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Design and Syntheses of Putative Bioactive Taxanes

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Abstract—Reduction of 5α-hydroxy-7β,9α,10β-triacetoxy-4(20), 11(12)-taxadien-13-one **1** with activated zinc in glacial acetic acid led to rearranged products, including compounds with double bonds at C3–C4, C10–C11 or with an epoxide at C11–C12. Molecular modeling studies suggested that addition of a side chain at C-20 or C-5 of the taxanes with a C3–C4 double bond might lead to bioactivity. Semi-syntheses and results of bioactivities are discussed.

Introduction

Taxus canadensis is a low trailing shrub very common in Quebec. Its composition of taxanes was shown to differ from other yews.[1–11] It is the only species producing 9-dihydro-13-acetylbaccatin III as the most abundant taxane.[1,2,11] In addition, taxinine and taxinine E are found in larger amounts than other taxanes.[8] Previous work in our laboratory suggested that a major conformational change of the core skeleton of a taxane can lead to unusual structure–activity-reactivities.[7,8,12] For example, taxuspine D, a taxane isolated from the Canadian yew needles[8] as well as from the Japanese yew,[13,14] was found to promote the polymerization of tubulin with a potency corresponding to half of the activity of paclitaxel.[13] This result was surprising since taxuspine D lacked all the key features essential for bioactivity: a C-13 side chain with a 2′-OH, a benzoyl group on C-2 and an oxetane on C4-C5.[15–17] We explained this result by molecular modeling studies.[12] The C12–C13 double bond caused a substantial change in the conformation of the core skeleton, and the C-5 cinnamoyl in taxuspine D is found to mimic part of the C-13 side chain of paclitaxel.

In this paper, we wanted to test this hypothesis by building different taxane core skeletons with side chains situated elsewhere than on C-13. We report the rearrangement reactions of a taxinine derivative: 5α-hydroxy-7β,9α,10β-triacetoxy-4(20), 11(12)-taxadien-13-one **1** when treated with activated zinc in glacial acetic acid. We obtained taxanes with C3–C4, or C10–C11 double bonds as well as a C11–C12 epoxide. Molecular modeling studies, semi-syntheses, and bioactivities of the C3–C4 double bond taxanes with C-5 or C-20 side chains are reported.

Results and Discussion

Rearrangement of 5α-hydroxy-7β, 9α, 10β-triacetoxy-4(20), 11(12)-taxadien-13-one, 1 (Scheme 1)

Initially, we intended to prepare taxinine derivatives with C12–C13 double bonds. We, therefore, used the reaction which had been successfully reported for taxanes.[18] We were surprised to obtain unusual taxinine derivatives, none of which had a C12–C13 double bond. Indeed, treatment of **1**[19,20] with activated Zn in glacial acetic acid gave three major peaks on the analytical HPLC. Two pure compounds were isolated upon purification by semi-preparative HPLC and were identified as compounds **2** (13%) and **3** (21%). ^{1}H NMR spectroscopy of the third peak isolated proved to be a mixture

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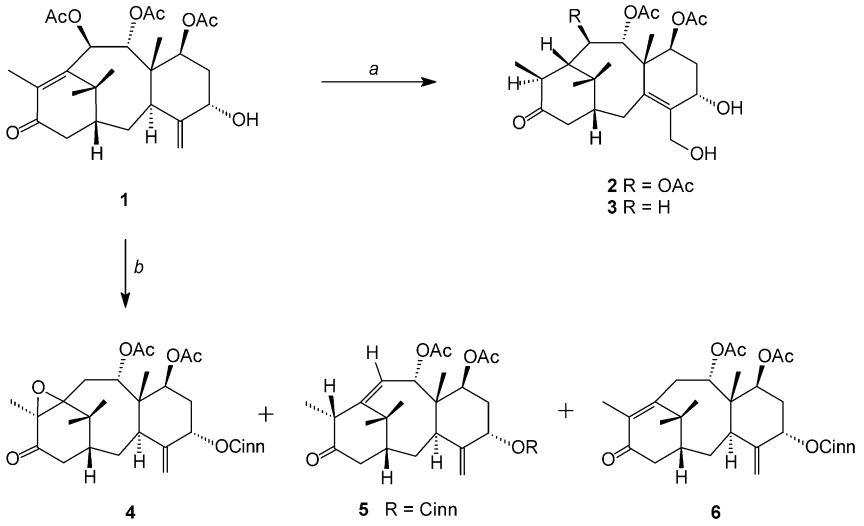
of three compounds. These peaks could not be purified either by HPLC or preparative TLC. Cinnamoylation proved useful as we were able to purify the cinnamoylated products. Upon purification by semi-preparative HPLC, three compounds were isolated and identified as compounds **4** (37%), **5** (19%), and **6** (38%). This reaction was repeated several times and depending on the length of the reaction, the ratio between compounds **2**–**6** could be changed. Upon treatment of **1** with Zn in glacial acetic acid for a longer period of time (42 h compared to 25 h) the amounts of **2** and **3** were decreased and we were able to isolate **5** as the major compound from the mixture. The ^{1}H NMR spectra of compounds **2** and **3** were very similar and only differed in the signals of H-9, H-10, and H-11. The chemical shift of H-9 changed from δ 6.20 in **2** to δ 5.90 in **3**. The C-10 proton doublet at δ 5.46 in **2** was replaced by two aliphatic proton signals at δ 2.22 and δ 1.72 in **3**. The chemical shift of H-11 changed from δ 1.64 in **2** to δ 1.46 in **3**. The upfield changes to these proton signals and the appearance of the two aliphatic protons confirm the deacetylation of **2** at C-10. This was also confirmed by the high-resolution mass spectrometry of the potassium adducts: *m*/*e* 533.2154 for **2** and *m*/*e* 475.2099 for **3**, a difference of 58 units confirming the loss of an acetyl group. The downfield shift of C-3 at δ 34.8 in **1** to δ 140.6 in **2** or δ 141.7 in **3** and the upfield shift of C-20 at δ 111.3 in **1** to δ 64.4 in **2** and δ 64.8 in **3** confirm the shift of the double bond from C4–C20 to C4–C3. The upfield shift of C-11 at δ 151.2 in **1** to δ 54.0 in **2** or δ 48.3 in **3** as well as the upfield shift of C-12 at δ 138.5 in **1** to δ 44.0 in **2** or δ 47.3 in **3** confirm the disappearance of the C12–C13 double bond. The new H-12 proton at δ 2.58 for **2** or δ 2.55 for **3** had a strong NOE correlation with H-7, thereby confirming its α-orientation, and indicating that Me-18 is β. The new H-11 proton at δ 1.64 for **2** or δ 1.46 for **3** had a strong correlation with Me-18, thereby confirming its β-orientation.

