0, 10.5	72.49	CH	1H	4.78/m	71.31	CH
29	1H	2.84/m	64.35	CH	1H	2.89/m	62.09	CH
30	2H	1.45/m, 140/m	31.17	CH_2	2H	1.54/m 1.79/m	29.73	CH_2
31	6H	2.28/s	40.82	$2CH_3$'s	6H	2.15/s	39.81	$2CH_3$s
32	–	–	166.15	–	–	–	162.80	–
33	–	–	130.17	–	–	–	170.00	–
34	1H	7.99/dd 1.5, 7.5	129.91	CH	1H	7.92/d 7.5	128.96	CH
35	1H	7.41/d7.5	128.26	CH	1H	7.52/t 8.0	128.21	CH
36	1H	7.53/t 7.5	132.74	CH	1H	7.63/t 7.5	132.79	CH
37	1H	7.41/d7.5	128.26	CH	1H	7.52/t 8.0	128.21	CH
38	1H	7.99/dd 1.5, 7.5	129.91	CH	1H	7.92/d 7.5	128.76	CH

* Refer Fig. 3 and Fig. 5 for numbering; ** J: This column gives the ^{1}H–^{1}H coupling constant, (br) broad, (dd) doublet of doublet, (s) Singlet, (t) triplet, (m) multiplet.

The ^{1}H NMR of **2** was compared with **3** (Figs. S3 and S4; Table 2). The absence of exchangeable proton at δ 3.76 ppm due to hydroxyl group in **3** and appearance of a quaternary carbon signal at δ 206.62 ppm in **3** as seen from gHMBC (Fig. S5) indicated the conversion of hydroxyl group to carbonyl group. This is in agreement with the expected conversion of hydroxyl group at position 8 (Table 1) in **2** to keto group in **3** as per the synthetic scheme (Scheme 1). Proton and carbon-13 NMR values of **2** and **3** are tabulated (Table 2).

Structure elucidation of by-product 4

The HR-MS spectrum of **4** (Fig. S6) displayed exact mass of the protonated molecular ion at *m/z* 656.3808 (Calc 656.3799 for $C_{37}H_{54}NO_9$), which corresponds, to the molecular formula $C_{37}H_{53}NO_9$.

The mass spectral data indicated that **4** could be obtained by the loss of two water molecules from **3.** Loss of two water molecules from **3** could be due to the loss of OH groups from positions 3 and 4. The ^{1}H NMR data of **4** (Fig. S7 and Table 3) shows a new olefinic proton singlet at δ 5.64 ppm and corresponding carbon chemical shift at δ 130.29 ppm (from gHSQC spectrum Fig. S8) indicated the presence of olefinic CH group. The presence of a ^{13}C resonance at δ103.50 ppm in **4** instead of resonance at δ 46.80 ppm in case of **2** may be due to the conversion of a methylene CH_2 to methine CH.

It is also interesting to note that C=O resonance at δ 221.0 ppm (from gHSQC spectrum) in **2** due to carbonyl group present at position 1 was absent in **4** instead a new carbon chemical shift at 154.02 ppm was observed corresponding to a C-O group. This carbon chemical shift shows correlation with protons at positions 12, 14 and 15 as seen by gHMBC spectrum (Fig. S9). This change in chemical shift can be rationalized as formation of an epoxide ring between positions 1 and 4. The predicted molecular structure of **4** is shown Figure 3.

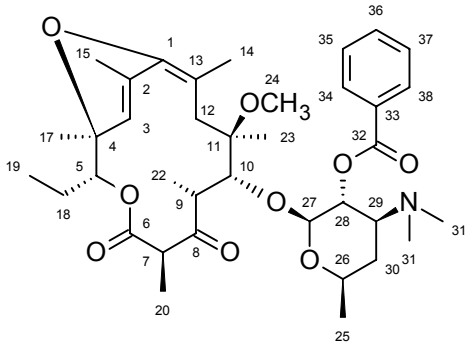


Fig. 3. Structure of by-product **4** with atom numbering.

