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Synthesis, isolation, stereostructure and cytotoxicity of paclitaxel analogs from cephalomannine

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1. Introduction

Paclitaxel, or Taxol® (1, Fig. 1), is a structurally peculiar diterpenoid compound that was isolated from the bark of the North American *Taxus brevifolia* by Wani et al. in 1971 [1]. Because of its significant anticancer activity, unique mechanism of action, complex molecular structure, and multiple chiral centers, research on its chemical synthesis, structural modification, and structure–activity relationship has long been a focus in the field of natural product chemistry [2]. At present, paclitaxel and its semisynthetic derivative, docetaxel (**2**, Fig. 1), are mainly used as clinical treatments for breast, ovarian, and non-small-cell lung cancer [3].

As can be seen from its name, cephalomannine (**3**, Fig. 1) was initially considered to have been isolated from *Cephalotaxus mannii*, but it was later discovered that there was an error in the plant classification and that cephalomannine was actually from *Taxus wallichiana*, a species of yew [4]. In later phytochemical studies, cephalomannine was found in several other yew species [5–7]. The structure of cephalomannine is very similar to that of paclitaxel, differing only in its C-13 side chain: paclitaxel has an

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ABSTRACT

Four paclitaxel derivatives were afforded by preparative HPLC separation of two pairs of diastereoisomers, which were obtained by catalytic hydrogenation and epoxidation of the C-13 side-chain double bond of cephalomannine, a naturally occurring paclitaxel analog. The four paclitaxel derivatives were analyzed using NMR, CD spectroscopy, and side-chain hydrolysis in order to measure their optical rotations and GC characteristics. In this way, the stereoconfigurations of the products were determined. Evaluation of the compounds' activity indicated that they had differing cytotoxic activities: compound **5** had superior activity in BCG-823 tumor cells compared to paclitaxel. These results indicate that the stereoconfiguration of the paclitaxel N-acyl side chain has a significant impact on its activity.

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N-benzoyl group in its C-13 side chain, while cephalomannine has an *N*-tigloyl group. Because of these structural similarities, the separation of cephalomannine from paclitaxel is very difficult, and several methods for effectively separating the two compounds have been developed [8–10]. Since the amount of cephalomannine might even be higher than that of paclitaxel in certain *Taxus* plants, it can also be a useful semi-synthetic raw material that can be converted into paclitaxel or docetaxel [11,12]. Furthermore, there have been reports of interesting structural modifications and biotransformations of cephalomannine [13–15]. Therefore, structural modification of readily available cephalomannine can contribute to a better understanding of the structure–activity relationship in the anticancer effects of taxanes.

The epoxidation of cephalomannine has been reported in a patent on the preparation of cephalomannine analogs, but the diastereomers were not separated in this report [16]. There is no stereoselectivity of hydrogenation and epoxidation reactions on the double bond of cephalomannine's sidechain and the afforded compounds in the patent were a pair of diastereoisomers. Therefore, in the current study, catalytic hydrogenation and epoxidation of cephalomannine were carried out separately on the side-chain *N*-tigloyl double bond to obtain two different diastereoisomers, which were then







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Fig. 1. Structures of paclitaxel (1), docetaxel (2) and cephalomannine (3).

separated using preparative high-performance liquid chromatography (HPLC). In addition, the three-dimensional structures as well as anticancer activities of the obtained diastereoisomers were studied.

2. Experimental

2.1. General

¹H and ¹³C NMR spectra were obtained on a Varian Unity INOVA 400/54 and Bruker ARX-500 spectrometer in CDCl₃ with TMS as the internal standard. Mass spectra were obtained on a VG Auto spec 3000 or on a Finnigan MAT90 instrument. Optical rotations were measured on a Perkin-Elmer 341, and CD spectra were measured on a JASCO J-810 spectropolarimeter. Preparative HPLC was performed on a Shimadzu LC-8A liquid chromatograph equipped with a Zorbax PrepHT GF (21.2 mm × 25 cm, 7 µm) or Venusil MP C18 (20 mm × 25 cm, 5 µm) column. GC–MS analysis was performed on a GC (Agilent, 6890N) interfaced with a mass selective detector (Agilent, 5973B). Silica gel H (Qingdao Sea Chemical Factory, Qingdao, People's Republic of China) was used for column chromatography.

Spots on TLC (silica gel G) were detected by spraying with H_2SO_4 -EtOH. Commercially available reagents and solvents were directly used without further purification.

2.2. Substrates

Cephalomannine (**3**) was purchased from Kunming Wuyi Biotechnology Co., Ltd., China. The structures were characterized on the basis of ¹H NMR and ¹³C NMR spectra, and the purities were determined to be >95% by HPLC analyses.

2.3. Preparation of compounds 4 and 5

A solution of cephalomannine (200 mg, 0.24 mmol) and Pd–C (10 mg, 5%) under H_2 atmosphere was strongly stirred for 24 h. Then the reaction solution was filtered and evaporated to give a mixture of compounds **4** and **5**. The mixture was

further subjected to preparative reversed-phase HPLC eluting with CH₃CN-H₂O (60/40, v/v) to give compounds **4** (85 mg, $t_{\rm R} = 16.7$ min) and **5** (82 mg, $t_{\rm R} = 18.6$ min).

2.3.1. N-debenzoyl-N-(S-2-methylbutanoyl)paclitaxel (4)

White powder, $[a]_D^{25} - 49$ ° (c 0.10, MeOH); IR ν_{max} 3438, 2961, 1730, 1714, 1638 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) and ¹³C NMR (125 MHz, CDCl₃): see Table 1. MS (ESI, MeOH) m/z 834 [M + H]⁺.

2.3.2. N-debenzoyl-N-(R-2-methylbutanoyl)paclitaxel (5)

White powder, $[a]_D^{25} - 25$ ° (c 0.10, MeOH); IR ν_{max} 3438, 2962, 1730, 1716, 1638 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) and ¹³C NMR (125 MHz, CDCl₃): see Table 1; MS (ESI, MeOH) m/z 834 [M + H]⁺. HR-ESI-MS: 834.3632 [M + H]⁺ (calcd. for C₄₅H₅₆NO₁₄ 834.3622).

2.4. Preparation of compounds 6 and 7

The solution of cephalomannine (250 mg, 0.30 mmol) in CH_3Cl_3 (50 mL) was added in *m*CPBA (3-chloroperbenzoic acid, 62 mg, 0.36 mol, 1.2 equiv.). The reaction mixture was stirred for 8 h under argon prior to being quenched with aqueous solution of Na₂S₂O₃. The organic phase was separated, washed with brine and dried over anhydrous Na₂SO₄. Then the organic solvent was removed under reduced pressure to give the mixture of compounds **6** and **7**. The mixture was further subjected to preparative reversed-phase HPLC eluting with CH₃CN-H₂O (60/40, v/v) to give compounds **6** (76 mg, $t_R = 12.7$ min) and **7** (72 mg, $t_R = 15.2$ min).

2.4.1. N-debenzoyl-N-[(2R,3S)-2,3-epoxy-2-methylbutanoyl] paclitaxel (**6**)

White powder, $[a]_{D}^{25} - 38$ ° (c 0.10, MeOH); IR ν_{max} 3422, 2988, 1710, 1632 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) and ¹³C NMR (125 MHz, CDCl₃): see Table 1; MS (ESI, MeOH) *m/z* 848 [M + H]⁺. HR-ESI-MS: 848.3466 [M + H]⁺ (calcd. for C₄₅H₅₄NO₁₅ 848.3415).

