Pergamon

Tetrahedron Vol. 50, No. 34, pp. 10289-10298, 1994

0040-4020/94 $7.00+0.00

0040-4020(94)00600-8

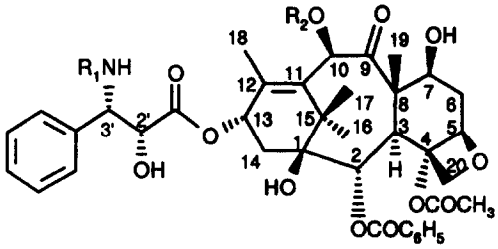
Partial Synthesis of Major Human Metabolites of Docetaxel

Alain Commerçon*, Jean-Dominique Bourzat, Daniel Bézard and Marc Vuilhorgne

Rhône-Poulenc Rorer S.A.- Centre de Recherches de Vitry-Alfortville
13, Quai Jules Guesde - BP14 - 94403 Vitry-sur-Seine (France)

Abstract: The structures and synthesis of the four major human metabolites of docetaxel are reported. These metabolites, *i.e.* compounds **3**, **4a,b** and **5**, are side-chain oxidation derivatives. They were prepared by partial synthesis from the previously described amino-taxoid **8** using mixed-carbonates as acylation reagents. *In vitro* biological activities are detailed.

Taxoids are new anticancer agents that interfere with the microtubule-tubulin system in eukariotic cells[1]. Docetaxel **1** (Taxotere®), like the related natural analog paclitaxel **2** (Taxol®), are currently considered as the most exciting leads of this new series of antitumor drugs. These two taxoids are currently in clinical trials for various types of cancer such as breast, ovarian and non-small cell lung cancers[2].



1, R_1 = tBuOCO, R_2 = H (docetaxel)

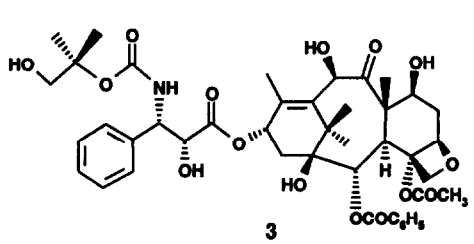
2, R_1 = C_6H_5CO, R_2 = Ac (paclitaxel)

In spite of widening clinical use, little information has been reported thus far on the biological fate of paclitaxel and docetaxel.

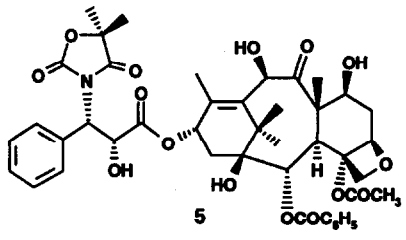
Paclitaxel metabolism in animals has been previously studied by Monsarrat *et al.*[3-5]. These authors showed that no metabolites of paclitaxel were detected in rat urine, and only 10% of the injected paclitaxel was recovered in urine over a 24-hr period. In contrast, 20% and 40% of the injected drug was recovered in the bile as unchanged paclitaxel and metabolites, respectively. Biotransformation leads to products oxidized at different sites such as the meta-position of the benzoate at C-2 and the para-position of the phenyl group at C-3'. Recently, *in vitro* metabolism of paclitaxel in freshly isolated rat hepatocytes has been investigated[6]. Two metabolites were separated and shown to be monohydroxylated on the side chain and the baccatin core. These results are in agreement with the findings made by Monsarrat *et al.* in the rat bile.

Regarding the metabolism of paclitaxel in humans, it has been reported that small amounts of unchanged drug are found in urine[7]. As in rats, paclitaxel (3%) and its metabolites (17%) were excreted in human bile until 24 hours post-treatment[5,6]. Five metabolites were detected and three of them were identified as products of mono- and dihydroxylation at the para-position of the phenyl group at C-3' and/or at the C-6 position[8] of the baccatin moiety. The same monohydroxylated metabolites were also generated *in vitro* by human liver microsomes[9].

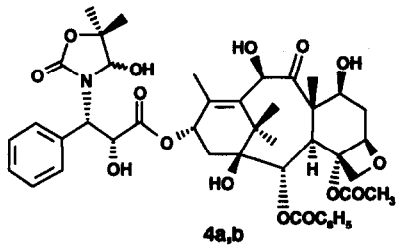
The principal structural difference between docetaxel **1** and paclitaxel **2** is the presence of a tert-butoxycarbonyl group instead of a benzoyl group on the nitrogen atom of the side-chain. This structural dissimilarity might induce different metabolic transformations. In the course of docetaxel clinical studies, an investigation of human docetaxel metabolites has been conducted. Several metabolites were isolated and structurally characterized in human feces[10]. Metabolism of docetaxel in humans leads mainly to side-chain-oxidative products. The major isolated metabolites are the side-chain-hydroxylated compound **3**, the oxazolidine-type compounds **4** (2 epimers, **4a** and **4b**) and **5** as well as, in much lower concentrations, the corresponding 7-epimerized compounds of **3-5**[11].



3



5

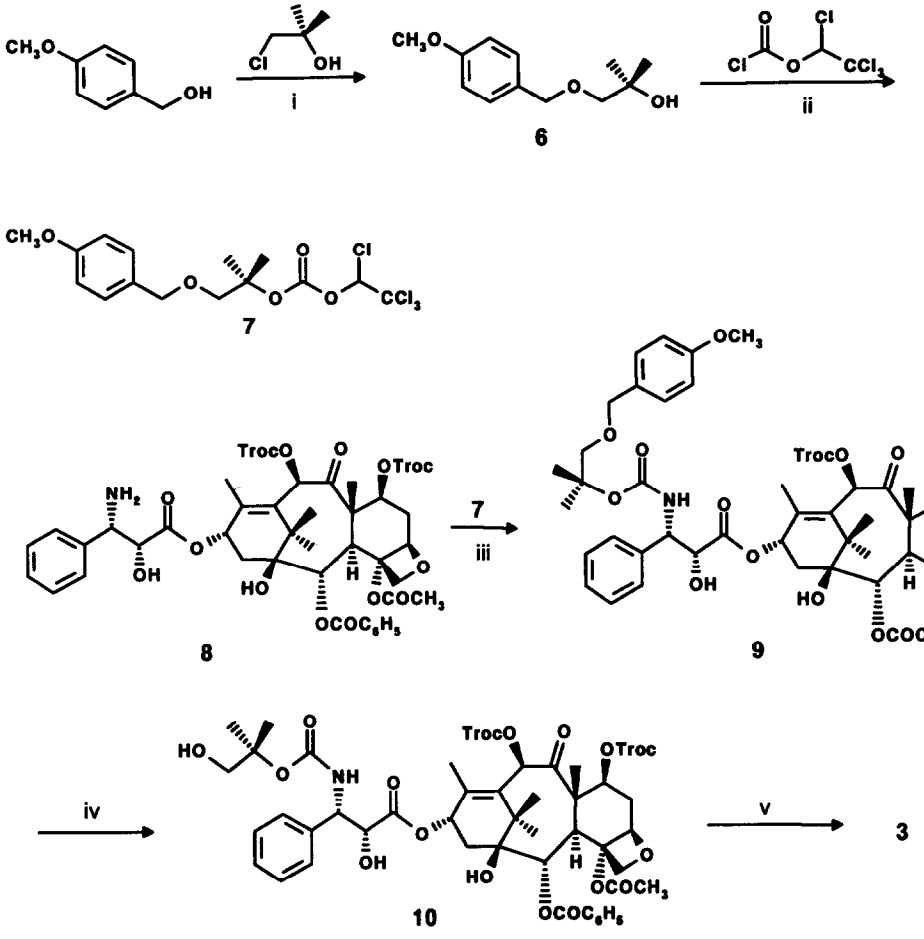


4a,b

As metabolites could be very toxic species, thus we synthesized these compounds in order to assess their biological and toxicological effects. This was undertaken using suitable acylation reagents with amino taxoid **8** which is available from 10-deacetyl baccatin III[12].