Single crystal X-ray diffraction of **4** has been carried out to confirm the structure unambiguously. Colourless blocks of **4** were grown from ethanol and petroleum ether (1:2 v/v) solvent mixture by slow evaporation method at ambient conditions. The crystal structure shows that the asymmetric unit consists of one molecule of **4**. The by-product has crystallized in the monoclinic crystal system (space group $P2_1$). The ORTEP is shown in Figure 4. The molecule consists of 10 chiral centres, since the C_{11} chiral centre will not

be changed during our synthetic procedure and during the refinement the configuration has been fixed as "R" as per the Clarithromycin crystal structure [8]. Thus, the chiral centres C_5, C_7, C_9, C_{10}, C_{24} and C_{26} are found to have "R", and chiral centres at C_4, C_{25} and C_{27} are found to have "S" configurations. The spectroscopic and single crystal X-ray diffraction elucidate the structure of **4** as (1*S*,2*R*,5*R*,7*R*,8*R*,9*R*)-2-ethyl-9-methoxy-1,5,7,9,11,13-hexamethyl-4,6-dioxo-3,15-dioxabicyclo[10.2.1]pentadeca-11,13-dien-8-yl 3,4,6-trideoxy-3-(dimethylamino)-2-*O*-(phenylcarbonyl)-β-D-*xylo*-hexopyranoside.

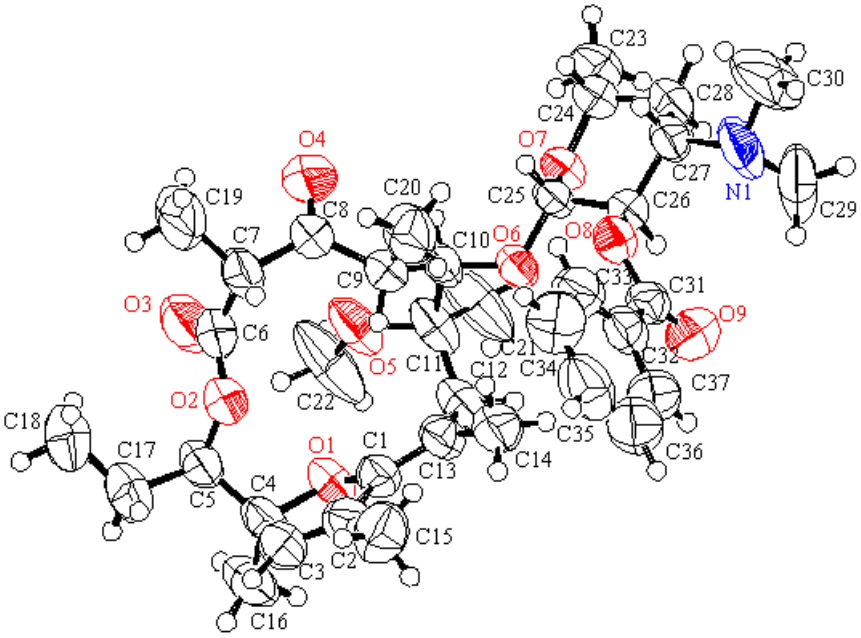


Fig. 4. The ORTEP of 4, showing the atom-labeling scheme, with displacement ellipsoids drawn at the 50 % probability level for non-hydrogen atoms. H atoms are represented by circles of arbitrary size.

Structure confirmation of 6

The HR-MS spectrum of **6** (Fig. S10) displayed exact mass of the protonated molecular ion at m/z 674.3917 (Calc. 674.3904 for $C_{37}H_{56}NO_{10}$), which corresponds to the molecular formula $C_{37}H_{55}NO_{10}$. This indicates that **6** has 18 mass units less than the parent **3**, **6** may be obtained by the loss of a water molecule from **3**.