2.4.2. N-debenzoyl-N-[(2S,3R)-2,3-epoxy-2-methylbutanoyl] paclitaxel (**7**)

White powder, $[a]_{D}^{25} - 66$ ° (c 0.10, MeOH); IR ν_{max} 3422, 2988, 1712, 1632 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) and ¹³C NMR (125 MHz, CDCl₃): see Table 1; MS (ESI, MeOH) *m/z* 848 [M + H]⁺. HR-ESI-MS: 848.3446 [M + H]⁺ (calcd. for C₄₅H₅₄NO₁₅ 848.3415).

2.5. Complete alkaline hydrolysis of compounds 4-7

A solution of **4** (or **5**, **6**, **7**) (50 mg) and KOH (100 mg) in MeOH (15 mL) was stirred overnight at room temperature. The reaction mixture was neutralized with 1 N HCl and extracted with EtOAc (10 mL × 3). Then the combined organic phase was dried over anhydrous Na₂SO₄ and evaporated to give the residue. The two residues, from compounds **4** and **5**, were dissolved in MeOH (5 mL) and treated with excess CH₂N₂. After evaporation, the residues were dissolved in CHCl₃ (5 mL) and filtered. An aliquot of the two filtrates was analyzed by GC using a chiral column (Astec Chiraldex G-TA G0012-08, 30 m, 50 °C), to give peaks at t_R 12.5 or 11.4 min, respectively. The standard methyl ester of (*R*)-or (*S*)-2-methylbutyric

Table 1			
¹ H and ¹³ C NMR data for composite	unds 3, 4, 5, 6,	7 (δ in p	pm, in $CDCl_3$).