Preparation of metabolite **3** was achieved as depicted in Scheme 1. Since the taxane skeleton is known to be sensitive to acidic and basic conditions[13], we chose for the additional hydroxy function a protective group able to be cleaved under either weakly acidic or oxidizing conditions. We selected the para-methoxybenzyl protective group which fulfils both criteria. Thus tertiary alcohol **6** was obtained in 64% yield from the corresponding chlorohydrin and para-methoxybenzyl alcohol under alkylating conditions. Subsequent reaction of **6** with 1,2,2,2-tetrachloroethyl chloroformate afforded the mixed-carbonate **7** (50% yield after filtration over silica gel). By analogy to the work of Barcelo *et al.*[14] with other mixed-carbonates (1,2,2,2-tetrachloroethyl and tertiary alkyl), carbonate **7** was reacted with amino-taxoid **8** in pyridine at room temperature to give within 7h carbamate **9** in 53% yield. Deprotections can be carried out in any order but the best overall yield was obtained by initial removal of the para-methoxybenzyl group with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in the presence of water (75% yield) and final cleavage of the trichloroethoxycarbonyl (Troc) groups with zinc in acetic acid (60% yield).

Scheme 1

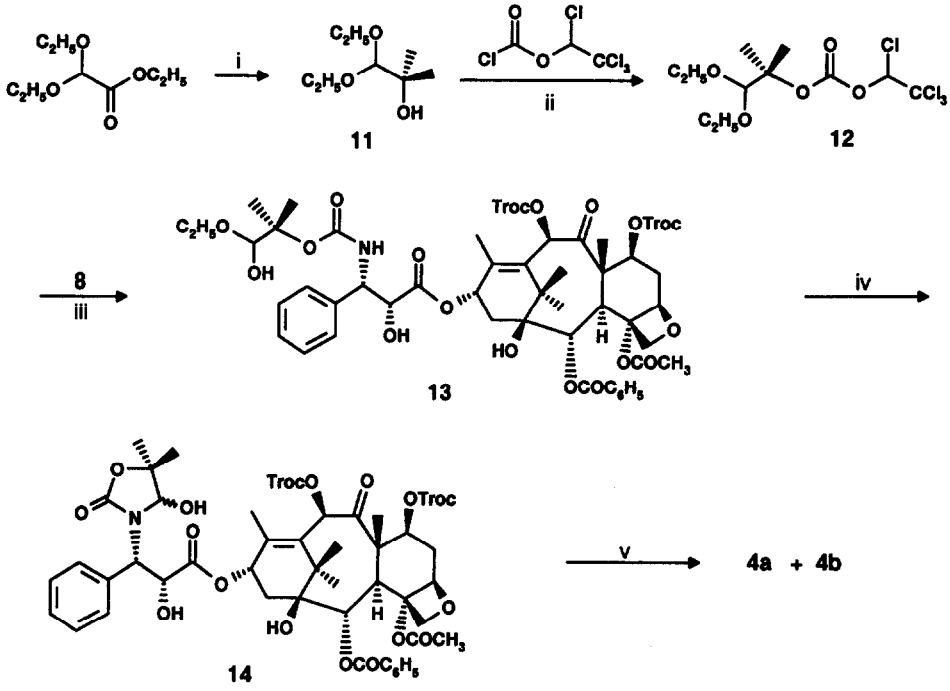


Reagents: i) NaH, DMF, r.t., 48h, 64%. ii) pyridine, CH_2Cl_2, r.t., 6h, 50%. iii) pyridine, r.t., 7h, 53%. iv) DDQ, CH_2Cl_2, H_2O, r.t., 1h, 75%. v) Zn, AcOH, MeOH, 60°C, 15min, 60%.

Metabolites **4a,b** likely come from metabolite **3** *via* a subsequent biochemical oxidation of the hydroxylated carbamate moiety. The putative aldehydic intermediate should be able to cyclize to give the two epimeric forms of hydroxyoxazolidinones **4**. We attempted the oxidation of **3** as a 2'-O-triethylsilyl-7,10-O-diTroc-protected compound. However in all cases we were unable to isolate any oxidation products of the hydroxy-carbamate moiety. So we undertook a partial synthesis based on an approach, similar to that outlined above, with another suitable mixed-carbonate.

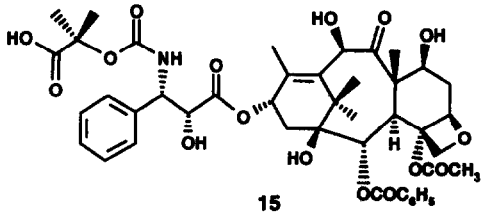
Alcohol **11** was prepared by condensation of methylmagnesium bromide with ethyl diethoxyacetate according to a known procedure[15] (66% yield). Formation of carbonate **12** by reaction with 1,2,2,2-tetrachloroethyl chloroformate in pyridine was achieved in nearly quantitative yield. Acylation of amino-taxoid **8** was performed at room temperature and the reaction was quenched after 24h to give the hemi-acetal **13** (in some experiments the acetal was recovered instead of the hemi-acetal).

Scheme 2



Reagents: i) MeMgBr, THF, 10°C, 66%. ii) pyridine, CH_2Cl_2, r.t., 48h, 100% crude. iii) pyridine, CH_2Cl_2, r.t., 24h, 58%. iv) HCOOH, H_2O, AcOEt, r.t., 24h, 61%. v) Zn, AcOH, MeOH, 60°C, 15min., 53% then separation by HPLC.