The ^{1}H NMR data of **6** (Fig. S11, Table 3) was compared with those of **3** (Fig. S4). The absence of the exchangeable signal at δ 3.90 ppm in **6** could be due to the loss of OH group from position 3 and H from position 2 of product ketolide **3** leading to intermediate **6**.

From the gHSQC spectrum of **6** (Fig. S12) and **3**, it is observed that in the macrocyclic lactone ring the number of oxygen attached methines in **6** is only two as against three in **3.** Further, from the gHMBC spectrum of **6** (Fig. S13) the carbon chemical shift at δ 206.89 ppm shows correlation with protons at position 3, 12 and 13. Thus from **3** there could be loss of hydroxyl group at position 3 and hydrogen atom at position 2 leading to the formation of **6** (Figure 5)

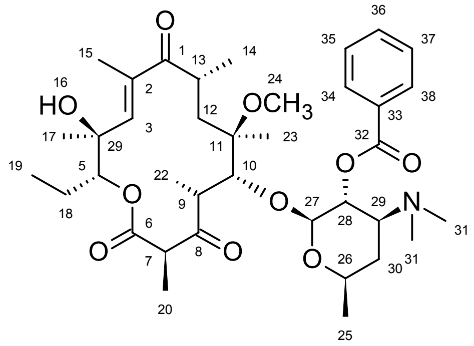


Fig. 5. Structure of intermediate **6** with atom numbering.

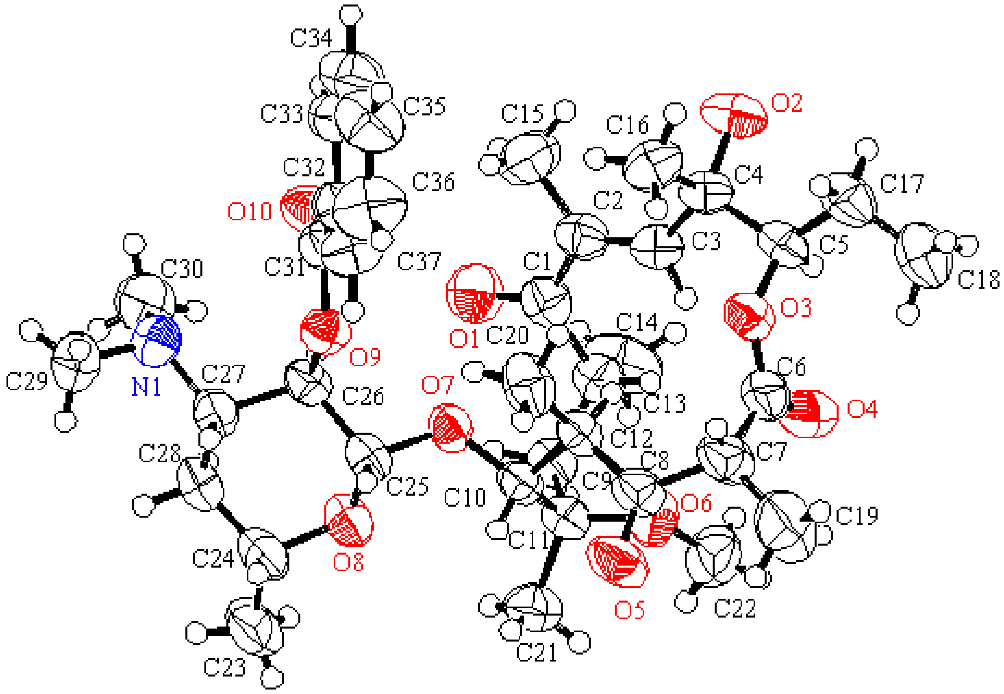


Fig. 6. The ORTEP of 6, showing the atom-labeling scheme, with displacement ellipsoids drawn at the 50 % probability level for non-hydrogen atoms. H atoms are represented by circles of arbitrary size.