nnnnnnnnnnnnnnn1700 s703 s700 s704 d704 d <th>Position</th> <th colspan="2">Cephalomannine (3)</th> <th colspan="2">Compounds 4/5^a</th> <th colspan="2">Compounds 6/7^b</th>	Position	Cephalomannine (3)		Compounds 4/5 ^a		Compounds 6 / 7 ^b	
1		δ _H (<i>J</i> in Hz)	δ _C	δ _H (<i>J</i> in Hz)	δ _C	$\delta_{\rm H}$ (J in Hz)	δ_{C}
2567 d(72)749 d568 d(72)749 d749 d754 d <td>1</td> <td>_</td> <td>79.0 s</td> <td>_</td> <td>78.7 s</td> <td>_</td> <td>79.0/78.9 s</td>	1	_	79.0 s	_	78.7 s	_	79.0/78.9 s
33.8 a4.5.4 d4.5.4 d5.7.4 (2.2)45.5 d5.7.7 (6.8)4.5.4 (5.0) <t< td=""><td>2</td><td>5.67 d (7.2)</td><td>74.9 d</td><td>5.68 d (7.2)</td><td>74.9 d</td><td>5.67 d (6.8)</td><td>74.9/74.8 d</td></t<>	2	5.67 d (7.2)	74.9 d	5.68 d (7.2)	74.9 d	5.67 d (6.8)	74.9/74.8 d
4-810 s-810 s-810 s-810 s100 s	3	3.78 d (7.2)	45.5 d	3.78 d (7.2)	45.5 d	3.77 t (6.8)	45.5/45.6 d
5494 (48)844 (48)843 (43)843 (48) </td <td>4</td> <td>_</td> <td>81.1 s</td> <td>_</td> <td>81.0 s</td> <td>_</td> <td>81.0/81.1 s</td>	4	_	81.1 s	_	81.0 s	_	81.0/81.1 s
6188 m25.4186 m35.4181 m35.43.5.535.43.5.574.40 m72.0 d4.39 m72.1 d8-55.5.s55.4.s-35.5.s9-203.8.s-203.6.s-203.5.s106.27 s75.6 d6.28 s-203.5.s-203.5.s11-133.1 s-75.5 d6.28 d (3.0)75.75.5-114.9.s12-133.1 s133.5 s-141.9.s13-13.1 s13.5 s-141.9.s142.34 m35.5 s2.28 m-2.28 m2.27 a2.27 a142.24 m2.35 m3.5 s2.36 m3.5 s2.36 m3.5 s161.5 s2.16 m1.26 s2.16 m2.16 m2.16 m2.16 m171.5 s2.16 m1.26 s1.05 m2.16 m2.16 m2.16 m1819.0 s1.34 n1.26 s1.04 n1.05 m2.16 m2.17 n191.5 s2.16 m1.26 s1.05 m2.16 m2.16 m2.17 n191.5 s2.16 m1.6 s1.6 s1.6 s1.6 s2.17 n101.6 s1.6 s1.6 s1.6 s1.6 s1.6 s1.6 s101.6 s1.6 s1.6 s1.6 s1.6 s1.6 s1.6 s101.6 s1.6 s1.6 s1.6 s1.6 s1.6 s<	5	4.94 d (8.0)	84.4 d	4.94 d (8.0)	84.3 d	4.93 d (10.0)	84.3 d
1.54 mi253 mi200 mi200 mi7.4 dom72.4 dom72.0	6	1.88 m	35.5 t	1.86 m	35.5 t	1.81 m	35.4/35.5 t
7440 m72.d d440 m72.d d439 m71.d d85554.d58.d d958.d d958.d d958.d d958.d d958.d d958.d d90.d d58.d d75.d d58.d d75.d d58.d d75.d d58.d d75.d d58.d d75.d d58.d d75.d		2.54 m		2.53 m		2.50 m	
8 9-58.5 s-58.4 s-58.4 s-58.5 s203.5 s106.27 s7.56 d6.28 s7.55 d6.28 s6.28 s7.55 s133.5 s12.27 s7.27 s7.37 s7.3	7	4.40 m	72.2 d	4.40 m	72.0 d	4.39 m	72.1 d
9-203,8 i-203,6 i-203,6 i203,7 is106.27 s756 d6.28 s75.6 d6.28 d75.6 d28.6 d75.7 s75.7 s7	8	_	58.5 s	_	58.4 s	_	58.5 s
10627 s756 d628 s75.5 d628 (36)75.6 d75.6 d75.6 d75.7 s75.7 s <td>9</td> <td>_</td> <td>203.8 s</td> <td>_</td> <td>203.6 s</td> <td>_</td> <td>203.5 s</td>	9	_	203.8 s	_	203.6 s	_	203.5 s
11-133.1 s-133.5 s-133.5 s-133.5 s112-142.2 s-149.s-149.s-149.s13621 (8.4)72.2 d6.18 (8.8)72.4 d6.18 (8.8)72.27.3 d142.24 m2.25 m2.26 m2.26 m35.t2.27.3 d152.8 m2.28 m2.29 m2.24 m43.1 s43.1 s161.15 s2.18 q1.15 s2.18 q1.15 s2.18 q171.26 s2.68 q1.26 s2.66 q1.26 s2.68 q181.20 s2.68 q1.28 s1.47 q1.79 s1.71/48 q191.68 s9.5 q2.68 q1.57 s2.68 q2.5 q201.68 s9.5 q2.68 q4.74 q1.79 s1.71/48 q21-7.27 s7.30 d7.30 d7.30 d7.30 d11'-7.33 d-7.30 d7.30 d7.30 d22'7.33 d-7.30 d2.5 q23'7.17 s7.41 d7.41 d7.41 d40Ac-1.74 s-7.17 s7.31 m7.30 d202.25 s2.5 q2.7 q2.3 s2.5 q2.5 q2'1.71 s-7.12 s7.12 s2'-1.74 s-1.72 s7.3 s1.31 s2'-1.74 s-1.71 s1.31 s1.	10	6.27 s	75.6 d	6.28 s	75.5 d	6.28 d (3.6)	75.6/75.5 s
12-142 s-141 s-141 s13621 t (8.4)722 d61 t (8.8)7272 d7272 d14234 m355 t236 m75 t26 m75 t28 m228 m229 m24 m75 t76 m75 t15-4.1 s-4.1 s76 m71 s71 m161.5 s218 q1.5 s218 q1.5 s218 q71 s17126 s268 q1.5 s218 q1.5 s28 q181.80 s9.5 q1.26 s85 q75 q75 q191.68 s9.5 q1.26 s85 q75 q75 q20 4 (84)9.5 q1.80 s76 d1.72 s75 q21 4 (19 q)-73 d-72 s70 d76 d21 4 (19 q)-73 d-72 s70 d70 d21 4 (19 q)-73 d-71 d (8)70 d70 d22 5 q23 d (24 s)25 q23 d (24 s)25 q23 d (34 s)25 q10 Ac-71 d s-71 d (8)70 d72 s72 s10 Ac-71 d s-71 d (8)72 s72 s10 Ac-71 d s-71 d (8)72 s72 s10 Ac-71 d (8)-71 d (8)72 s72 s10 Ac-71 d (8)-71 d (8)72 s72 s10 Ac-10 d (7) d (7) s1	11	_	133.1 s	_	133.5 s	_	133.7 s
13621 (84)72.2 d618 (88)72.4 d618 (88)72.4 d1423a'm35.t236'm20'm20'm152.8 m2.9 m24'm11s161.5 s2.18 q1.5 s2.18 q1.5 s171.26 s2.8 q1.5 s2.8 q1.5 s2.8 q181.80 s2.6 8 q1.6 s2.6 6 q1.5 s2.6 8 q181.80 s9.5 q1.6 8 s9.5 q1.6 7 s9.5 q201.68 s9.5 q1.6 8 s9.5 q1.6 8 s9.5 q217.2 8 s7.2 8 s7.3 7 s217.2 8 s7.3 0 s7.3 7 s22-7.3 3 d-7.3 0 s7.3 7 s237.3 1 s7.3 0 s7.3 1 s341.1 2 s-7.0 7 s247.1 7 s2.5 q2.2 1 s252.3 5 s2.5 q2.2 5 q2.2 5 q2.2 5 q262.5 1 0.5 s2.5 1 0.5 s1.6 5 s1.6 5 s271.1 5 s-1.6 2 s262.5 1 0.5 s2.5 1 0.5 s2.5 1 0.5 s2.5 1 0.5 s271.5 1 0.5 s1.6 1 0.5 s281.5 1 0.5 s1.5 1 0.5 s291.5 1 0.5 s1.5 1 0.5 s291.5 1 0.5 s1.5 1 0.5 s20- <td>12</td> <td>_</td> <td>142.2 s</td> <td>-</td> <td>141.9 s</td> <td>_</td> <td>141.9 s</td>	12	_	142.2 s	-	141.9 s	_	141.9 s
14 24 m 35 t 23 m 35 t 26 m 224 m 15 - 43.1 s - - 43.1 s - <t< td=""><td>13</td><td>6.21 t (8.4)</td><td>72.2 d</td><td>6.18 t (8.8)</td><td>72.4 d</td><td>6.18 g (8.8)</td><td>72.2/72.3 d</td></t<>	13	6.21 t (8.4)	72.2 d	6.18 t (8.8)	72.4 d	6.18 g (8.8)	72.2/72.3 d