The characterization of **5**, albeit without the cinnamoyl group at C-5, was discussed in our previous publication.[20] The downfield shift of H-5 at δ 4.26 in **1** to δ 5.43 in **6** confirmed the positioning of the cinnamoyl group at C-5. Only two acetyls were observed and they are located on C-7 and C-9, as confirmed by HMBC correlations of H-7 (δ 5.64) and H-9 (δ 5.57) with carbonyl acetyls δ 170.1 and δ 170.1, respectively. The disappearance of the H-10 doublet at δ 6.32 in **1** and the appearance of the aliphatic protons H-10a and H-10b in **6** at δ 3.37 and δ 2.53 is consistent with deacetylation at C-10. When comparing the NMR data of **4** to **6** we observe that the major differences occur in ring A where the double bond between C-11 and C-12 in **6** has been replaced by an epoxide in **4**. Me-18 is correlated to two relatively deshielded carbons, C-11 (δ 65.2) and C-12 (δ 59.4) and to a carbonyl, C-13 (δ 210.5). The NOE correlations between H-3, H-7 and Me-18 confirm the α orientation of Me-18. The NMR spectral data as well as the HMBC and NOESY correlations of taxanes **2**, **4** and **6** are shown in Tables 1–3.

The chemistry of taxanes is unusual and we did not predict these rearrangements. Once the structures were confirmed, mechanisms could be postulated. For example, the conversion of compound **1** to **2** involved attack of OAc^- on C-20 followed by migration of the C4–C20 double bond to its more stable tetrasubstituted C3–C4. The transfer of the C3 hydride to C-11 was aided by the U-configuration of the taxane. The C11–C12 double bond picked up H^+ to give taxane **2**. Taxane **3** was obtained from additional reduction of compound **2**. Similarly, the formation of compound **5** can be easily rationalized by a hydride attack on C12 of taxane **1** triggering migration of the C12–C11 double bond to C11–C10 and elimination of OAc^-. Taxane **6** was obtained by reduction of **1**. We were puzzled by the transformation of taxane **1** to **4**. The existence of the epoxide is unambiguously confirmed: two quaternary carbons at C11-C12 from the ^{13}C NMR and the extra oxygen atom in the FAB-HR-MS. The only possibility is the transfer of an oxygen either from the neighboring acetyl group or from the acetic acid.



Scheme 1. (a) Zn, glacial acetic acid, 23 °C, 25 h, taxane **2** 13%, taxane **3** 21%; (b) (i) Zn, glacial acetic acid, 23 °C, 25 h; (ii) *trans*-cinnamic acid, DCC, 4-DMAP, toluene, 90 °C, 5 h, taxane **4** 37%, taxane **5** 19%, taxane **6** 38%.

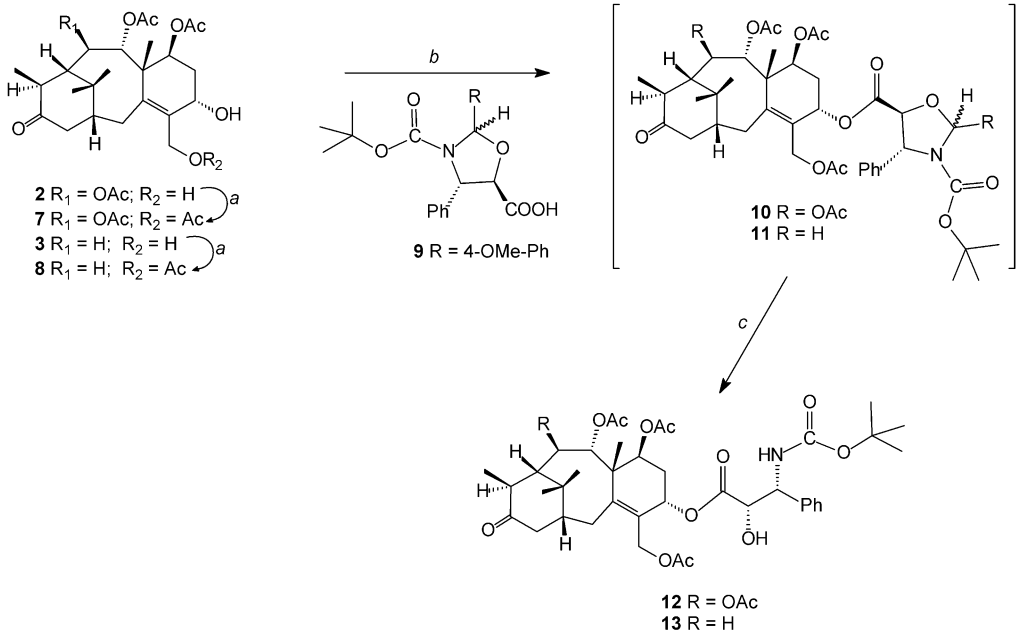
Semi-syntheses of taxanes 12, 13, 15 (Schemes 2 and 3)

The semi-syntheses of compounds **12** and **13** (Scheme 2) were very similar and provided compounds having a C-5 side chain. In order to esterify the C5-OH of compounds **2** or **3** with the docetaxel side chain, it was first necessary to protect the primary C-20 alcohol. Acetylation of compounds **2** and **3** with acetyl chloride in pyridine, afforded compounds **7** (59% yield) and **8** (36% yield), respectively. Esterification of the free C5-OH was