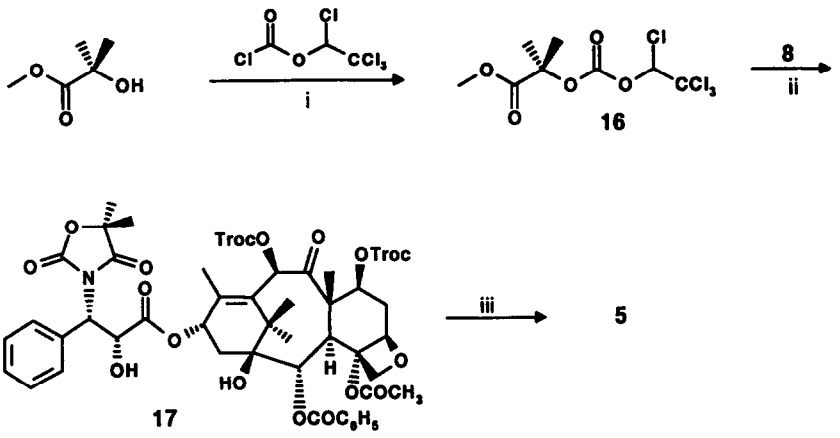
Hydrolysis of the hemi-acetal was carried out with formic acid at room temperature to give both cyclized epimers in nearly equal amounts (60% total yield)[16]. Final cleavage of the Troc groups was performed on the mixture of epimers with zinc in acetic acid. The recovered crude material was purified on silica gel and the two epimeric forms **4a** (first epimer eluted) and **4b** were separated in 53% yield (ratio 44:56). We have not been able thus far to determine the configuration at the newly formed stereocenter of these two metabolites (efforts of characterization by X-ray crystallographic analysis are in progress).



15

The last metabolite, *i.e.* compound **5**, is likely produced from acid **15**[10] formed by further biochemical oxidation. We were able to obtain by partial synthesis acid **15**[17]. This compound has proved to be rather unstable, cyclizing easily to give the oxazolidinedione derivative **5**.

Scheme 3



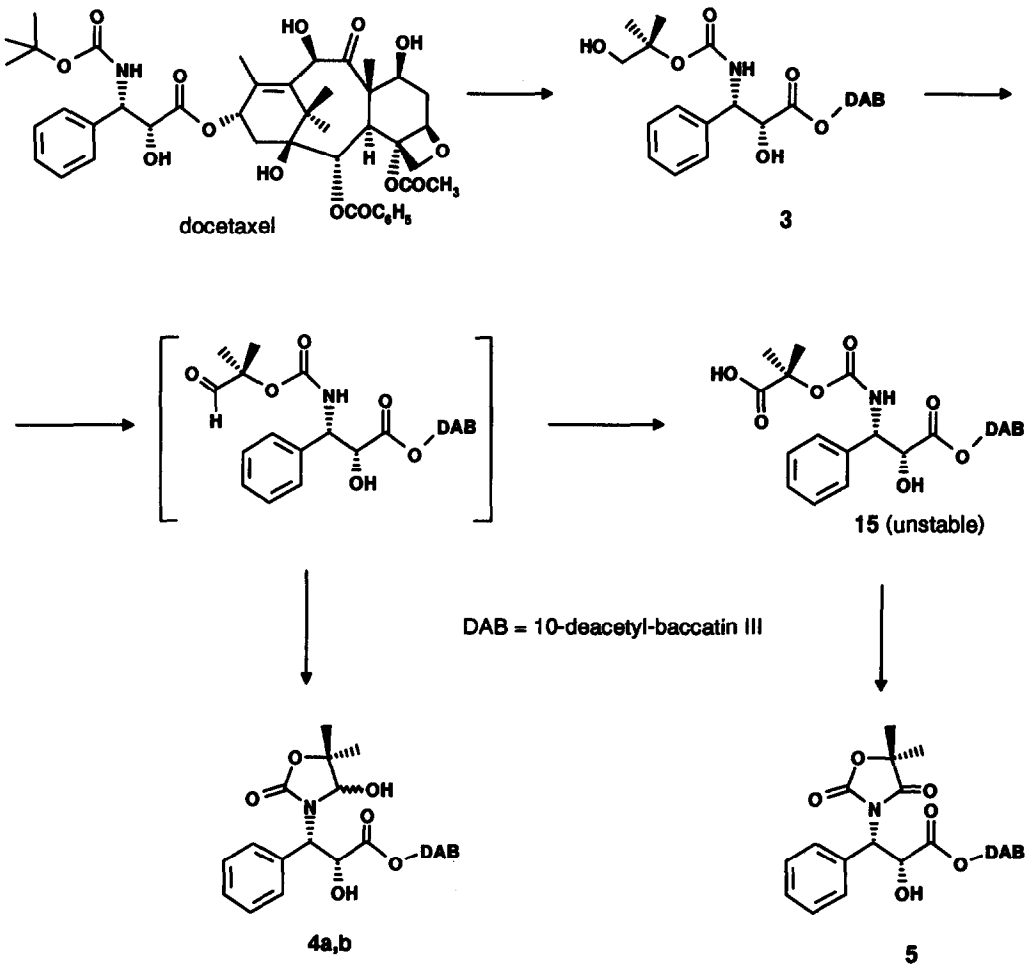
Reagents: i) pyridine, CH_2Cl_2, r.t., 24h, 70% crude. ii) aq. K_2CO_3 (5M), THF, r.t., 24h, 78%. iii) Zn, AcOH, MeOH, 60°C, 15min., 53%.

A direct access to compound **5** was realized as follows. The mixed-carbonate **16** was prepared from methyl 2-hydroxyisobutyrate and 1,2,2,2-tetrachloroethyl chloroformate in pyridine (70% yield). Reaction of carbonate **16** with amino-taxoid **8** in a mixture of THF and potassium carbonate (5M) in water led directly to the oxazolidinedione derivative **17** (78% yield)[18]. Cleavage of the Troc groups under standard conditions gave metabolite **5** in 53% yield.

The human metabolites have been biologically evaluated in *in vitro* experimental models. Metabolites **4a**, **4b** and **5** are poor inhibitors of depolymerization in the microtubule disassembly assay[19] (50T, 2.4T and 100T respectively, T being the IC_{50} value for paclitaxel in the same assay). Metabolite **3** retains a high level of activity in the microtubule disassembly assay (0.65T). This is in good agreement with its close structural analogy with docetaxel. However **3** showed diminished cytotoxicity against P388 leukemia cells[20] with an IC_{50} of 0.45 μg/ml (IC_{50} for docetaxel was 0.04 μg/ml) while metabolites **4a**, **4b** and **5** proved to be completely inactive in the same cytotoxicity assay (IC_{50} >10 μg/ml).

The chemical reactivities observed during the preparation of these major human metabolites of docetaxel are in agreement with the putative metabolic process depicted in Scheme 4. The biochemical sequence likely starts from an hydroxylation of the tert-butoxycarbonyl moiety (metabolite **3**) followed by the oxidation of the newly formed hydroxy function to give metabolites **4a,b**. We observed by chemical synthesis that the open-aldehydic form of compounds **4a,b** could not be isolated while both cyclized forms were recovered. Further metabolic oxidation of the putative intermediate aldehydic metabolite leads to the oxazolidine-dione derivative (metabolite **5**). Based on our chemical observations, it is postulated that carboxylic acid **15** might be the real biochemical precursor of compound **5** although a biochemical oxidation of hydroxyoxazolidines **4a,b** cannot be ruled out. We were also able to detect compound **15** at very low concentrations in human feces using LC-MS experiments[10].

Scheme 4 (Postulated metabolic pathway for docetaxel)



Acknowledgements: We thank Drs. C. Combeau, J.F. Riou, M-C. Bissery, P. Vrignaud and F. Lavelle for biological evaluation, Dr. C. Gaillard for the human metabolites isolation and Dr. C.J. Burns for critical reading of the manuscript.