152.28 m2.29 m2.24 m2.24 m15-43.1 s-43.1 s-43.1 s161.15 s2.18 q1.15 s2.18 q1.15 s2.18 q171.26 s2.68 q1.26 s2.66 q1.26 s2.68 q181.80 s1.47 q1.82 s2.64 q1.79 s1.47/148 q191.68 s5.5 q1.88 s9.5 1 q1.67 s9.5 q204.29 d (8.4)7.65 t4.29 d (8.4)7.64 d4.28 d (8.4)7.64 d21-1.22 s7.3 d-7.30 d7.30 d7.30 d1'-1.72 s-7.30 d-1.72 s2'-7.30 d-1.72 s-1.70 s2'-1.70 s1.72 s-2.65 s2.5 q2.24 s2.5 q2.3 s2.5 q2.25 q2.06x1.72 s-1.72 s-2.072.5 s2.08 q2.1 s2.16 s2.08 q2.66 s1.30 d1.30 d7.50 m1.28 s-1.23 s1.0-0Ac1.67 s-1.66 s-2.08 q1.72 s-1.72 s2.09 z-1.61 s1.28 s-1.0-0Ac1.67 s-1.66 sPh-1-1.67 s-1.66 s-1.66 sPh-2,68.13 d (7.2)	14	2.34 m	35.5 t	2.33 m	35.5 t	2.36 m	35.5 t
15-43.1 s-43.1 s-43.1 s-43.1 s161.15 s21.8 q1.15 s21.8 q1.15 s21.8 q1.15 s21.8 q161.26 s26.6 q1.26 s26.6 q1.26 s26.8 q18180 s14.7 q1.28 s14.7 q7.9 s14.7/14.8 q191.68 s9.5 q1.67 s9.5 q1.67 s9.5 q204.29 d (8.4)7.6 s4.29 d (8.4)7.6 d4.28 d (8.4)7.6 d21-7.3 q-7.3 q7.3 q7.3 q2'-7.3 d-7.3 q7.3 q7.3 q2'-7.3 d-7.3 q7.3 q7.3 q2'-7.3 d-7.1 q7.3 q7.3 q2'-7.3 q-7.1 q7.3 q7.3 q2'-7.3 d-7.1 q7.3 q7.3 q4'17.2 s-7.3 q/7.3 d7.3 q10-0Ac-17.4 s-1.7 s2.2 s q2.2 s q20 s2.5 q2.5 q2.2 s q2.5 q2.2 s q2.5 q10-0Ac-16.1 s-1.2 s1.6 s1.1 s2-0B2-1.6 s-1.2 s1.3 d1.3 d10-1Ac-1.6 s-1.6 s1.6 s1.6 s10-1Ac-1.6 s-1.6 s1.6 s1.6 s11-1-1		2.28 m		2.29 m		2.24 m	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	15	_	43.1 s	_	43.1 s	_	43.1 s
17126 s268 q126 s266 q126 s266 q126 s268 q18180 s14.7 q122 s14.7 q1.79 s14.7 l43 q191.68 s9.5 q1.68 s9.5 q1.68 s9.5 q1.67 s9.5 q204.29 d (8.4)76.5 t4.29 d (8.4)76.4 d4.28 d (8.4)76.4 t21-172.9 s-4.18 d (8.4)4.7 d (8.4)76.1 t11'-73.3 d-73.0 d-73.0 d73.0 d2'-73.3 d-73.0 d-73.0 d-2'-73.3 d171.2 s-73.0 d3'-170.4 s171.2 s-170.1 s2.36 s2.5 q2.24 s2.5 q2.23 s2.5 q2.5 q2.06 c-171.4 s-172.8 s2.25 s2.08 q2.17 s2.06 s-172.8 s-2.06 z-167.1 s-166.8 s2.07 s10.3 d8.10 d (7.2)130.1 d8.10 d (7.2)130.3 d2.98 d-129.1 s-128.3 d3.3 d2.94 d7.60 m138.3 d7.60 m128.8 d7.60 m138.3 d3'-Ph-1-127.0 d7.30 m128.6 d7.60 m138.3 d3'-Ph-2.67.40 br. s128.3 d7.60 m138.0 d7.60 m138.3 d3'-Ph-3.5<	16	1.15 s	21.8 g	1.15 s	21.8 g	1.15 s	21.8/21.7 g
18180 s147 q182 s147 q179 s147/148 q191.68 s9.5 q1.68 s9.5 q1.67 s9.5 q20429 d (8.4)76.5 t4.29 d (8.4)76.4 d4.28 d (8.4)7.5 q11'-4.19 d (8.4)-4.18 d (8.4)-4.17 d (8.4)12'73.3 d-73.0 d73.0/7.3 d-73.0/7.3 d3'-54.8 d-54.1 d170.1 s2'-73.3 d-73.0 d-170.1 s3'-1.71.2 s170.1 s-2.00 c-170.4 s-171.2 s10-0Ac-171.4 s-172.8 s-171.2 s2.08 q2.25 s20.8 q2.17 s20.8 q2.16 s20.8 q2.00 z-167.1 s-122.1 s-122.2 s2.00 z-1.23 s-129.1 s-122.2 s2.00 z-1.23.1 s-1.29.1 s-122.2 s2.00 z-1.33.3 d7.60 m133.8 d7.50 m128.8 d7.50 m133.8 d3'-Ph-267.40 br. s1.28.3 d7.50 m128.4 d7.38 br. s131.3 s3'-Ph-1-1.28.3 d7.60 m133.8 d7.60 m133.8 d3'-Ph-1-1.28.3 d7.60 m133.8 d7.60 m133.8 d3'-Ph-1-1.28.3 d7.60 m </td <td>17</td> <td>1.26 s</td> <td>26.8 g</td> <td>1.26 s</td> <td>26.6 g</td> <td>1.26 s</td> <td>26.8 g</td>	17	1.26 s	26.8 g	1.26 s	26.6 g	1.26 s	26.8 g
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	18	1.80 s	147 a	1.82 s	147 a	1795	147/148 a
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	19	1.68 s	950	1 68 s	9510	167 s	95 a
419 d (8.4) $418 d (8.4)$ $417 d (8.4)$ 1'-172.9 s-172.9 s-172.8 s2'73.0 d73.0 d-73.073.3 d3'73.0 d-73.073.3 d4'OAc-54.8 d73.0 d170.4 s70.173.5 d2.36 s22.5 q2.24 s2.5 q2.23 s22.5 q2.36 s22.5 q2.24 s2.5 q2.23 s2.5 g2.08 q2.17 s0.8 q2.16 s0.8 q2-0Bz-167.1 s-166.8 s-2-0Bz-167.1 s-129.1 s-Ph-2,68.13 d (7.2)130.3 d8.10 d (7.2)130.1 d8.10 d (7.2)9h-3,57.5 Im128.3 d7.50 m128.8 d7.50 m138.3 d3'-Ph-2,67.40 br. s127.0 d7.39 m128.4 d7.88 br. s131.3 s d3'-Ph-3,57.42 br. s128.8 d7.40 m126.7 d7.31 m128.8 d3'-Ph-3,57.42 br. s128.8 d7.40 m126.7 d7.31 m128.8 d3'-Ph-46.51 d (8.8)NH6.51 d (8.8)17.5 s17.5 s17.5 s17.5 s1'152 s122.9 d138 m17.5 q18.0 s13.5/13.4 q2''-138.2 s2.34 m43.1 q-600.599.s s3'-Ph-2,6138.2 s </td <td>20</td> <td>4.29 d (8.4)</td> <td>76.5 t</td> <td>4.29 d (8.4)</td> <td>76.4 d</td> <td>4.28 d (8.4)</td> <td>76.4 t</td>	20	4.29 d (8.4)	76.5 t	4.29 d (8.4)	76.4 d	4.28 d (8.4)	76.4 t
1' - 172.9 s - 172.8 s - 172.7 s 2' 73.3 d 73.0 d 73.0 d 73.0 /3.3 d 4'-0Ac - 548.d - 541.d - 541./54.3 d 4-0Ac - 170.4 s - 171.2 s - 170.1 s 236 s 22.5 q 2.24 s 22.5 q 2.23 s 22.5 q 10-0Ac - 171.4 s - 172.8 s - - 2.25 s 20.8 q 2.17 s 20.8 q 2.16 s 20.8 q 2-OBz - 167.1 s - 129.1 s - - 129.2 s Ph-2,6 8.13 d (7.2) 130.3 d 8.10 d (7.2) 130.1 d 8.10 d (7.2) 130.3 d Ph-3,5 751 m 128.3 d 7.50 m 128.8 d 7.60 m 128.3 d 3'-Ph-1 - 133.8 d 7.61 m 133.0 d 7.60 m 133.8 d 3'-Ph-3,5 751 m 128.8 d 7.60 m 128.4 d 7.30 m 128.4 d 3'-Ph-3,5 740 br. s 127.0		4.19 d (8.4)		4.18 d (8.4)		4.17 d (8.4)	
2' $733 d$ $730 d$ $730 d$ $730 d$ $3'$ $ 541 d$ $ 730 d$ $730 d$ $3'$ $ 541 d$ $ 730 d$ $730 d$ $740 d$ $730 d$ $740 d$ $740 d$ $730 d$	1′	_	172.9 s	_	172.8 s	_	172.7 s
3'-54.8 d-54.1 d-54.1 d4-OAc-170.4 s-171.2 s-170.1 s236 s22.5 q2.24 s2.5 q2.23 c2.5 q10-OAc-171.4 s-172.8 s2.25 s2.08 q2.17 s2.08 q2.16 s2.08 q2-OBz-16.0 s-16.6 s-16.9 sPh-1-129.2 s-129.1 s-129.2 sPh-2,68.13 d (7.2)130.3 d8.10 d (7.2)130.1 d8.10 d (7.2)130.3 dPh-3,57.51 m128.3 d7.61 m138.0 s-131.3 s3'-Ph-1-131.3 s-138.0 s-131.3 s3'-Ph-2,67.40 br. s129.0 d7.39 m128.4 d7.38 br. s127.0 d3'-Ph-1-0.8 c7.40 br. s128.0 c-138.0 s127.0 d3'-Ph-2,67.40 br. s128.0 d7.40 m128.7 d128.8 d127.0 d3'-Ph-1-0.8 c7.40 m128.7 d128.8 d127.0 d3'-Ph-2,61.40 br. s128.9 d7.40 m128.7 d128.8 d127.0 d3'-Ph-2,61.40 br. s128.9 d7.40 m128.7 d128.8 d127.0 d3'-Ph-2,61.40 br. s128.8 d7.40 m128.7 d128.8 d127.0 d3'-Ph-2,61.40 br. s128.8 d7.40 m128.7 d128.8 d127.0 d1	2′		73.3 d		73.0 d		73.0/73.3 d