Table 1. ^{1}H and ^{13}C NMR for taxane **2** in $CDCl_3$

Position	δ ^{1}H mult.[a] *J* (*J* in Hz)	δ ^{13}C[b]	HMBC	NOESY[c]
1	2.05 o.m	44.0		$2a^{s}$, $14a^{s}$, 16^{s}, 17^{s}, 19^{s}, (5^{s}, $6b^{s}$)
2a	2.80 dd (*15.3, 6.5*)	26.7	1, 3, 4, 8	1^{w}, 2b/$14a^{s}$, $20a^{s}$
2b	2.65 o.m			
3	—	140.6		
4	—	134.5		
5	4.39 br. s	67.7		$6a^{m}$, $20b^{m}$
6a	2.04 o.m	33.2		See 1
6b	1.93 o.m			5^{w}, $6a^{w}$, 7^{w}
7	5.81 dd (*12.0, 4.6*)	66.3	6, 9, 19, 169.0	$6b^{w}$, 10^{s}, 12^{s}
8	—	46.2		
9	6.20 d (*11.0*)	75.7	3, 7, 8, 10, 19, 169.9	17^{s}, 19^{m}, 2b/$14a^{s}$
10	5.46 d (*11.0*)	72.9	9, 15, 168.5	7^{s}, 11^{s}, 12^{s}, 18^{s}
11	1.64 d (*8.0*)	54.0		10^{s}, 16^{s}, 18^{s}
12	2.58 qu (*7.0*)	44.0		7^{s}, 10^{m}, 18^{s}
13	—	215.2		
14a	2.66 o.m	39.2	1, 2, 13	
14b	2.51 d (*19.9*)		1, 2, 13, 15	$14a^{s}$, $20a^{s}$
15	—	36.5		
16	0.91 s	35.0	1, 11, 15, Me-17	1^{m}, $14a^{m}$, 11^{m}, 17^{s}
17	1.48 s	25.8	1, 11, 15, Me-16	1^{m}, 9^{s}, 16^{s}
18	1.27 d (*6.4*)	15.0	11, 12, 13	10^{s}, 11^{s}, 12^{s}
19	1.18 s	22.6	3, 7, 8, 9	1^{s}, 9^{s}, $2b^{w}$
20a	4.52 d (*11.6*)	64.4	3, 4, 5	$2a^{s}$, $14b^{m}$, $20b^{s}$
20b	4.20 d (*11.6*)		3, 4, 5	5^{s}, $20a^{s}$
OAc	2.06 s	21.1	169.0 (C-7)	
OAc	2.02 s	20.5	169.9 (C-9)	
OAc	1.97 s	20.9	168.5 (C-10)	

[a]Mult. multiplicity: s, singlet; d, doublet; qu, quintet; dd, doublet of doublet; br, broad; m, multiplet; o, overlapping. The precision of the coupling constants is ±0.5 Hz.
[b]The ^{13}C chemical shifts were extracted from the HSQC and HMBC experiments (for quaternary carbons) (±0.2 ppm).
[c]NOESY intensities are marked as strong (s), medium (m) or weak (w).



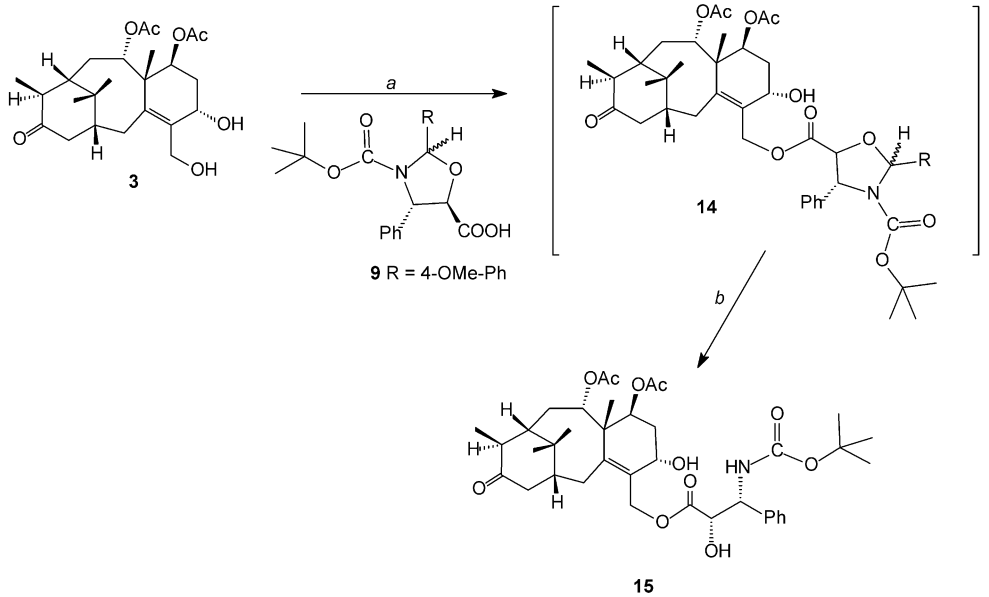
Scheme 2. (a) Acetyl chloride, pyridine, 4 °C, 18 h, taxane **7** 59%, taxane **8** 36%; (b) **9**, DCC, 4-DMAP, CH_2Cl_2–toluene, 75 °C, 5 h, taxane **10** (C-10 R = OAc, side chain R = 4-OMe-Ph) 29%, taxane **11** (C-10 R = H, side chain R = 4-OMe-Ph) 28%; (c) *p*-toluenesulfonic acid, methanol, 23 °C, taxane **12** 57%, taxane **13** 58%.

realized with 2-(4-OMe)phenyl-1,3-oxazolidine of *N*-Boc phenylisoserine (**9**),[21] dicyclohexylcarbodiimide and 4-(dimethylamino)pyridine, in dichloromethane–toluene at 75 °C affording diastereomers **10** (29%) and **11** (28%), respectively. Deprotection of **10** and **11** with a catalytic amount of *p*-toluenesulfonic acid in methanol afforded compounds **12** (57%) and **13** (58%), respectively. The major differences in the NMR spectral data between compounds **2** and **12** occur in ring C. A fourth acetyl group is observed in **12** and is located on C-20 as