Experimental Section

NMR spectra were determined with either a Bruker AC-200, AC-300 or AM-400 spectrometer. Chemical shifts (δ) are in ppm relative to TMS (0.00). Mass spectra were produced using FINNIGAN 3300 (70ev; Electron Impact), VG AUTOSPEC (LSIMS Liquid Secondary Ion Mass Spectrometry; matrix: NBA *m*-Nitro Benzyl Alcohol); SCIEX API III (Electrospray); FINNIGAN TSQ45 (DCI; reactant gas: NH_3). Silica gel Merck 60 (0.063-0.20 mm) was used for column chromatography. Preparative thin layer chromatography was carried out on Silica gel Merck 60S-254 plates coated with 0.5 mm layer of silica gel.

2-Methyl-1-(4-methoxyphenyl)methoxy-2-propanol, 6: A solution of 10.9 g (0.1 mol) of 1-chloro-2-methyl-2-propanol in 20 mL of DMF was added dropwise at 10°C over 0.5 h to 9.6 g (0.2 mol) of NaH (50% dispersion in oil) in 120 mL of DMF. After 2 h of additional stirring at 20°C, a mixture of 12.5 mL (0.1 mol) of 4-methoxy-benzylalcohol in 20 mL of DMF was added dropwise over 0.5 h. The reaction mixture was stirred at room temperature for 2 days and then heated at 60°C for 8 h to complete the reaction. Then the reaction mixture was poured in 1.2 L of water. The pH was adjusted to 5 by adding 175 mL of hydrochloric acid (1N). The aqueous solution was extracted with three portions of 250 mL of CH_2Cl_2. The solvent was dried with $MgSO_4$ and evaporated. The crude product was purified on 500 g of silica gel with CH_2Cl_2. This gave 13.6 g (64%) of compound **6** as a pale yellow oil. ^{1}H-NMR ($CDCl_3$, 200MHz); δ: 1.24 [s, 6H: -O-C(CH_3)$_2$], 2.42 (s, 1H: -OH), 3.30 (s, 2H: -OCH_2-), 3.83 (s, 3H: Ar-OCH_3), 4.52 (s, 2H: Ar-CH_2O-), 6.94 (d, J= 8.5 Hz, 2H: Aromatics in ortho of -OCH_3), 7.30 (d, J= 8.5 Hz, 2H: Aromatics in meta of -OCH_3). MS(EI): M/z = 210: $M^{+\cdot}$, 138 = $M^{+\cdot}$ -OtBu.

2-[2-Methyl-1-(4-methoxyphenyl)methoxy]propyl-1',2',2',2'-tetrachloroethyl-carbonate, 7: By analogy to ref.[14], to a mixture of 5.88 g (28 mmol) of alcohol **6** in 40 mL of CH_2Cl_2 was added at 5°C (ice bath) a solution of 7.59 g (30.8 mmol) of 1,2,2,2-tetrachloroethyl-chloroformate in 40 mL of CH_2Cl_2. Then 2.43 g (30.8 mmol) of pyridine in 20 mL of CH_2Cl_2 were added dropwise over 0.5 h.. The reaction mixture was stirred for 6 h at 5°C and then washed by three portions (3x50 mL) of water. The organic solvent was dried with $MgSO_4$ and evaporated. The crude product was filtered on 50 g of silica gel with CH_2Cl_2/cyclohexane: 1/1 to give 6.1 g (50%) of **7** as a pale yellow oil used freshly prepared and without further purification.

N-De-t-butoxycarbonyl-N-2-[2-Methyl-1-(4-methoxyphenyl)methoxy]propyloxycarbonyl-7,10-O-diTroc-docetaxel, 9: A solution of 3.36 g (8 mmol) of mixed-carbonate **7** in 25 mL of pyridine was added at 5°C (ice bath) to 4.24 g (4 mmol) of 10-deacetyl-7,10-diTroc-baccatin III **8** in 25 mL of pyridine. The reaction mixture was stirred 8 h at 5°C then concentrated in vacuo. The crude product was chromatographed on 250 g of silica gel with CH_2Cl_2 to give 2.1 g (41 %) of pure compound **9** as a white foam followed by 1.1 g (26%) of recovered **8**. ^{1}H-NMR ($CDCl_3$, 400MHz); δ: 1.19 (s, 3H: -CH_3 16 or 17), 1.25 (s, 3H: -CH_3 16 or 17), 1.36 [s, 6H: -O-C(CH_3)$_2$], 1.88 (s, 3H: -CH_3 19), 1.96 (s, 3H: -CH_3 18), 2.08 [ddd, J= 16, 11 and 2 Hz, 1H: -(CH)-H 6], 2.31 (d, J= 9 Hz, 2H: -CH_2- 14), 2.40 (s, 3H: -$COCH_3$), 2.63 [m, 1H: -(CH)-H 6], 2.92 (bb, 1H: -OH in 2'), 3.46 (s, 2H: -OCH_2-), 3.80 (s, 3H: Ar-OCH_3), 3.91 (d, J= 7 Hz, 1H: -H 3), 4.19 [d, J= 8 Hz, 1H: -(CH)-H 20], 4.34 [d, J= 8 Hz, 1H: -(CH)-H20], 4.41 (ab, J= 12 Hz, 2H: Ar-CH_2O-), 4.61 and 4.93 (2d, J= 12 Hz, 1H each: -OCOO-CH_2-CCl_3 in 7), 4.65 (bs, 1H: -H 2'), 4.78 (limit AB, 2H: -OCOO-CH_2-CCl_3 in 10), 4.96 (bd, J= 10 Hz, 1H: -H 5), 5.26 (bd, J= 10 Hz, 1H: -H 3'), 5.52 (d, J= 10 Hz, 1H: -CONH-), 5.55 (dd, J= 11 and 7.5 Hz, 1H: -H 7), 5.70 (d, J= 7 Hz, 1H: -H 2), 6.22 (bt, J= 9 Hz, 1H: -H 13), 6.24 (s, 1H: -H 10), 6.84 (d, J= 8.5 Hz, 2H: Aromatics in ortho of -OCH_3), 7.20 (d, J= 8.5 Hz, 2H: Aromatics in meta of -OCH_3), from 7.30 to 7.45 (m, 5H: -C_6H_5 in 3'), 7.51 [t, J= 8 Hz, 2H: -$OCOC_6H_5$ (-H 3 and -H 5)], 7.63 [t, J= 8 Hz, 1H: -$OCOC_6H_5$(-H 4)], 8.11 [d, J= 8 Hz, 2H: -$OCOC_6H_5$ (-H 2 and -H 6)]. MS(LSIMS): M/z = 1292: MH^+.