4 - 0 Ac $ 170.4 s$ $ 171.2 s$ $ 171.2 s$ $236 s$ $22.5 q$ $22.5 q$ $22.3 s$ $22.5 q$ $10 - 0 Ac$ $ 171.4 s$ $ 172.8 s$ $ 2.25 s$ $20.8 q$ $2.17 s$ $20.8 q$ $2.16 s$ $20.8 q$ $2 - 0 Bz$ $ 167.1 s$ $ 166.8 s$ $ 166.9 s$ $Ph - 1$ $ 129.2 s$ $ 166.8 s$ $ 166.9 s$ $Ph - 2.6 s$ $8.13 d (7.2)$ $130.3 d$ $8.10 d (7.2)$ $130.1 d$ $8.10 d (7.2)$ $130.3 d$ $Ph - 2.6 s$ $5.1 m$ $128.3 d$ $7.0 m$ $128.8 d$ $7.50 m$ $128.3 d$ $Ph - 4$ $7.62 m$ $133.8 d$ $7.61 m$ $133.0 d$ $7.60 m$ $133.8 d$ $3' - Ph - 1$ $ 131.3 s$ $ 138.0 s$ $ 3' - Ph - 3.5$ $7.40 br. s$ $128.8 d$ $7.40 m$ $133.8 d$ $7.20 d$ $3' - Ph - 3.5$ $7.40 br. s$ $128.8 d$ $7.40 m$ $126.7 d$ $7.31 m$ $128.8 d$ $3' - Ph - 3.5$ $7.40 br. s$ $128.8 d$ $7.40 m$ $126.7 d$ $7.1 m$ $128.9 d$ $3' - Ph - 3.5$ $7.40 br. s$ $128.8 d$ $7.40 m$ $126.7 d$ $7.1 m$ $128.9 d$ $3' - Ph - 3.5$ $7.40 br. s$ $128.8 d$ $7.40 m$ $126.7 d$ $7.1 m$ $129.0 d$ $3' - Ph - 3.5$ $1.62 s$ $12.9 d$ $1.38 m$ $12.5 q$ $ NH - CO$	3′	_	54.8 d	-	54.1 d	_	54.1/54.3 d
$10-0\Delta$ 2.36 s $22.5 q$ 2.24 s $22.5 q$ 2.23 s $22.5 q$ $10-0\Delta$ $ 171.4$ s $ 172.8$ s $ 171.2$ s 2.25 s $20.8 q$ 2.17 s $20.8 q$ 2.16 s $20.8 q$ $2-0Bz$ $ 165.8$ $ 166.9$ sPh-1 $ 129.2$ s $ 129.1$ s $ 2-0Bz$ $1.3 d (7.2)$ $130.3 d$ $8.10 d (7.2)$ $130.1 d$ $8.10 d (7.2)$ $130.3 d$ Ph-2.6 $8.13 d (7.2)$ $130.3 d$ $8.10 d (7.2)$ $130.1 d$ $8.10 d (7.2)$ $130.3 d$ Ph-3.5 $7.51 m$ $128.3 d$ $7.50 m$ $128.8 d$ $7.50 m$ $128.8 d$ Ph-4 $7.62 m$ $133.8 d$ $7.61 m$ $133.0 d$ $7.60 m$ $133.8 d$ $3'-Ph-1$ $ 133.8 d$ $7.61 m$ $128.4 d$ $7.60 m$ $133.8 d$ $3'-Ph-1,5$ $7.40 h s. s$ $127.0 d$ $7.39 m$ $128.4 d$ $7.60 m$ $128.8 d$ $3'-Ph-3,5$ $7.42 h s. s$ $128.0 d$ $7.30 m$ $128.4 d$ $7.61 m$ $128.4 d$ $3'-Ph-3,5$ $7.40 h s. s$ $129.0 d$ $7.33 m$ $129.1 d$ $7.21 m$ $129.0 d$ NH $6.51 d (8.8)$ $ NH-CO$ $ 60.15 m$ $ NH$ $6.52 d (8.8)$ $ NH-CO$ $ NH$ $6.52 $	4-OAc	_	170.4 s	-	171.2 s	_	170.1 s
10-OAc - 171.4 s - 172.8 s - 171.2 s 2.25 s 20.8 q 2.17 s 20.8 q 2.16 s 20.8 q 2-OBz - 167.1 s - 166.8 s - 166.9 s Ph-1 - 129.2 s - 129.1 s - 129.2 s Ph-2,6 8.13 d (7.2) 130.3 d 8.10 d (7.2) 130.1 d 8.10 d (7.2) 130.3 d Ph-3,5 7.51 m 128.3 d 7.50 m 128.8 d 7.50 m 128.8 d 3'-Ph-4 7.62 m 131.3 s - 138.0 s - 131.3 s 3'-Ph-2,6 7.40 br. s 127.0 d 7.39 m 128.4 d 7.38 br. s 127.0 d 3'-Ph-3,5 7.42 br. s 128.8 d 7.40 m 126.7 d 7.31 m 128.8 d 3'-Ph-4, 7.34 m 129.0 d 7.33 m 129.1 d 7.21 m 129.0 d NH 6.51 d (8.8) - - - - - - NH 6.52 d (8.8) - 17.5 q 1.80 s 13.5/13.4 q <t< td=""><td></td><td>2.36 s</td><td>22.5 g</td><td>2.24 s</td><td>22.5 g</td><td>2.23 s</td><td>22.5 g</td></t<>		2.36 s	22.5 g	2.24 s	22.5 g	2.23 s	22.5 g
2.25 s20.8 q2.17 s20.8 q2.16 s20.8 q2-OBz-167.1 s-166.8 s-166.9 sPh-1-129.2 s-129.1 s-129.2 sPh-2.68.13 d (7.2)130.3 d8.10 d (7.2)130.1 d8.10 d (7.2)130.3 dPh-3.57.51 m128.3 d7.50 m128.8 d7.50 m128.3 dPh-47.62 m133.8 d7.61 m133.0 d7.60 m133.8 d3'-Ph-1-131.3 s-138.0 s-131.3 s3'-Ph-2.67.40 br. s127.0 d7.39 m128.4 d7.38 br. s127.0 d3'-Ph-3.57.42 br. s128.0 d7.40 m126.7 d7.31 m128.8 d3'-Ph-46.51 d (8.8)NH-C0-6.01 s-165.5 s-115.5 s1''1.62 s122 q1.38 m17.5 q1.80 s13.5/13.4 q2''-138.2 s2.34 m43.1 q-600/59.9 s3''6.33 q (6.8)132.0 d1.08 m27.1 q2.98/300 q (4.4)59.6/59.9 s3''1.72 d (6.8)132.0 q0.86 t (7.2)11.8 q1.42 d (4.4)12.6/12.7 q	10-0Ac	_	171.4 s	_	172.8 s	_	171.2 s
2-OBz - 167.1 s - 166.8 s - 166.8 s - 166.9 s Ph-1 - 129.2 s - 129.1 s - 129.2 s Ph-2.6 8.13 d (7.2) 130.3 d 8.10 d (7.2) 130.1 d 8.10 d (7.2) 130.3 d Ph-3.5 7.51 m 128.3 d 7.50 m 128.8 d 7.50 m 128.3 d Ph-4 7.62 m 133.8 d 7.61 m 133.0 d 7.60 m 133.8 d 3'-Ph-1 - 131.3 s - 138.0 s - 131.3 s 3'-Ph-2.6 7.40 br. s 127.0 d 7.39 m 128.4 d 7.38 br. s 127.0 d 3'-Ph-3.5 7.42 br. s 128.0 d 7.40 m 126.7 d 7.31 m 128.8 d 3'-Ph-4 7.40 br. s 128.0 d 7.40 m 128.7 d 129.0 d 128.8 d 127.0 d 127.0 d 3'-Ph-4 7.40 br. s 128.0 d 7.40 m 128.0 d 129.0 d 128.0 d 129.0 d 128.0 d 129.0 d 128.7 d 129.0 d 129.0 d 129.0 d 129.0 d 129.0		2.25 s	20.8 g	2.17 s	20.8 g	2.16 s	20.8 g
Ph-1-129.2 s-129.1 s-129.2 sPh-2.68.13 d (7.2)130.3 d8.10 d (7.2)130.1 d8.10 d (7.2)130.3 dPh-3.57.51 m128.3 d7.50 m128.8 d7.50 m128.3 dPh-47.62 m133.8 d7.61 m133.0 d7.60 m133.8 d3'-Ph-1-131.3 s-138.0 s-131.3 s3'-Ph-1,57.40 br. s127.0 d7.39 m128.4 d7.31 m128.8 d3'-Ph-3,57.42 br. s128.8 d7.40 m126.7 d7.31 m128.8 d3'-Ph-47.34 m129.0 d7.33 m129.1 d7.21 m129.0 dNH-CO6.28 d (8.8)NH-CO-15.1 s12.2 q1.38 m17.5 q1.80 s13.5/13.4 q2"-12.2 q1.38 m17.5 q1.80 s13.5/13.4 q2"-132.0 d1.08 m27.1 q2.98/3.00 q (4.4)5.96/5.9 s3"1.72 d (6.8)13.2 q0.86 t (7.2)11.8 q1.42 d (4.4)12.6/12.7 q	2-OBz	_	167.1 s	_	166.8 s	_	166.9 s