Table 2. ^{1}H and ^{13}C NMR for taxane **4** in $CDCl_3$

Position	δ ^{1}H mult.[a] *J* (*J* in Hz)	δ ^{13}C[b]	HMBC	NOESY[c]
1	1.99 o.m	42.1		
2a/**2b**	1.98/1.95 o.m	24.5		
3	2.75 o.m	36.9		2^{w}, 7^{s}, $14b^{s}$, 16^{m}, 18^{m} (o.14a)
4	—			
5	5.60 br.dd (*4.0, 1.6*)	74.8		$20a^{s}$
6a	2.07 o.m	33.6		
6b	1.91 o.m			
7	5.75 dd (*11.3, 5.2*)	68.4	19, 170.4	3^{w}, $6a^{w}$, 18^{w}
8	—	47.0		
9	5.66 dd (*12.3, 4.5*)	77.2	8, 170.4	$2/10^{s}$, 17^{s}, 19^{m}
10a	2.16 dd (*14.9, 4.5*)	30.9		
10b	2.09 o.dd (*14.9, 12.3*)			
11	—	65.2		
12	—	59.4		
13	—	210.5		
14a	2.72 dd (*19.4, 8.5*)	41.2	2, 12, 13	See H-3
14b	1.90 o.d (*19.4*)			
15	—	40.6		
16	0.78 s	28.2	1, 11, 15, Me-17	1^{m}, $14a^{m}$, 17^{s}
17	1.58 s	23.9	1, 11, 15, Me-16	1^{m}, 9^{s}, $10a^{w}$, 16^{s}
18	1.98 s	14.7	11, 12, 13	
19	0.88 s	12.8	3, 7, 8, 9	$6b/2^{s}$, 9^{m}, $20b^{w}$
20a	5.45 s	116.6	5	5^{s}, $20b^{s}$
20b	5.10 s		3, 5	2^{m}, $20a^{s}$
OAc	2.05 s	21.1	170.4 (C-7)	
OAc	2.05 s	21.1	170.4 (C-9)	
1′(CO)	—	165.9		
2′ CH=	6.25 d (*15.7*)	117.1	134.1, 165.9	Me-18 (overlap)
3′=CH	7.62 d (*15.7*)	146.7		
4′-Ph	—	134.1		
o	7.59 br.d (∼*7.3*)	128.0		
m*/*p	7.41 m	129.3		
		130.0		

[a]Mult. multiplicity: s, singlet; d, doublet; dd, doublet of doublet; br, broad; m, multiplet; o, overlapping. The precision of the coupling constants is ±0.5 Hz.
[b]The ^{13}C chemical shifts were extracted from the HSQC and HMBC experiments (for quaternary carbons) (±0.2 ppm).
[c]NOESY intensities are marked as strong (s), medium (m) or weak (w).



Scheme 3. (a) **9**, DCC, 4-DMAP, CH_2Cl_2–toluene, 4 °C, 18 h, taxane **14** (side chain R = 4-OMe-Ph) 12%; (b) *p*-toluenesulfonic acid, methanol, 23 °C, 18 h, taxane **15** 58%.

confirmed by HMBC correlations of H-20 (δ 5.12) with the carbonyl acetyl at δ 170.0. The downfield shift of H-5 at δ 4.39 in **2** to δ 5.69 in **12** confirmed the positioning of the docetaxel group at C-5. Similar comparisons can be made between compounds **3** and **13**. A third acetyl group is observed in **13** and is located on C-20 as confirmed by HMBC correlations of H-20 (δ 5.14) with the carbonyl acetyl at δ 170.3. The downfield shift of H-5 at δ 4.39 in **3** to δ 5.73 in **13** confirmed the positioning of the docetaxel group at C-5. The NMR spectral data as well as the HMBC and NOESY correlations of taxane **12**, are shown in Table 4.

The semi-synthesis of compound **15** from **3** afforded a compound with a C-20 side chain. We postulated that the primary C-20 alcohol of **3** would be more reactive than the secondary alcohol at C-5 especially at lower temperatures. Direct esterification of **3** was realized with 2-(4-OMe)phenyl-1,3-oxazolidine of *N*-Boc phenylisoserine (**9**),[21] dicyclohexylcarbodiimide and 4-(dimethylamino)pyridine, in dichloromethane–toluene at 4 °C affording diastereomers **14** (12%). Deprotection of **14** with a catalytic amount of *p*-toluenesulfonic acid in methanol afforded compound **15** (58%). The downfield shift of H-20 at δ 4.20 in **3** to δ 4.80 in **15** confirmed the positioning of the docetaxel group at C-20.

Molecular modeling of taxanes

In an attempt to rank the priority of compounds to be synthesized, we built a database of 28 virtual compounds, including the known bioactive compounds paclitaxel, docetaxel and taxuspine D. The database was docked against the refined structure of β-tubulin[12] using the DOCK program.[22] According to the intermolecular energy dock score (Table 5), compounds **12** (−38.4 kcal/mol) and **13** (−36.1 kcal/mol) were predicted to be bioactive since their dock scores were similar to that of docetaxel (−37.3 kcal/mol). In addition, compounds **15** (−33.5 kcal/mol) and a virtual analogue of compound **5** (Scheme 1) wherein the cinnamoyl group had been replaced by the paclitaxel side chain, compound **16** (−33.0 kcal/mol) had a similar dock score to taxuspine D (−33.8 kcal/mol) and, therefore, were predicted to be bioactive. However, after compounds **12**, **13**, and **15** were synthesized, the tubulin assembly assay showed no bioactivity at 10 μM concentrations.[23] This prompted us to investigate the reason for the false positives in the docking study. It is well known that solvation free energies are omitted in the DOCK scoring function although they can be taken into account implicitely.[22] We therefore calculated the solvation free energy using the AM1-SM5.4PDA model.[24] In order to speed up the