N-De-t-butoxycarbonyl-N-2-(1-hydroxy-2-methyl) propyloxycarbonyl-7,10-O-diTroc-docetaxel, 10: To a solution of 5 g (3.86 mmol) of compound **9** in 75 mL of CH_2Cl_2 were added successively 1.5 mL of water and 2.6 g (11.6 mmol) of DDQ. The black reaction mixture was stirred at room temperature for 1 h. The insoluble material was filtered off and the organic solution was washed successively twice with 50 mL of saturated aqueous $NaHCO_3$ solution and twice with 50 mL of water. The organic solution was dried with $MgSO_4$ and evaporated. The crude product was purified on 130 g of silica gel with CH_2Cl_2/MeOH : 99/1 to give 3.4 g (75%) of **10** as a white foam. ^{1}H-NMR ($CDCl_3$, 400MHz); δ: 1.22 (s, 3H: -CH_3 16 or 17), 1.30 [s, 9H: -CH_3 16 or 17 and -O-C(CH_3)$_2$], 1.88 (s, 3H: -CH_3 19), 1.99 (s, 3H: -CH_3 18), 2.09 [ddd, J= 15, 11 and 2 Hz, 1H: -(CH)-H 6], 2.30 and 2.37 (2 dd, J= 16 and 9 Hz, 1H each: -CH_2- 14), 2.43 (s, 3H: -$COCH_3$), 2.57 (s, 1H: -OH in 1), 2.63 [m, 1H: -(CH)-H 6], 3.29 (d, J= 5 Hz, 1H: -OH in 2'), 3.40 [dd, J= 14 and 5 Hz, 1H: -O-(CH)H)], 3.59 [dd, J= 14 and 7 Hz, 1H: -O-(CH)H)], 3.86 (dd, J= 7 and 5 Hz, 1H: -CH_2-OH), 3.92 (d, J= 7 Hz, 1H: -H 3), 4.22 [d, J= 8.5 Hz, 1H: -(CH)-H 20], 4.35 [d, J= 8.5 Hz, 1H: -(CH)-H 20], 4.61 and 4.92 (2d, J= 12.5 Hz, 1H each: -OCOO-CH_2-CCl_3 in 7), 4.71 (dd, J= 5 and 1 Hz, 1H: -H 2'), 4.79 (limit AB, J= 11 Hz, 2H: -OCOO-CH_2-CCl_3 in 10), 4.97 (bd, J= 9 Hz, 1H: -H 5), 5.33 (bd, J= 10 Hz, 1H: -H 3'), 5.57 (dd, J= 11 and 7.5 Hz, 1H: -H 7), 5.63 (d, J= 10 Hz, 1H: -CONH-), 5.74 (d, J= 7 Hz, 1H: -H 2), 6.24 (s, 1H: -H 10),

6.31 (bt, J= 9 Hz, 1H: -H 13), from 7.30 to 7.50 (m, 5H: -C_6H_5 in 3'), 7.51 [t, J= 8 Hz, 2H: -$OCOC_6H_5$ (-H 3 and -H 5)], 7.62 [t, J= 8 Hz, 1H: -$OCOC_6H_5$ (-H 4)], 8.12 [d, J= 8 Hz, 2H: -$OCOC_6H_5$ (-H 2 and -H 6)].

N-De-t-butoxycarbonyl-N-2-(1-hydroxy-2-methyl) propyloxycarbonyl-docetaxel, 3: To a solution of 3.4 g (2.89 mmol) of 7,10-O-diprotected compound **10** in a mixture of 68 mL of acetic acid and 68 mL of methanol was added at 60°C and under vigorous stirring 6.8 g of zinc powder. Stirring was maintained for 15 min then the reaction mixture was cooled to room temperature, filtered and concentrated in vacuo. The crude product was chromatographed on 90 g of silica gel with CH_2Cl_2/MeOH : 95/5 to give 1.2 g (60%) of metabolite **3** as a white foam, $[\alpha]_D^{20}$ -32.8 (c 0.519, MeOH); ^{1}H-NMR ($CDCl_3$, at temperature of 323°K, 400MHz); δ: 1.16 (s, 3H: -CH_3 16 or 17), 1.28 (s, 3H: -CH_3 16 or 17), 1.31 and 1.33 [s, 6H: -O-C(CH_3)$_2$], 1.78 (s, 3H: -CH_3 19), 1.87 [m, 1H: -(CH)-H 6], 1.89 (s, 3H: -CH_3 18), 2.26 and 2.33 (2 dd, J= 15 and 9 Hz, 1H each: -CH_2- 14), 2.38 (s, 3H: -$COCH_3$), 2.59 [m, 1H: -(CH)-H 6], 3.26 (bs, 1H: -OH in 2'), 3.44 and 3.6O (2d, J= 14 Hz, 1H each: -CH_2O-), from 3.50 to 3.70 (bb, 1H: -OH), 3.94 (d, J= 7 Hz, 1H: -H 3), 4.19 (bs, 1H: -OH in 10), 4.23 (m, 1H: -H 7), 4.25 [d, J= 8.5 Hz, 1H: -(CH)-H 20], 4.33 [d, J= 8.5 Hz, 1H: -(CH)-H 20], 4.67 (bb, 1H: -H 2'), 4.97 (dd, J= 10 and 2 Hz, 1H: -H 5), 5.21 (s, 1H: -H 10), 5.31 (bd, J= 10 Hz, 1H: -H 3'), 5.62 (d, J= 10 Hz, 1H: -CONH-), 5.72 (d, J= 7 Hz, 1H: -H 2), 6.29 (bt, J= 9 Hz, 1H: -H 13), from 7.30 to 7.45 (m, 5H: -C_6H_5 in 3'), 7.50 [t, J= 8 Hz, 2H: -$OCOC_6H_5$ (-H 3 and -H 5)], 7.61 [t, J= 8 Hz, 1H: -$OCOC_6H_5$ (-H 4)], 8.12 [d, J= 8 Hz, 2H: -$OCOC_6H_5$ (-H 2 and -H 6)]. MS(LSIMS): M/z = 824: MH^+.

2-(1,1-Diethoxy-2-methyl-propyl)-1',2',2',2'-tetrachloroethyl-carbonate, 12: By analogy to ref.[14], with the use of a similar procedure as for **7**, 0.81 g (50 mmol) of 2-(1,1-diethoxy-2-methyl)propanol **11** gave 1.8 g (100%) of carbonate **12** as a pale yellow oil used freshly prepared in the next step.