Ph-2.68.13 d (7.2)130.3 d8.10 d (7.2)130.1 d8.10 d (7.2)130.3 dPh-3.57.51 m128.3 d7.50 m128.8 d7.50 m128.3 dPh-47.62 m133.8 d7.61 m133.0 d7.60 m133.8 d3'-Ph-1-131.3 s-138.0 s-131.3 s3'-Ph-2,67.40 br. s127.0 d7.39 m128.4 d7.38 br. s127.0 d3'-Ph-3,57.42 br. s128.8 d7.40 m126.7 d7.31 m128.8 d3'-Ph-47.34 m129.0 d7.33 m129.1 d7.21 m129.0 dNH-C0NH-C0-169.1 s-6.28 d (8.8)NH-C0-162.2 q1.38 m17.5 q1.80 s13.5/13.4 q2"-1.88 c2.34 m43.1 q-60.0/59.9 s3"-182.2 q0.86 t (7.2)11.8 q1.42 d (4.4)59.6/59.5 s	Ph-1	_	129.2 s	_	129.1 s	_	129.2 s
Ph-3,5 7,51 m 128.3 d 7,50 m 128.8 d 7,50 m 128.3 d Ph-4 7,62 m 133.8 d 7,61 m 133.0 d 7,60 m 133.8 d 3'-Ph-1 - 131.3 s - 138.0 s - 131.3 s 3'-Ph-2,6 7,40 br. s 127.0 d 7.39 m 128.4 d 7.38 br. s 127.0 d 3'-Ph-3,5 7,42 br. s 128.8 d 7,40 m 126.7 d 7.31 m 128.8 d 3'-Ph-4 7,34 m 129.0 d 7.33 m 126.7 d 7.31 m 128.8 d 3'-Ph-4 7.34 m 129.0 d 7.33 m 129.1 d 7.21 m 128.8 d NH-CO - - - - - - - - NH-CO - 169.1 s - 176.5 s - - - - 1" 1.62 s 12.2 q 1.38 m 17.5 q 1.80 s 13.5/13.4 q 2" - 138.2 s 2.34 m 43.1 q - 600/59.9 s 3" 6.43 q (6.8) 132.0 d 1.08 m </td <td>Ph-2,6</td> <td>8.13 d (7.2)</td> <td>130.3 d</td> <td>8.10 d (7.2)</td> <td>130.1 d</td> <td>8.10 d (7.2)</td> <td>130.3 d</td>	Ph-2,6	8.13 d (7.2)	130.3 d	8.10 d (7.2)	130.1 d	8.10 d (7.2)	130.3 d
Ph-47.62 m133.8 d7.61 m133.0 d7.60 m133.8 d3'-Ph-1-131.3 s-138.0 s-131.3 s3'-Ph-2,67.40 br. s127.0 d7.39 m128.4 d7.38 br. s127.0 d3'-Ph-3,57.42 br. s128.8 d7.40 m126.7 d7.31 m128.8 d3'-Ph-47.34 m129.0 d7.33 m129.1 d7.21 m129.0 dNH6.51 d (8.8)NH-CO-169.1 s-6.28 d (8.8)1"1.62 s12.2 q1.38 m17.5 q1.80 s13.5/13.4 q2"-138.2 s2.34 m43.1 q-6.0/59.9 s3"6.43 q (6.8)132.0 d1.08 m27.1 q2.98/3.00 q (4.4)59.6/59.3 s4"1.72 d (6.8)13.9 q0.86 t (7.2)11.8 q1.42 d (4.4)12.6/12.7 q	Ph-3,5	7.51 m	128.3 d	7.50 m	128.8 d	7.50 m	128.3 d
3'-Ph-1 - 131.3 s - 138.0 s - 131.3 s 3'-Ph-2,6 7.40 br. s 127.0 d 7.39 m 128.4 d 7.38 br. s 127.0 d 3'-Ph-3,5 7.42 br. s 128.8 d 7.40 m 126.7 d 7.31 m 128.8 d 3'-Ph-4 7.34 m 129.0 d 7.33 m 129.1 d 7.21 m 129.0 d NH 6.51 d (8.8) - - - - - - NH-CO - 160.1 s - 170.5 s - 171.5 s - 1" 1.62 s 122.2 q 1.38 m 175.9 q 1.80 s 13.5/13.4 q 2" - 1.38 m 17.5 q 1.80 s 13.5/13.4 q 2" - 1.38 n 2.34 m 43.1 q - 60.0/59.9 s 3" - 1.80 s 132.0 d 1.08 m 7.1 q 298/3.00 q (4.4) 59.6/59.3 s 4" 1.72 d (6.8) 13.9 q 0.86 t (7.2) 11.8 q 1.42 d (4.4) 12.6/12.7 q	Ph-4	7.62 m	133.8 d	7.61 m	133.0 d	7.60 m	133.8 d
3'-Ph-2,6 7.40 br. s 127.0 d 7.39 m 128.4 d 7.38 br. s 127.0 d 3'-Ph-3,5 7.42 br. s 128.8 d 7.40 m 126.7 d 7.31 m 128.8 d 3'-Ph-4 7.34 m 129.0 d 7.33 m 129.1 d 7.21 m 129.0 d NH 6.51 d (8.8) - 6.28 d (8.8) - - - NH-CO - 160.1 s - 171.5 s 171.5 s 171.5 s 1" 1.62 s 12.2 q 1.38 m 17.5 q 1.80 s 13.5/13.4 q 2" - 1.38 m 17.5 q 1.80 s 3.5/13.4 q 2" - 1.38 m 17.5 q 1.80 s 3.5/13.4 q 2" - 1.38 m 2.34 m 43.1 q - 6.0/59.9 s 3" 6.43 q (6.8) 132.0 d 1.08 m 27.1 q 2.98/3.00 q (4.4) 59.6/59.3 s 4" 1.72 d (6.8) 13.9 q 0.86 t (7.2) 11.8 q 1.42 d (4.4) 12.6/12.7 q	3′-Ph-1	-	131.3 s	_	138.0 s	_	131.3 s
3'-Ph-3,5 7.42 br. s 128.8 d 7.40 m 126.7 d 7.31 m 128.8 d 3'-Ph-4 7.34 m 129.0 d 7.33 m 129.1 d 7.21 m 129.0 d NH 6.51 d (8.8) - 6.28 d (8.8) - - - NH-CO - 169.1 s - 176.5 s - 171.5 s 1" 1.62 s 12.2 q 1.38 m 17.5 q 1.80 s 13.5/13.4 q 2" - 1.38 m 17.5 q 1.80 s 13.5/13.4 q 2" - 1.38 m 17.5 q 1.80 s 13.5/13.4 q 2" - 1.38 m 17.5 q 1.80 s 13.5/13.4 q 2" - 1.32 nd 1.08 m 27.1 q 2.98/3.00 q (4.4) 59.6/59.3 s 3" 6.43 q (6.8) 13.9 q 0.86 t (7.2) 11.8 q 1.42 d (4.4) 12.6/12.7 q	3′-Ph-2,6	7.40 br. s	127.0 d	7.39 m	128.4 d	7.38 br. s	127.0 d
3'-Ph-4 7.34 m 129.0 d 7.33 m 129.1 d 7.21 m 129.0 d NH 6.51 d (8.8) - 6.28 d (8.8) - - - NH-CO - 169.1 s - 176.5 s - 171.5 s 1" 1.62 s 12.2 q 1.38 m 17.5 q 1.80 s 13.5/13.4 q 2" - 38.2 s 2.34 m 43.1 q - 60.0/59.9 s 3" 6.43 q (6.8) 132.0 d 1.08 m 27.1 q 2.98/3.00 q (4.4) 59.6/59.3 s 4" 1.72 d (6.8) 13.9 q 0.86 t (7.2) 11.8 q 1.42 d (4.4) 12.6/12.7 q	3'-Ph-3,5	7.42 br. s	128.8 d	7.40 m	126.7 d	7.31 m	128.8 d
NH 6.51 d (8.8) - 6.28 d (8.8) - <td>3'-Ph-4</td> <td>7.34 m</td> <td>129.0 d</td> <td>7.33 m</td> <td>129.1 d</td> <td>7.21 m</td> <td>129.0 d</td>	3'-Ph-4	7.34 m	129.0 d	7.33 m	129.1 d	7.21 m	129.0 d
NH-CO - 176.5 s - 171.5 s 1" 1.62 s 12.2 q 1.38 m 17.5 q 1.80 s 13.5/13.4 q 2" - 138.2 s 2.34 m 43.1 q - 60.0/59.9 s 3" 6.43 q (6.8) 132.0 d 1.08 m 27.1 q 2.98/3.00 q (4.4) 59.6/59.3 s 4" 1.72 d (6.8) 13.9 q 0.86 t (7.2) 11.8 q 1.42 d (4.4) 12.6/12.7 q	NH	6.51 d (8.8)	_	6.28 d (8.8)	_	_	-
1" 1.62 s 12.2 q 1.38 m 17.5 q 1.80 s 13.5/13.4 q 2" - 138.2 s 2.34 m 43.1 q - 600/59.9 s 3" 6.43 q (6.8) 132.0 d 1.08 m 27.1 q 2.98/3.00 q (4.4) 59.6/59.3 s 4" 1.72 d (6.8) 13.9 q 0.86 t (7.2) 11.8 q 1.42 d (4.4) 12.6/12.7 q	NH-CO	_	169.1 s	_	176.5 s	_	171.5 s
2" - 138.2 s 2.34 m 43.1 q - 60.0/59.9 s 3" 6.43 q (6.8) 132.0 d 1.08 m 27.1 q 2.98/3.00 q (4.4) 59.6/59.3 s 4" 1.72 d (6.8) 13.9 q 0.86 t (7.2) 11.8 q 1.42 d (4.4) 12.6/12.7 q	1″	1.62 s	12.2 a	1.38 m	17.5 a	1.80 s	13.5/13.4 a
3" 6.43 q (6.8) 132.0 d 1.08 m 27.1 q 2.98/3.00 q (4.4) 59.6/59.3 s 4" 1.72 d (6.8) 13.9 q 0.86 t (7.2) 11.8 q 1.42 d (4.4) 12.6/12.7 q	2″	_	138.2 s	2.34 m	43.1 g	_	60.0/59.9 s
4" 1.72 d (6.8) 13.9 q 0.86 t (7.2) 11.8 q 1.42 d (4.4) 12.6/12.7 q	3″	6.43 g (6.8)	132.0 d	1.08 m	27.1 g	2.98/3.00 g (4.4)	59.6/59.3 s
	4″	1.72 d (6.8)	13.9 q	0.86 t (7.2)	11.8 g	1.42 d (4.4)	12.6/12.7 a