Table 3. ^{1}H and ^{13}C NMR for taxane **6** in $CDCl_3$

Position	δ ^{1}H mult.[a] *J* (*J* in Hz)	δ ^{13}C[b]	HMBC	NOESY[c]
1	2.23 br.t (*5.7*)	40.1		$2a^{w}$, $2b^{m}$, $14a^{s}$, 16^{s}, 17^{s}
2a	1.95 o.m	25.4		See 14a
2b	1.85 o.m			1^{w}, $2a^{w}$, 19^{w} (see 6b)
3	3.22 br.d (*4.4*)	36.3		7^{m}, $2b^{m}$
4	—	147.1		
5	5.43 br.t (∼*2.9*)	74.6		$6a^{w}$, $6b^{w}$, $20a^{s}$
6a	2.07 o.m	34.0		5^{s}, $6b^{s}$, 7^{s}
6b	1.79 o.m			5^{s}, $6a^{s}$, 7^{m}, 19^{w} (see 2b)
7	5.64 dd (*11.7, 5.5*)	69.7	6, 8, 19, 170.1	3^{s}, $6a^{s}$, 10^{s}, 18^{s}
8	—	48.0		
9	5.57 dd (*11.7, 4.7*)	76.5	7, 8, 10, 19, 170.1	$2a^{s}$, $10b^{s}$, 17^{s}, 19^{m}
10a	3.37 t (*12.3*)	33.1	9, 11, 12, 16	7^{m}, $10b^{s}$, 18^{s}
10b	2.53 dd (*12.7, 4.9*)			9^{m}, $10a^{s}$, 17^{m}
11	—	157.2		
12	—	135.1		
13	—	200.0		
14a	2.91 dd (*19.4, 7.0*)	39.7		1^{w}, $14b^{s}$, 16^{w}, 19^{w}, $20b^{w}$
14b	1.94 o.d (*19.4*)		1/15, 2, 13	$14a^{w}$
15	—	40.1		
16/17	1.16 s	36.6	1/15, 11, Me	1^{m}, 17^{s}
17/16	1.57 s	24.6	1/15, 11, Me	1^{m}, $2a^{m}$, 9^{s}, $10b^{m}$, 16^{s}
18	2.19 s	13.9	11, 12, 13	3^{w}, 7^{s}, $10a^{s}$, $3'^{m}$
19	0.83 s	12.9	3, 7, 8, 9	9^{w}
20a	5.26 s	114.2	3, 5	5^{s}, $20b^{s}$
20b	4.91 s		3, 5, 4 (weak)	$2b^{s}$, $20a^{s}$
OAc	2.07 s	21.2	170.1	
OAc	2.05 s	20.9	170.1	
1′ (CO)	—	166.2		
2′ CH=	6.44 d (*16.1*)	118.1	134.4, 166.2	Me-18
3′=CH	7.63 d (*16.1*)	145.6	128.6, 118.1	
4′ Ph	—	134.4		
o	7.74 d (*7.7*)	128.6	145.6, 130.4	
m	7.43 m	128.9		
p	7.38 m	130.4		

[a]Mult. multiplicity: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; br, broad; m, multiplet; o, overlapping. The precision of the coupling constants is ±0.5 Hz.
[b]The ^{13}C chemical shifts were extracted from the HSQC and HMBC experiments (for quaternary carbons) (±0.2 ppm).
[c]NOESY intensities are marked as strong (s), medium (m) or weak (w).

Table 4. ^{1}H and ^{13}C NMR for taxane **12** in $CDCl_3$

Position	δ ^{1}H mult.[a] *J* (*J* in Hz)	δ ^{13}C[b]	HMBC	NOESY[c]
1	2.14 o.t (*7.4*)	44.2		$2a^{m}$, $14a^{w}$, 16^{m}, 17^{s}
2a	2.99 dd (*15.5, 7.2*)	27.5		1^{w}, $2b^{s}$, $20a^{s}$
2b	2.76 d (*15.8*)			$2a^{s}$, 9^{s}, 17^{m}, 19^{w}
3	—	147.6		
4	—	127.9		
5	5.69 br. s	68.4		6^{w}
6a/b	2.0 o.m	30.7		5^{s}, 7^{w}, 19^{s}
7	5.74 t (8.8)	65.7		6^{s}, 10^{s}, 12^{s}
8	—	46.2		
9	6.19 d (*11.2*)	75.5	7, 8, 10, 169.9	$2b^{s}$, 17^{s}, 19^{m},
10	5.46 br.d (*11.2*)	73.1		7^{s}, 11^{s}, 12^{w}, 18^{w}
11	1.70 br.d (*6.5*)	53.3		10^{w}, 16^{s}, 18^{s}
12	2.29 qu (*7.0*)	44.0		7^{s}, 10^{m}, 18^{s}
13	—	217.5		
14a	2.66 br.dd (*20.3, 7.3*)	38.8		1^{m}, $14b^{s}$, 16^{s}
14b	2.02 o.m			$14a^{s}$, $20a^{w}$
15	—	36.2		
16	0.92 s	34.9	1, 11, 15, Me-17	1^{w}, 11^{s}, 17^{s}
17	1.48 s	26.1	1, 11, 15, Me-16	1^{w}, $2b^{w}$, 9^{s}
18	1.31 br.d	17.2		10^{m}, 11^{s}, 12^{w}
19	1.18 s	22.8	3, 7, 8, 9	6^{w}, 9^{w}
20a	5.12 d (*11.9*)	61.7	3, 4, 5, 170.0	$2a^{w}$, $14b^{w}$, $20b^{s}$
20b	4.01 br.d (*11.9*)			$20a^{s}$
2′	4.52 br	74.4		$3'^{w}$
OH-2′	3.81 br			
3′	5.09 o.br	56.9		
3′-Ph				
o	7.43 d (*7.5*)	126.5		
m	7.35 t (*7.8*)	128.5		
p	7.26 o.t	127.3		
4′(NH)	5.81 br.d (*8.9*)			
8′ *t*-Bu(Me)	1.39 br	28.3		
OAc	2.06 s	21.2		
OAc	2.04 s	20.6		
OAc	2.02 s	20.55		
OAc	2.98 s	21.0		