Single crystal X-ray diffraction of **6** has been carried out to confirm the structure unambiguously. Colorless blocks of **6** were grown from acetone and hexane (1:2, *v*/*v*) solvent mixture by slow evaporation method at ambient conditions. The crystal structure shows that the asymmetric unit consists of one molecule of **6**. The by-product has crystallized in the orthorhombic crystal system (space group $P2_12_12_1$). The ORTEP is shown in Figure. 6. The molecule consists of 11 chiral centers, since the C_{11} chiral centre will not be changed during our synthetic procedure and during the refinement the configuration has been fixed as “R” as per the Clarithromycin crystal structure [8]. Thus, the chiral centres C_5, C_7, C_9, C_{10}, C_{13}, C_{24} and C_{26} are found to have “R”, and chiral centres at C_4, C_{25} and C_{27} are found to have “S” configurations. The spectroscopic and single crystal X-ray diffraction elucidate the structure of **6** as

(3*R*,5*R*,6*R*,7*R*,9*R*,11*E*,13*S*,14*R*)-14-ethyl-13-hydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-2,4,10-trioxooxacyclotetradec-11-en-6-yl 3,4,6-trideoxy-3-(dimethylamino)-2-*O*-(phenylcarbonyl)-β-D-*xylo*-hexopyranoside.

Experimental

Synthesis of (3R,4S,5S,6R,7R,9R,11R,12R,13S,14R)-14-Ethyl-4,12,13-trihydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-2,10-dioxooxacyclotetradecan-6-yl 3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranoside (3-O-De(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)-6-O-methylerythromycin, 1)

To a solution of 200 mL of water and 20 mL of 12 N hydrochloric acid (HCl), small quantities of Clarithromycin (20 g, 26.77 mmol) were added. The reaction was stirred for 2 hrs at room temperature, saturated with sodium chloride and pH was adjusted to 8 with aqueous ammonium hydroxide. This solution was extracted with ethyl acetate and dried over anhydrous sodium sulphate (Na_2SO_4). The product was purified by column chromatography by eluting with 96:4 ethyl acetate/triethyl amine to afford 5 g (yield 25%) of **1** as white colored compound.

Synthesis of (3R,4S,5S,6R,7R,9R,11R,12R,13S,14R)-14-Ethyl-4,12,13-trihydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-2,10-dioxooxacyclotetradecan-6-yl 3,4,6-trideoxy-3-(dimethylamino)-2-O-(phenylcarbonyl)-β-D-xylo-hexopyranoside (3-O-De(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)-6-O-methylerythromycin 2'-benzoate, 2)

To a solution of **1** (7 g, 11.8 mmol) in dichloromethane (100 mL), triethyl amine (4.9 g, 48.66 mmol) and benzoic anhydride (6.7 g, 29.65 mmol) were added, the mixture was stirred at room temperature for 16 hrs. The solution was diluted with water and extracted with dichloromethane (200 mL). The organic layer was collected over anhydrous Na_2SO_4. The product (yield 42%) was purified by column chromatography by eluting with 96:4 ethyl acetate/triethyl amine to afford 5.2 g of **2** as white colored compound.

Synthesis of (3R,5R,6R,7R,9R,11R,12R,13S,14R)-14-Ethyl-12,13-dihydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-2,4,10-trioxooxacyclotetradecan-6-yl 3,4,6-trideoxy-3-(dimethylamino)-2-O-(phenylcarbonyl)-β-D-xylo-hexopyranoside (3-De[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-6-O-methyl-3-oxoerythromycin 2'-benzoate, 3) and (1S,2R,5R,7R,8R,9R)-2-Ethyl-9-methoxy-1,5,7,9,11,13-hexamethyl-4,6-dioxo-3,15-dioxabicyclo[10.2.1]pentadeca-11,13-dien-8-yl 3,4,6-trideoxy-3-(dimethylamino)-2-O-(phenylcarbonyl)-β-D-xylo-hexopyranoside (3-De[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-8,9,10,11-tetradehydro-9-deoxo-11,12-dideoxy-9,12-epoxy-6-O-methyl-3-oxoerythromycin 2'-benzoate, 4)