N-De-t-butoxycarbonyl-N-2-(1-ethoxy-1-hydroxy-2-methyl)propyloxycarbonyl-7,10-O-diTroc-docetaxel, 13: With the use of a similar procedure as for **8**, 1.06 g (1 mmol) of amino-taxoid **6** and 0.41 g (1.1 mmol) of carbonate **12** in 10 mL of pyridine led after purification on 50 g of silica gel with CH_2Cl_2/MeOH : 98/2 0.53 g of compound **13** as a white foam. ^{1}H-NMR ($CDCl_3$, at temperature of 323°K, 400MHz); δ: from 1.10 to 1.40 (m: -CH_3 of the ethyl group), 1.21 (s, 3H: -CH_3 16 or 17), 1.29 (s, 3H: -CH_3 16 or 17), 1.35 [s, 6H: -O-C(CH_3)$_2$], 1.70 (s, 1H: -OH in 1), 1.88 (s, 3H: -CH_3 19), 1.98 (s, 3H: -CH_3 18), 2.08 [ddd, J= 16, 11 and 2 Hz, 1H: -(CH)-H 6], 2.33 (limit ab, 2H: -CH_2- 14), 2.38 (s, 3H: -$COCH_3$), 2.64 [m, 1H: -(CH)-H 6], 2.81 (d, J= 11 Hz, 1H: -OH), 3.32 (d, J= 5.5 Hz, 1H: -OH in 2'), from 3.50 to 3.90 (m: -OCH_2- of the ethyl group), 3.94 (d, J= 7 Hz, 1H: -H 3), 4.21 [d, J= 8 Hz, 1H: -(CH)-H 20], 4.33 [d, J= 8 Hz, 1H: -(CH)-H 20], 4.62 and 4.91 (2d, J= 13 Hz, 1H each: -OCOO-CH_2-CCl_3 in 7), 4.64 (dd, J= 5 and 2.5 Hz, 1H: -H 2'), 4.79 (limit AB, J= 14 Hz, 2H: -OCOO-CH_2-CCl_3 in 10), 4.87 (d, J= 11 Hz, 1H: -O-CH-O-), 4.97 (bd, J= 9.5 Hz, 1H: -H 5), 5.25 (bd, J= 10 Hz, 1H: -H 3'), 5.45 (d, J= 10 Hz, 1H: -CONH-), 5.55 (dd, J= 11 and 7 Hz, 1H: -H 7), 5.72 (d, J= 7 Hz, 1H: -H 2), 6.25 (bt, J= 9 Hz, 1H: -H 13), 6.28 (s, 1H: -H 10), from 7.30 to 7.45 (m, 5H: -C_6H_5 in 3'), 7.51 [t, J= 8 Hz, 2H: -$OCOC_6H_5$ (-H 3 and -H 5)], 7.62 [t, J= 8 Hz, 1H: -$OCOC_6H_5$ (-H 4)], 8.12 [d, J= 8 Hz, 2H: -$OCOC_6H_5$ (-H 2 and -H 6)]. MS(LSIMS) : M/z = 1198: MH^+- H_2O.

3'-De-t-butoxycarbonylamino-3'-[3-(5,5-dimethyl-4-hydroxy-2-oxo-1,3-oxazolidinyl)]-7,10-O-diTroc-docetaxel, 14, as a mixture of diastereomers: To a mixture of 0.22 g of compound **13** in 5 mL of ethylacetate was added a solution of 2 mL of formic acid in 2 mL of water. The reaction mixture was stirred at room temperature for 24 h then diluted with water and extracted with ethyl acetate. The organic solution was dried with $MgSO_4$ and evaporated. The crude product was purified on 10 g of silica gel with CH_2Cl_2/MeOH : 97/3 to give 0.183 g (87%) of **14** (mixture 44:56 of diastereomers) as a white foam. ^{1}H-NMR ($CDCl_3$, at temperature of 323°K, 400MHz); δ: 1.18, 1.21, 1.24, 1.27, 1.33, 1.40 and 1.42 [7s, 12H: -CH_3 16, -CH_3 17 and -O-C(CH_3)$_2$], from 1.80 to 2.20 [m, 1H: -(CH)-H 6], 1.84 and 1.86 (2s, 3H: -CH_3 19), 2.01 and 2.05 (2s, 3H: -CH_3 18), 2.10 and 2.19 (2 dd, J= 14 and 9 Hz, 1H: -CH_2- 14 of one diastereoisomer], 2.24 and 2.30 (2s, 3H: -$COCH_3$), 2.38 (d, J= 9 Hz, 1H: -CH_2- 14 of the other diastereoisomer), 2.62 [m, 1H: -(CH)-H 6], 3.90 (d, J= 7 Hz, 1H: -H 3), 4.16 and 4.30 (2d, J= 8 Hz, 1H: -CH_2- 20 of one diastereoisomer), 4.22 and 4.31 (2d, J= 8.5 Hz, 1H: -CH_2- 20 of the other diastereoisomer), 4.40 and 4.78 (2s, 1H: CH-O), 4.60 and 4.90 (2d, J= 12 Hz, 1H each: -OCOO-CH_2-CCl_3 in 7), 4.72 (bs, 0.5 H: -H 2' of one diastereoisomer), 4.79 (limit AB, J= 14

Hz, 2H: -OCOO-CH_2-CCl_3 in 10), 4.95 (bd, J= 9.5 Hz, 1H: -H 5), 4.97 (d, J= 6 Hz, 0.5 H: -H 2' of the other diastereoisomer), 5.02 (d, J= 6 Hz , 0.5 H: -H 3' of one diastereoisomer), from 5.50 to 5.60 (m, 1H: -H 7), 5.55 (bs, 1H: -H 3' of the other diastereoisomer), 5.67 and 5.71 (2d, J= 7 Hz, 1H: -H 2), 6.13 and 6.25 (2 bt, J= 9 Hz, 1H: -H 13), 6.26 (s, 1H: -H 10), from 7.30 to 7.70 [m, 8H: -C_6H_5 in 3' and -$OCOC_6H_5$ (-H 3, -H 4 and -H 5)], 8.06 and 8.10 [2d, J= 8 Hz, 2H: -$OCOC_6H_5$ (-H 2 and -H 6)].

3'-De-t-butoxycarbonylamino-3'-[3-(5,5-dimethyl-4-hydroxy-2-oxo-1,3-oxazolidinyl)]-docetaxel, 4a and 4b: By analogy to ref.[14], with the use of a similar procedure as for **3**, 0.95 g (0.085 mmol) of 7,10-O-diTroc derivative **14** in 2 mL and acetic acid and 2 mL of methanol treated with 0.2 g of zinc powder led, after purification on 10 g of silica gel with CH_2Cl_2/MeOH : 95/5, 0.035 g (56%) of compound **4** as a mixture of diastereomers (**4a**:**4b** = 44:56). After further purification using preparative TLC (CH_2Cl_2/MeOH : 9/1), **4a** (first product eluted) and then **4b** were obtained as white foams:

4a: $[\alpha]_D^{20}$ -13.1 (c 0.610, MeOH); ^{1}H-NMR ($CDCl_3$, 400 MHz); δ: 1.13 (s, 3H: -CH_3 16 or 17), 1.18, 1.26 and 1.42 [3s, 9H: -CH_3 16 or 17 and -O-C(CH_3)$_2$], 1.75 (s, 3H: -CH_3 19), 1.85 [ddd, J=16, 11 and 2 Hz, 1H: -(CH)-H 6], 1.89 (s, 1H: -OH in 1), 1.92 (s, 3H: -CH_3 18), 2.17 (s, 3H: -$COCH_3$), 2.31 and 2.37 (dd, J= 16 and 9 Hz, 1H each: -CH_2- 14), 2.53 [m, 1H: -(CH)-H 6], 3.86 (d, J= 7 Hz, 1H: -H 3), 4.20 (d, J= 8.5 Hz, 1H: -(CH)-H 20), 4.23 (dd, J= 11 and 7 Hz, 1H: -H 7), 4.30 [d, J= 8.5 Hz, 1H: -(CH)-H 20], 4.34 (s, 1H: -OH in 10), 4.38 (bs, 1H: CH-O-), 4.97 (dd, J= 10 and 2 Hz, 1H: -H 5), 5.00 (d, J= 2.5 Hz, 1H: -H 2'), 5.31 (s, 1H: -H 10), 5.56 (d, J= 2.5 Hz, 1H: -H 3'), 5.68 (d, J= 7 Hz, 1H: -H 2), 6.26 (bt, J= 9 Hz, 1H: -H 13), 7.40 [t, J= 8 Hz, 1H: -C_6H_5 in 3' (-H 4)], 7.46 [t, J= 8 Hz, 2H: -C_6H_5 in 3' (-H 3 and -H 5)], 7.53 [t, J= 8 Hz, 2H: -$OCOC_6H_5$ (-H 3 and -H 5)], 7.58 [d, J= 8 Hz, 2H: -C_6H_5 in 3'(-H 2 and -H 6)], 7.63 [t, J= 8 Hz, 1H: -$OCOC_6H_5$ (-H 4)], 8.11 [d, J= 8 Hz, 2H: -$OCOC_6H_5$ (-H 2 and -H6)]. MS(DCI) : M/z = 839: MNH_4^+.

4b: $[\alpha]_D^{20}$ -46.2 (c 0.515, MeOH); ^{1}H-NMR ($CDCl_3$, 400MHz); δ: 1.12 (s, 3H: -CH_3 16 or 17), 1.22 (s, 3H: -CH_3 16 or 17), 1.34 and 1.44 [3s, 6H: -O-C(CH_3)$_2$], 1.72 (s, 1H: -OH in 1), 1.75 (s, 3H: -CH_3 19), 1.85 [ddd, J= 16, 11 and 2 Hz, 1H: -(CH)-H 6], 1.98 (s, 3H: -CH_3 18), 2.01 and 2.11 (2 dd, J= 16 and 9 Hz, 2H: -CH_2- 14), 2.30 (s, 3H: -$COCH_3$), 2.30 (s, 3H: -$COCH_3$), 2.57 [m, 1H: -(CH)-H 6], 3.66 (bb, 1H: -OH in 2'), 3.90 (d, J= 7 Hz, 1H: -H 3), 4.15 [d, J= 8 Hz, 1H: -(CH)-H 20], 4.26 (dd, J= 11 and 7 Hz, 1H: -H 7), 4.30 [d, J= 8 Hz, 1H: -(CH)-H 20], 4.31 (bs, 1H: -OH in 10), 4.68 (bs, 1H: CH-O-), 4.93 (dd, J= 10 and 2 Hz, 1H: -H 5), 4.96 (bd, J= 6.5 Hz, 1H: -H 2'), 5.01 (d, J= 6.5 Hz, 1H: -H 3'), 5.24 (s, 1H: -H 10), 5.31 (bb, 1H: -OH), 5.64 (d, J= 7 Hz, 1H: -H 2), 6.14 (bt, J= 9 Hz, 1H: -H 13), 7.33 [t, J= 8 Hz, 1H: -C_6H_5 in 3' (-H 4)], 7.41 [t, J= 8 Hz, 2H: -C_6H_5 in 3' (-H 3 and -H 5)], 7.45 [d, J= 8 Hz, 2H: -C_6H_5 in 3' (-H 2 and -H 6)], 7.50 [t, J= 8 Hz, 2H: -$OCOC_6H_5$ (-H 3 and -H 5)], 7.64 [t, J= 8 Hz, 1H: -$OCOC_6H_5$ (-H 4)], 8.06 [d, J= 8 Hz, 2H: -$OCOC_6H_5$ (-H 2 and -H 6)].MS(DCI) : M/z = 839: MNH_4^+.

2-(2-methoxycarbonyl)propyl)-1',2',2',2'-tetrachloroethyl-carbonate, 16: With the use of a similar procedure as for **7**, 2.36 g (0.2 mol) of methyl 2-hydroxyisobutyrate gave 4.57 g (70%) of carbonate **16** as white powder (m.p. <50°C) used freshly prepared in the next step.

3'-De-t-butoxycarbonylamino-3'-[3-(5,5-dimethyl-2,4-dioxo-1,3-oxazolidinyl)]-7,10-O-diTroc-docetaxel, 17: To a mixture of 0.264 g (0.25 mmol) of amino-taxoid **8** in 5 mL of THF were added successively 0.082 g (1.1 mmol) of carbonate **16** and 1 mL of an aqueous solution of K_2CO_3 (5M). The reaction mixture was stirred at room temperature for 24 h. Then 15 mL of water were added and extraction was performed with ethyl acetate (3x10 mL). The organic solution was dried with $MgSO_4$ and evaporated in vacuo. The crude product was purified on 15 g of silica gel with CH_2Cl_2/MeOH : 99/1 to give 0.228 g (78%) of **17** as a white foam. ^{1}H-NMR ($CDCl_3$, 400 MHz); δ: 1.17 (s, 3H: -CH_3 16 or 17), 1.21 (s, 3H: -CH_3 16 or 17), 1.60 and 1.63 [2s, 3H each: -O-C(CH_3)$_2$], 1.83 and 2.03 (2 dd, J= 14 and 9 Hz, 1H each: -CH_2- 14), 1.84 (s, 3H: -CH_3 19), 1.98 (s, 3H: -CH_3 18), 2.05 [ddd, J= 16, 11 and 2 Hz, 1H: -(CH)-H 6], 2.39 (s, 3H: -$COCH_3$), 2.63 [m, 1H: -(CH)-H 6], 3.85 (d, J= 7 Hz, 1H: -H 3), 4.00 (bb, 1 H: -OH in 2'), 4.14 [d, J= 8 Hz, 1H: -(CH)-H 20], 4.31 [d, J= 8 Hz, 1H: -(CH)-H 20], 4.60 and 4.92 (2d, J= 11 Hz, 1H each: -OCOO-CH_2-CCl_3 in 7), 4.77 (limit AB, J= 12 Hz, 2H: -OCOO-CH_2-CCl_3 in 10), 4.96 (bd, J= 9.5 Hz, 1H: -H 5), 5.26 (bd, J= 8 Hz, 1H: -H 2'), 5.40 (d, J= 8 Hz, 1H: -H 3'), 5.54 (dd, J= 11 and 7 Hz, 1H: -H 7), 5.64 (d, J= 7 Hz, 1H: -H 2), 6.07 (bt, J= 9 Hz, 1H: -H 13), 6.22 (s, 1H: -H 10), 7.31 [t, J= 8 Hz, 1H: -C_6H_5 in 3' (-H 4)], 7.40 [t, J= 8 Hz, 2H: -C_6H_5 in 3' (-H 3 and -H 5)], 7.50 [d, J= 8 Hz, 2H: -C_6H_5 in 3' (-H 2 and -H 6)], 7.53 [t, J= 8 Hz, 2H: -$OCOC_6H_5$ (-H 3 and -H 5)], 7.67 [t, J= 8 Hz, 1H: -$OCOC_6H_5$ (-H 4)], 8.06 [d, J= 8 Hz, 2H: -$OCOC_6H_5$ (-H 2 and -H 6)]. MS(DCI) : M/z = 1185: MNH_4^+.