[a]Mult. multiplicity: s, singlet; d, doublet; t, triplet; qu, quintet; dd, doublet of doublet; br, broad; m, multiplet; o, overlapping. The precision of the coupling constants is ±0.5 Hz.
[b]The ^{13}C chemical shifts were extracted from the HSQC and HMBC experiments (for quaternary carbons) (±0.2 ppm).
[c]NOESY intensities are marked as strong (s), medium (m) or weak (w).

process, only single point calculations were performed on the docked conformations. Consequently, the change of conformational energy on binding were omitted. The results were reported in Table 5. Our results clearly show that solvation free enegies are an important factor in ligand binding. As indicated by the corrected dock scores, compounds **12** (−7.4 kcal/mol), **13** (−8.1 kcal/mol) and **15** (−7.1 kcal/mol) should be inactive since their corrected dock scores were much lower than that of taxuspine D (−12.3 kcal/mol). Interestingly, the ranking of taxane **16** (−14.5 kcal/mol), remained above that of taxuspine D after the correction of the solvation free energy. This virtual compound has a double bond at C10–C11 and a paclitaxel side chain at C-5. The synthesis of this compound is in progress.

Table 5. Docks intermolecular energy score, the solvation free energy (ΔG_{solv}) and the corrected dock score (kcal/mol)

Compounds	Dock score	ΔG_{solv}	Corrected dock score
Paclitaxel	−44.4	−26.1	−18.3
Docetaxel	−37.3	−22.5	−14.8
Taxane **16**[a]	−33.0	−18.5	−14.5
Taxuspine D	−33.8	−21.5	−12.3
Taxane **12**	−38.4	−31.0	−7.4
Taxane **13**	−36.1	−28.0	−8.1
Taxane **15**	−33.5	−26.4	−7.1

[a]A virtual analogue of compound **5** (Scheme 1) wherein the cinnamoyl group had been replaced by the paclitaxel side chain.

Conclusion

A multidisciplinary approach combining bioorganic mechanisms, synthetic chemistry and molecular modeling led to interesting taxane structures with double bonds at C3–C4, C10–C11 or with a C11–C12 epoxide. Addition of side chains situated elsewhere than on C-13 produced very different conformations which may act on different cell targets.

Experimental

Instrumentation

The instrumentation used was as previously described.[20,25]

Rearrangement of 5α-hydroxy-7β,9α,10β-triacetoxy-4(20),11(12)-taxadien-13-one, 1. 5α-Hydroxy-7β,9α,10β-triacetoxy-4(20),11(12)-taxadien-13-one **1** (69 mg, 0.15 mmol) was dissolved in glacial acetic acid (0.89 mL) and treated with activated Zn[18] (0.83 g, 12.7 mmol) at 23 °C for 25 h. The mixture was diluted with EtOAc and filtered through Celite. Heptane was added and the mixture was evaporated under reduced pressure. The residue was purified by semi-preparative HPLC (25–100% CH_3CN in H_2O, 50 min gradient at a flow rate of 3 mL/min) affording **2** (9 mg, 13%, $t_R = 24.76$ min), **3** (13 mg, 21%, $t_R = 27.38$ min) and a mixture [11 mg, 18% (based on the average molecular weight of the three compounds before cinnamoylation), $t_R = 37.89$ min]. The mixture (4 mg, 0.010 mmol) was dissolved in toluene (0.5 mL) and was treated with *trans*-cinnamic acid (8 mg, 0.054 mmol), DCC (11 mg, 0.053 mmol) and DMAP (6 mg, 0.049 mmol) at 90 °C for 5 h. The solution was diluted with EtOAc, washed with a saturated $NaHCO_3$ solution and brine. The organic phase was dried, filtered and evaporated. The residue was purified by semi-preparative HPLC (25–100% CH_3CN in H_2O, 50 min gradient at a flow rate of 3 mL/min) affording **4** (2 mg, 37%, $t_R = 52.00$ min), **5** (1 mg, 19%, $t_R = 53.50$ min) and **6** (2 mg, 38%, $t_R = 54.75$ min). The NMR data of **2** is shown in Table 1. FAB-HR-MS for **2**: $C_{26}H_{38}O_9K$ $[M+K]^+$ requires: 533.2153; found: 533.2154; ^1H NMR (500 MHz, $CDCl_3$) for **3**: δ 5.90 (dd, $J = 12.6$, 3.4 Hz, 1H, H-9), 5.82 (dd, $J = 11.9$, 4.7 Hz, 1H, H-7), 4.54 (d, $J = 11.6$ Hz, 1H, H-20a), 4.39 (br.dd, $J = 4.1$, 2.0 Hz, 1H, H-5), 4.20 (d, $J = 11.6$ Hz, 1H, H-20b), 2.74 (dd, $J = 15.6$, 6.6 Hz, 1H, H-2a), 2.65 (dd, $J = 19.8$, 8.3 Hz, 1H, H-14a), 2.58 (o.d, $J = 15.6$ Hz, 1H, H-2b), 2.55 (qu, $J = 6.8$ Hz, 1H, H-12), 2.48 (d, $J = 19.5$ Hz, 1H, H-14b), 2.22 (br.t, 1H, H-10a), 2.07 (s, 3H, OAc), 2.04 (o.m, 1H, H-1), 2.04 (o.m, 1H, H-6a), 2.02 (s, 3H, OAc), 1.90 (ddd, $J = 13.3$, 4.2, 1.6 Hz, 1H, H-6b), 1.72 (ddd, $J = 14.5$, 7.4, 4.3 Hz, 1H, H-10b), 1.46 (o.m, 1H, H-11), 1.39 (s, 3H, H-17), 1.13 (o.d, 3H, H-18), 1.13 (o.s, 3H, H-19), 0.91 (s, 3H, H-16); ^{13}C NMR (125 MHz, $CDCl_3$) for **3**: δ 217.2 (C-13), 141.7 (C-3), 134.1 (C-4), 77.7 (C-9), 68.1 (C-5), 66.4 (C-7), 64.8 (C-20), 48.3 (C-11), 47.3 (C-12), 46.1 (C-8), 43.9 (C-1), 39.8 (C-14), 37.0 (C-15), 34.7 (C-16), 33.4 (C-6), 31.9 (C-10), 26.7 (C-2), 25.0 (C-17), 22.4 (C-19), 21.6 (OAc), 20.2 (OAc), 15.3 (C-18). FAB-HR-MS for **3**: $C_{24}H_{36}O_7K$ $[M+K]^+$ requires: 475.2098; found: 475.2099. The NMR data for **4** is shown in Table 2; FAB-HR-MS for **4**: $C_{33}H_{40}O_8K$ $[M+K]^+$ requires: 603.2360; found: 603.2359. The NMR data for **5** was identical to literature;[20] FAB-HR-MS for **5**: $C_{33}H_{40}O_7K$ $[M+K]^+$ requires: 587.2411; found: 587.2410. The NMR data for **6** is shown in Table 3; FAB-HR-MS for **6**: $C_{33}H_{40}O_7K$ $[M+K]^+$ requires: 587.2411; found: 587.2410.