To a solution of **2** (2 g, 2.88 mmol), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC HCl) (3.68 g, 19.22 mmol) and dimethyl sulphoxide (DMSO) (3.96 mL, 51.84 mmol) in 50 mL of methylene chloride were added. To this mixture a solution of pyridinium trifluoroacetate (3.71 g, 19.22 mmol) in 20 mL of methylene chloride was added drop wise at 15 °C. The reaction was stirred for 4 hr at room temperature, and 4 mL of water was added. After stirring for about 10 min, the mixture was taken in 20 mL of methylene chloride, followed by washing with water and dried over Na_2SO_4. The solvent

was evaporated to obtain the product. The product was purified by column chromatography by eluting with 90:10 isopropyl ether/triethyl amine to afford 900 mg (yield = 9.7 %) of **3** as white colored compound along with 700 mg (yield 7.5%) of a crystalline white colored solid as by-product **4**, this was isolated in pure form.

Synthesis of (3*R*,5*R*,6*R*,7*R*,9*R*,11*E*,13*S*,14*R*)-14-Ethyl-13-hydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-2,4,10-trioxooxacyclotetradec-11-en-6-yl 3,4,6-trideoxy-3-(dimethylamino)-2-O-(phenylcarbonyl)-β-D-xylo-hexopyranoside ((10*E*)-3-De[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-10,11-didehydro-11-deoxy-6-O-methyl-3-oxoerythromycin 2'-benzoate, 6)

To a solution of **3** (230 mg, 0.33 mmol) in 5 mL of dry pyridine under nitrogen at 10 °C, 139 mg (0.796 mmol) of methanesulfonic anhydride was added. The reaction mixture was stirred for 5 hrs at room temperature. The resultant solution was filtered, concentrated, extracted with ethyl acetate and dried over Na_2SO_4 to obtain **5** (not isolated). To **5** 5 mL of acetone, 0.047 mL (0.321 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were slowly added at room temperature. After stirring for 20 hrs, the reaction was diluted with methylene chloride, washed with water, dried over Na_2SO_4, and concentrated under vacuum to obtain the crude product. The crude product was further purified by column chromatography by eluting with 40: 60 acetone / hexane to afford 130 mg (yield 0.4%) of **6** as white solid.

High performance liquid chromatography

The reagents used for HPLC analysis were potassium dihydrogen *ortho*-phosphate (AR grade) and acetonitrile (Gradient grade) purchased from (SD fine chemicals, India), Ranbaxy Laboratories, India respectively. Water used was purified using Milli-Q plus purification system.

A Waters Model Alliance 2695 Separations module equipped with a Waters 2996 photo diode array UV detector was used. A simple in-house gradient LC method was developed for the analysis of ketolides, where a Inertsil C8 3, 250 x 4.6mm I.D., 5µ (GL Sciences, UK) with a mobile phase consisting of A: 0.01 M potassium hydrogen orthophosphate adjusted to pH 6.5 with dilute KOH; B: Acetonitrile were used with UV detection at 230 nm at a flow rate of 1.2ml/min using the following gradient program Time/% B: 0/65, 5/65, 10/80, 35/80, 40/65, 45/65 for the separation of ketolide, by-product and intermediate. The data was recorded using Waters Empower software. All the samples were dissolved in acetonitrile and water premixed in the ratio of 70:30, sonicated and filtered with 0.22 µ syringe filter then injected into HPLC.