^a The ¹H and ¹³C NMR data of the diastereoisomers 4 and 5 were completely identical.
^b The diastereoisomers 6 and 7 had a weak difference in ¹³C NMR data.

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Table 2Cytotoxicity of paclitaxel and compounds 4, 5, 6 and 7.

Compd.	IC ₅₀ (nM)						
	HCT-8	Bel-7402	BGC-823	A549	A2780		
Paclitaxel	51	6.0	3.29	16	7.86		
4	460	12.5	16.5	240	18.6		
5	680	7.2	2.14	22	9.45		
6	220	10.5	9.45	86	12.8		
7	25	5.2	2.66	5.0	7.05		

acid gave a peak at t_R 11.4 and 12.5 min. The other two residues, from compounds **6** and **7**, were separated via column chromatography over silica gel eluting with EtOAc followed by CHCl₃–MeOH (1:2) to yield (2R, 3S)- or (2S, 3R)-2,3epoxy-2-methylbutanoic acid, respectively, for measuring optical rotations.

2.6. Cytotoxicity bioassays

The cytotoxicity assays against the HCT-8 human colorectal adenocarcinoma cell line, Bel-7402 human liver cancer cell line, BGC-823 human gastric cancer cell line, A549 human lung carcinoma cell line and the A2780 human ovarian were accessed using MTT method.