Taxane 12. Taxane **2** (14 mg, 0.028 mmol) dissolved in pyridine (0.5 mL) was treated with acetyl chloride (10 μL, 0.14 mmol) at 4 °C for 18 h. The reaction was quenched with brine and extracted with dichloromethane. The organic phase was dried, filtered and evaporated. The residue was purified by flash chromatography (CH_2Cl_2/acetone, 8:2) to give compound **7** (9 mg, 59%). Taxinine **7** (4 mg, 0.008 mmol) in toluene (0.2 mL) was treated with the chiral acid **9** (4.8 mg, 0.012 mmol) in 0.1 mL CH_2Cl_2. A catalytic amount of 4-DMAP and DCC (2.5 mg, 0.012 mmol) were added and the solution was stirred at 75 °C for 5 h. The reaction mixture was diluted with CH_2Cl_2, washed with brine, dried, filtered and evaporated. The residue was purified by semi-preparative HPLC (25–100% CH_3CN in H_2O, 50 min gradient at a flow rate of 3 mL/min) affording taxane **10** (2 mg, 29%, $t_R = 50.01$ min). The protected taxinine ester **10** (2 mg, 0.002 mmol) in MeOH (1 mL) was treated with a catalytic amount of *p*-toluenesulfonic acid (PTSA) at 23 °C for 3 h. The solution was diluted with EtOAc, washed with brine to neutrality, dried, filtered and evaporated. The residue was purified by semi-preparative HPLC (25–100% CH_3CN in H_2O, 50 min gradient at a flow rate of 3 mL/min) affording taxane **12** (1 mg, 57%, $t_R = 44.16$ min). The NMR data for **12** is shown in Table 4. FAB-HR-MS: $C_{42}H_{57}N_1O_{14}K$ $[M+K]^+$ requires: 838.3416; found: 838.3412.

Taxane 13. Taxane **3** (10 mg, 0.023 mmol) dissolved in pyridine (0.5 mL) was treated with acetyl chloride (11 μL, 0.16 mmol) at 4 °C for 18 h. The reaction was quenched with brine and extracted with dichloromethane. The organic phase was dried, filtered and evaporated. The residue was purified by semi-preparative HPLC (25–100% CH_3CN in H_2O, 50 min gradient at a flow rate of 3 mL/min) affording taxane **8** (4 mg, 36%, $t_R = 34.47$ min). Taxinine **8** (4 mg, 0.008 mmol) in toluene (0.2 mL) was treated with the chiral acid **9** (5.3 mg, 0.013 mmol) in 0.1 mL CH_2Cl_2. A catalytic amount of 4-DMAP and DCC (2.8 mg, 0.014 mmol) were added and the solution was stirred at 75 °C for 5 h. The reaction mixture was diluted with CH_2Cl_2, washed with brine, dried, filtered and evaporated. The residue was purified by semi-preparative HPLC (25–100% CH_3CN in H_2O, 70 min gradient at a flow rate of 3 mL/min) affording taxane **11** (2 mg, 28%, $t_R = 67.22$ min). The protected taxinine ester **11** (2 mg, 0.002 mmol) in MeOH (1 mL) was treated with a catalytic amount of *p*-toluenesulfonic acid (PTSA) at 23 °C for 18 h. The solution was diluted with EtOAc, washed with brine to neutrality, dried, filtered and evaporated. The residue was purified by semi-preparative HPLC (25–100% CH_3CN in H_2O, 50 min gradient at a flow rate of 3 mL/min) affording taxane **13** (1 mg, 58%, $t_R = 47.31$ min). ^1H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, $J = 7.7$ Hz, 2H, H-2,6 of OBz), 7.35 (t, $J = 7.7$ Hz, 2H, H-3,5 of OBz), 7.26 (o.t, 1H, H-4 of OBz), 5.87 (dd, $J = 15.5$, 4.0 Hz, 1H, H-9), 5.83 (br.d, $J = 9.0$ Hz, 1H, NH-4′), 5.75 (o.dd, $J = 11.2$, 5.2 Hz, 1H, H-7), 5.73 (o.br.s, 1H, H-5), 5.14 (o.m, 1H, H-3′), 5.14 (o.d, $J = 11.9$ Hz, 1H, H-20a), 4.51 (br.s, 1H, H-2′), 4.02 (br.d, $J = 11.7$ Hz, 1H, H-20b), 3.75 (br.s, 1H, OH-2′), 2.92 (dd, $J = 15.2$, 6.9 Hz, 1H, H-2a), 2.69 (d, $J = 15.5$ Hz, 1H, H-2b), 2.66 (o, 1H, H-14a), 2.28 (qu, $J = 6.3$ Hz, 1H, H-12), 2.23 (br.m, 1H, H-10a), 2.14 (o.br.t, 1H, H-1), 2.10 (o.m, 1H, H-6a), 2.08 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.97 (o.m, 1H, H-14b), 1.77 (ddd, $J = 14.8$, 7.3, 4.3 Hz, 1H, H-10b), 1.53 (o.m, 1H, H-11), 1.40 (o.s, 3H, H-17), 1.40 (o.s, 9H, *t*-Bu), 1.18 (br.d, $J = 6.4$ Hz, 3H, H-18), 1.13 (s, 3H, H-19), 0.92 (s, 3H, H-16); ^{13}C NMR