3'-De-t-butoxycarbonylamino-3'-[3-(5,5-dimethyl-2,4-dioxo-1,3-oxazolidinyl)]-docetaxel, **5**: With the use of a similar procedure as for **3**, 0.15 g (0.128 mmol) of 7,10-O-diTroc derivative **17** in 3 mL of acetic acid and 3 mL of methanol treated with 0.3 g of zinc powder at 60°C led, after purification on 5 g of silica gel with CH_2Cl_2/MeOH : 98/2, 0.055 g (53%) of pure compound **5** as a white foam, $[\alpha]_D^{20}$ -49.3 (c 0.531, MeOH); ^{1}H-NMR ($CDCl_3$, 400 MHz); δ : 1.10 (s, 3H: -CH_3 16 or 17), 1.18 (s, 3H: -CH_3 16 or 17), 1.60 and 1.63 [2s, 3H each: -O-C(-CH_3)$_2$], 1.74 (s, 3H: -CH_3 19), 1.80 (m, 2H: H 14 and H 6), 1.9 (s, 3H: -CH_3 18), 1.98 (dd, J= 14 and 9 Hz, 1H: H 14), 2.15 (s, 3H: -$COCH_3$), 2.60 [m, 1H: H 6], 3.86 (d, J= 7 Hz, 1H: -H 3), 4.02 (bd, 1 H: -OH in 2'), 4.15 to 4.30 [m, 4H: 2xH 20, H 7 and OH 7), 4.94 (bd, J= 9.5 Hz, 1H: -H 5), 5.20 (s, 1H: -H 10), 5.22 (t, J= 8 Hz, 1H: -H 2'), 5.40 (d, J= 8 Hz, 1H: -H 3'), 5.61 (d, J= 7 Hz, 1H: -H 2), 6.04 (bt, J= 9 Hz, 1H: -H 13), 7.28 [t, J= 8 Hz, 1H: -C_6H_5 in 3' (-H 4)], 7.40 [t, J= 8 Hz, 2H: -C_6H_5 in 3' (-H 3 and -H 5)], 7.50 [d, J= 8 Hz, 2H: -C_6H_5 in 3' (-H 2 and -H 6)], 7.53 [t, J= 8 Hz, 2H: -$OCOC_6H_5$ (-H 3 and -H 5)], 7.65 [t, J= 8 Hz, 1H: -$OCOC_6H_5$ (-H 4)], 8.06 [d, J= 8 Hz, 2H: -$OCOC_6H_5$ (-H 2 and -H 6)]. MS(LSIMS) : M/z = 820: MH^+.

References and notes:

1. a) Schiff P.B., Fant J., Horwitz S.B., *Nature*, **1979**, 665. b) Manfredi J.J., Horwitz S.B., *Pharmac. Ther.*, **1984**, 83.
2. a) For a recent review on preclinical studies on docetaxel, see: Lavelle F., Guéritte-Voegelein F., Guénard D., *Bull. Cancer*, **1993**, *80*, 326. b) For clinical results on docetaxel and paclitaxel, see: Rothenberg M.L., *Curr. Opin. Invest. Drugs*, **1993**, *2 (12)*, 1269. c) Paclitaxel has been recently approved by the FDA for ovarian cancer treatment.
3. Monsarrat B., Mariel E., Cros S., Garès M., Guénard D., Guéritte-Voegelein F., Wright M., *Drug Metab. Dispos.*, **1990**, *18*, 895.
4. Monsarrat B., Alvinerie P., Garès M., Wright M., Dubois J., Guéritte-Voegelin F., Guénard D., Donehower R., Rowinsky E., *Cell. Pharmacol.*, **1993**, *1* (suppl. I), S77.
5. Monsarrat B., Alvinerie P., Wright M., Dubois J., Guéritte-Voegelin F., Guénard D., Donehower R., Rowinsky E., *J. Natl. Cancer Inst. Monograph.*, **1994**, in press.
6. Walle T., Kumar G.N., McMillan J.M., Thornburg K.R., Walle U.K., *Biochem. Pharmacol.*, **1993**, *46 (9)*, 1661.
7. Rowinsky E.K., Cazenave I.A., Donehower R.C., *J. Natl. Cancer Inst.*, **1990**, *82*, 1247.
8. Harris J.W., Katki A., Anderson L.W., Chmurny G.N., Paukstelis J.V., Collins J.M., *J. Med. Chem.*, **1994**, *37*, 706.
9. Cresteil T., Monsarrat B., Alvinerie P., Tréluyer J.M., Vieira I., Wright M., *Cancer Res.*, **1994**, *54*, 386.
10. a) Vuilhorgne M. *et al.*, presented at the *207 th ACS National Meeting*, San Diego, March 13-17, 1994. b) Vuilhorgne M. *et al.*, ACS Symposium Series, in preparation. c) Monegier B., Gaillard C., Sablé S., Vuilhorgne M., *Tetrahedron Lett.*, **1994**, in press.
11. The epimerization of paclitaxel at C-7 in cell culture medium has been previously observed, see: Ringel I., Horwitz S.B., *J. Pharmacol. Exp. Ther.*, **1987**, *242*, 692.
12. Commerçon A., Bézard D., Bernard F., Bourzat J.D., *Tetrahedron Lett.*, **1992**, *33*, 5185.
13. For very recent reviews about taxoid chemistry, see: Kingston D.G.I., Molinero A.A., Rimoldi J.M., *Progress in the Chemistry of Organic Natural Products*, **1993**, *61*, 1; Nicolaou K.C., Dai W.M., Guy R.K., *Angew. Chem. Int. Ed. Engl.*, **1994**, *33*, 15.
14. Barcelo G., Senet J.P., Sennyey G., Bensoam J., Loffet A., *Synthesis*, **1986**, 627; Olofson R.A. *Pure Appl. Chem.*, **1988**, *60*, 1715..
15. Avy M.A., *Bull. Soc. Chim. Fr.*, **1931**, *49*, 12.
16. For another example of preparation of 5-hydroxy-2-oxo-1,3-oxazolidines, see: Kano S., Yuasa Y., Yokomatsu T., Shibuya S., *Chem. Lett.*, **1983**, 1475.
17. Bourzat J.D. *et al.*, unpublished results.
18. For a previous preparation of oxazolidine-2,4-diones, see: Rekker R.F., Verleur H., Nauta W.Th., *Rec. Trav. Chim. Pays-Bas*, **1951**, *70*, 5; Rekker R.F., Faber A.C., Tom D.H.E., Verleur H., Nauta W.Th., *Rec. Trav. Chim. Pays-Bas*, **1951**, *70*, 113.
19. Chauvière G., Guénard D., Picot F., Sénilh V., Potier P., *C. R. Acad. Sciences, Série II*, **1981**, *293*, 501.
20. Riou J.F., Naudin A., Lavelle F., *Biochem. Biophys. Res. Commun.*, **1992**, *187*, 164.

(*Received in Belgium* 7 *March* 1994; *accepted* 19 *May* 1994)