Cells were plated in the RPMI 1640 with 10% fetal calf serum media on 96-well plates in a total volume of 100 μ L with a density of 1×10^{-4} cells mL⁻¹. Triplicate wells were treated with media and tested compounds. The plates were incubated at 37 °C in 5% CO₂ for 72 h. Cell viability was determined based on mitochondrial conversion of 3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma) to formazan. The amount of MTT converted to formazan is a sign of the number of viable cells. Each well was supplemented with 50 μ L of a 1 mg mL⁻¹ solution of MTT in uncompleted media. The plates were incubated in 37 °C, 5% CO₂ for an additional 4 h. The media was carefully removed from each well and then 200 µL of DMSO was added. The plates were gently agitated until the reaction color was uniform and the OD₅₇₀ was determined using a micro-plate reader (Wellscan MK3, Labsystems Dragon). Microsoft® Excel 2000 was used to analyze data. Media-only treated cells served as the indicator of 100% cell viability. The 50% inhibitory concentration (IC₅₀) was defined as the concentration that reduced the absorbance of the untreated wells by 50% of the control in the MTT assay.



Fig. 2. Synthesis of paclitaxel analogs from cephalomannine.

3. Results and discussion

Compounds **4** and **5** were produced by catalytic hydrogenation of the N-tigloyl double bond of cephalomannine (Fig. 2). The ¹H-NMR results showed that the product of the hydrogenation appeared to be a single compound. In particular, the signal for the vinyl proton on the cephalomannine side-chain double bond disappeared ($\delta_{\rm H}$, 6.43, 1H, q, J = 6.8 Hz, Table 1) and the signal for the 4'-methyl group appeared as a triplet signal ($\delta_{\rm H}$, 0.86, 3H, t, J = 7.2 Hz, Table 1). However, HPLC results showed that there was a pair of products present in a 1:1 ratio, which indicates that there was no stereoselectivity of the hydrogenation of the cephalomannine $\Delta^{2^{*},3^{*}}$ double bonds, and a pair of diastereoisomers was formed. Therefore, optically pure compounds **4** and **5** were obtained by separating the hydrogenation products using preparative HPLC.

Next, analysis was performed to confirm the stereoconfiguration of the newly-formed C-2" chiral carbon in compounds **4** and **5**. There were no significant differences between the circular dichroism (CD) spectra of compounds 4 and 5, and hence, the absolute configurations of these two compounds could not be determined using CD spectroscopy. Gabetta et al. isolated *N*-debenzoyl-*N*-(2-methylbutanoyl) paclitaxel from Taxus media cv. Hicksii and confirmed that the stereoconfiguration of its 2-methylbutanoyl structural unit was S, and the optical rotation of this compound is $[a]_{D}^{25} - 48$ ° (c 0.10, MeOH) [17]. In this study, the optical rotations of **4** and **5** are $[a]_D^{25} - 49^\circ$ (c 0.10, MeOH) and $[a]_D^{25} - 25^\circ$ (c 0.10, MeOH), respectively. Hence, it can be deduced that compound 4 and the N-debenzoyl-N-(2-methylbutanoyl) paclitaxel isolated by Gabetta et al. are the same. Therefore, the 2-methylbutanoyl structural unit in compound **4** has the S configuration, while C2" in compound 5 has the R configuration. In order to further confirm the stereoconfigurations of compounds 4 and 5, gas chromatography (GC) was used to analyze the hydrolyzates of both compounds in comparison to a standard control. The $t_{\rm R}$ values of the 2-methylbutyric acids obtained from complete hydrolysis of compounds 4 and 5 were 12.5 min and 11.4 min, respectively, which correspond to (S)- and (*R*)-2-methylbutyric acid. Thus, the structures of compounds **4** and **5** were *N*-debenzoyl-*N*-(*S*-2-methylbutanoyl)paclitaxel and N-debenzoyl-N-(R-2-methylbutanoyl)paclitaxel, respectively. All ¹H and ¹³C NMR data for compounds **4** and **5** were assigned by comparison with cephalomannine and paclitaxel NMR data (Table 1).

Compounds **6** and **7** were produced by the epoxidation of cephalomannine (Fig. 2). The ¹H NMR results showed that the products were a pair of diastereomers, in which the signal of the proton on the double bond of the side chain of cephalomannine ($\delta_{\rm H}$, 6.43, 1H, q, J = 6.8 Hz, Table 1) was replaced with a proton on an epoxy carbon ($\delta_{\rm H}$, 2.98/3.00, 1H, q, J = 4.4 Hz). The HPLC results also showed that the epoxidation products were a pair of compounds with a ratio of 1:1, which again indicates that that there was no stereoselectivity of the epoxidation of the double bonds. Optically pure compounds **6** and **7** were obtained using preparative HPLC, and there were negligible differences between the ¹H and ¹³C NMR signals of the two compounds (Table 1).

In order to determine the stereo configuration of the newly formed epoxy group in compounds **6** and **7**, complete hydrolysis was carried out. A pair of 2,3-epoxy-2-methylbutanoic acid enantiomers with rotation values of $[a]_D^{25} - 5^{\circ}$ and $+5^{\circ}(c \ 0.10, CHCl_3)$ was obtained, respectively, which is consistent with the optical rotation values of $(2R_3S)$ - and $(2S_3R)$ -2,3-epoxy-2-methylbutanoic acid obtained from an asymmetric synthesis [18]. Thus, it can be deduced that the structures of compounds **6** and **7** were *N*-debenzoyl-*N*-[($2R_3S$)-2,3-epoxy-2-methylbutanoyl]paclitaxel and *N*-debenzoyl-*N*-[($2S_3R$)-2,3-epoxy-2-methylbutanoyl]paclitaxel, respectively. All ¹H and ¹³C NMR data for compounds **4** and **5** were assigned by comparison with cephalomannine, paclitaxel, and 2,3-epoxy-2-methylbutanoic acid NMR data (Table 1).

With paclitaxel and as a positive control, the cytotoxic activities of compounds **4**, **5**, **6**, and **7** were evaluated in five types of tumor cells, and the results are shown in Table 2. The activities of compounds **4** and **6** are weaker than that of paclitaxel, while the activities of compounds **5** and **7** are comparable to those of paclitaxel. Furthermore, the activity of compound **5** in the BGC-823 tumor cell line was superior to those of paclitaxel, and the activities of compound **7** in HCT-8 and A549 cells were 2 and 3 times higher than those of paclitaxel, respectively.

In summary, four paclitaxel derivatives were obtained by preparative HPLC separation of two pairs of diastereoisomers, which were obtained from catalytic hydrogenation and epoxidation of the C-13 side-chain double bond of cephalomannine and their stereostructures were confirmed by chemical and spectral methods. Various cytotoxic activities of these two pairs of diastereoisomers indicated that the stereoconfiguration of the *N*-acyl in the paclitaxel side chain has a significant impact on its activity, and increasing the oxygenation of *N*-acyl side chains improves the activity. This result can further enrich our understanding on the structure–activity relationship in paclitaxel.

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Appendix A. Supplementary data

Supplementary data associated with this article, including ¹H and ¹³C NMR spectra, CD and HPLC spectra, can be found in the online version, at doi: http://dx.doi.org/10.1016/j.fitote. 2013.07.011.

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