(125 MHz, $CDCl_3$) δ 217.2 (C-13), 170.3 (CO–OAc), 169.9 (CO–OAc), 169.2 (CO–OAc), 148.5 (C-3), 128.5 (C-3,5 of OBz), 127.3 (C-4 of OBz), 127.2 (C-4), 126.6 (C-2,6 of OBz), 77.4 (C-9), 74.4 (C-2′), 68.7 (C-5), 65.6 (C-7), 61.8 (C-20), 56.7 (C-3′), 47.5 (C-11), 47.0 (C-12), 46.0 (C-8), 43.8 (C-1), 39.3 (C-14), 36.6 (C-15), 34.4 (C-16), 31.9 (C-10), 30.6 (C-6), 28.1 (*t*-Bu), 27.3 (C-2), 24.7 (C-17), 22.4 (C-19), 21.4 (OAc), 21.0 (OAc), 20.6 (OAc), 17.2 (C-18). FAB-HR-MS: $C_{40}H_{55}N_1O_{12}K$ $[M+K]^+$ requires: 780.3361; found: 780.3364.

Taxane 15. Taxinine **3** (9 mg, 0.021 mmol) in toluene (0.4 mL) was treated with the chiral acid **9** (13 mg, 0.033 mmol) in 0.2 mL CH_2Cl_2. 4-DMAP (2 mg, 0.016 mmol) and DCC (6.8 mg, 0.033 mmol) were added and the solution was stirred at 4 °C for 18 h. The reaction mixture was diluted with CH_2Cl_2, washed with brine, dried, filtered and evaporated. The residue was purified by semi-preparative HPLC (25–100% CH_3CN in H_2O, 70 min gradient at a flow rate of 3 mL/min) affording taxane **14** (2 mg, 12%, $t_R = 52.21$ min). The protected taxinine ester **14** (2 mg, 0.002 mmol) in MeOH (1 mL) was treated with a catalytic amount of *p*-toluenesulfonic acid (PTSA) at 23°C for 18 h. The solution was diluted with EtOAc, washed with brine to neutrality, dried, filtered and evaporated. The residue was purified by semi-preparative HPLC (25–100% CH_3CN in H_2O, 50 min gradient at a flow rate of 3 mL/min) affording taxane **15** (1 mg, 58%, $t_R = 41.68$ min). ^1H NMR (500 MHz, $CDCl_3$) δ 7.43 (o.d, 2H, H-2,6 of OBz), 7.36 (o.t, 2H, H-3,5 of OBz), 7.28 (o.t, 1H, H-4 of OBz), 5.98 (o.m, 1H, H-7), 5.94 (o.m, 1H, H-9), 5.77 (o.m, 1H, NH-4′), 5.20 (o.d, 1H, H-3′), 4.84 (br.d, $J = 12.4$ Hz, 1H, H-20a), 4.80 (br.d, $J = 12.4$ Hz, 1H, H-20b), 4.53 (br.s, 1H, H-2′)), 4.18 (br.s, 1H, H-5), 2.68 (o.d, 1H, H-2b), 2.68 (o.m, 1H, H-14a), 2.61 (o.m, 1H, H-12), 2.22 (br.o.m, 1H, H-10a), 2.10 (o.m, 1H, H-14b), 2.07 (s, 3H, OAc), 2.05 (o.m, 1H, H-1), 2.05 (o.m, 1H, H-6a), 2.04 (dd, $J = 15.4$, 6.0 Hz, 1H, H-2a), 2.03 (s, 3H, OAc), 1.75 (o.m, 1H, H-10b), 1.48 (o.m, 1H, H-11), 1.41 (o.s, 9H, *t*-Bu), 1.40 (o.s, 3H, H-17), 1.19 (s, 3H, H-19), 1.15 (o.d, 3H, H-18), 0.89 (s, 3H, H-16); ^{13}C NMR (125 MHz, $CDCl_3$) δ 216.9 (C-13), 170.1 (CO–OAc), 169.6 (CO–OAc), 144.8 (C-3), 128.3 (C-3,5 of OBz), 127.6 (C-4), 127.3 (C-4 of OBz), 126.5 (C-2,6 of OBz), 77.4 (C-9), 74.2 (C-2′), 67.1 (C-5), 67.1 (C-20), 65.9 (C-7), 56.0 (C-3′), 47.8 (C-11), 47.3 (C-12), 45.7 (C-8), 44.1 (C-1), 36.7 (C-15), 34.6 (C-16), 33.7 (C-6), 28.2 (*t*-Bu), 27.3 (C-2), 24.9 (C-17), 22.0 (C-18), 15.1 (C-19). FAB-HR-MS: $C_{38}H_{53}N_1O_{11}K$ $[M+K]^+$ requires: 738.3256; found: 738.3256.

Molecular modeling

Based on the novel rearranged taxane skeletons **2**, **3**, and **5**, a series of novel side chains including those of paclitaxel and docetaxel were virtually attached either at C-5 or C-20 to generate a database of 28 virtual compounds. Paclitaxel, docetaxel and taxuspine D were added to the database as references. This database was docked against the refined β-tubulin structure[12] by using the DOCK program.[22] The docking procedure is the same as our previous work[26] except that the configurations saved at each cycle of pruning was set to 30 and the number of anchor positions to explore was set at 300. The solvation of free energy was calculated by performing single-point calculations on the docked conformations using the AMI-SM5. 4PDA model as implemented in the AMSOL program.[24] The correlated dock scores were obtained by subtracting the solvation free energy from the dock scores.

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