ESI-MS

The MS experiments were performed on a PESCIEX API 3000. The sample was introduced through a turbo ion spray interface in positive ionization mode using infusion pump. The nebulizer and curtain gases used were zero air and nitrogen respectively; ion spray voltage was maintained at 4500V. Focusing potential, declustering potential and entrance potential were kept at 180V, 70V and 10V respectively.

NMR

The ^{1}H, ^{13}C, DEPT (theta variable pulse angles used were 45°, 90° and 135° for DEPT)

and 2D NMR experiments gradient double quantum correlation spectroscopy (gs-DQCOSY), gradient hetero single quantum correlation spectroscopy (gs-HSQC) and gradient hetero multiple bond correlation spectroscopy (gs-HMBC) for **2, 3, 4** and **6** were performed in $CDCl_3$ solvent using Mercury plus 400MHz FT NMR spectrometer. The ^{13}C experiment for ketolide was performed in $CDCl_3$. The ^{1}H chemical shift values were reported on the δ scale in ppm, relative to TMS (δ=0.00ppm) and the chemical shift values were reported relative to $CDCl_3$ (δ=77.00ppm) and DMSO-d_6 (δ=39.50ppm) as internal standards respectively.

Single crystal X-Ray Diffraction (SXRD)

X-ray data for the single crystals **4** and **6** have been collected on Rigaku AFC-7S diffractometer equipped with Mercury CCD detector using graphite monochromated Mo-K_α radiation (λ = 0.7107 Å). The structures were solved with direct methods (SIR-2004) and refined using least squares procedure (CRYSTALS) using the crystal structure 3.8.1 software. The non-hydrogen atoms were refined anisotropically and hydrogen atoms bonded to O were located in the difference electron density map and refined isotropically. The C–H hydrogen atoms were positioned geometrically and refined in the riding model approximation with C–H = 0.95 Å, and with *U* (H) set to $1.2U_{eq}$(C). CCDC 725165 and CCDC 725166 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033).

HR-MS

All the samples were analyzed on UPLC-TOF-MS system consisted of an ACQUITY™ Ultra Performance Liquid Chromatography system and Micromass LCT Premier XE Mass Spectrometer (High sensitivity orthogonal time-of-flight instrument; Waters, Milford, USA) equipped with an ESI lock spray source for accurate mass values. Leucine-enkephalin was used as reference compound, was introduced via the lock spray channel. The mass range was calibrated with the cluster ions of sodium formate using a fifth order polynomial fit. Data were acquired using the high resolution (W) mode. operating in either the positive or negative ion mode. The source temperature was set at 110 °C with a cone gas flow of 10 L/h, a desolvation gas temperature of 240 °C, and a desolvation gas flow of 430 L/h. The capillary voltage was set at 2300V and the cone voltage to 30 V. All analyses were acquired using the lock spray to ensure accuracy and reproducibility; leucine-enkephalin was used as the lock mass. High resolution (W mode, FWHM 10500) positive polarity with Dynamic Range Enhancement (DRE) function was used and scan responses were collected from *m/z* 100 to 1000 at a rate of 1.0 s/scan. The lock spray frequency was set at 5s and data were averaged over 10 scans. The samples were dissolved in methanol at a concentration 0.02 mg/mL in methanol and infused in TOF-MS at a flow rate of 10 µL/min.

Conclusions

During the synthesis of telithromycin intermediate **6**, an unexpected by-product **4** was obtained. The HR-MS, 2D-NMR and single crystal X-ray diffraction techniques have been utilized to elucidate molecular structures of **4** and **6**, unambiguously.

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Supporting Information

The scanned HR-MS spectrum of **2–4** and **6**, the scanned ^{1}H NMR spectrum of **2–4** and **6**, the scanned gHMBC spectrum of **3**, **4** and **6**, and the scanned gHSQC spectrum of of **4** and **6** are available in the online version (Format: PDF, Seize: ca. 0.3 MB): http://dx.doi.org/10.3797/scipharm.0907-01.

Authors' Statement

Competing Interests

The authors declare no conflict of interest.

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