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Effects of the Hydrophobicity of Taxoids on their Interaction with Tubulin

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Abstract—Modifications of the hydrophobic character at the 7 and 10 positions of the taxoids greatly modified the effect of these drugs on the tubulin–microtubule system. The presence of an alkyl chain at these positions decreased the activity while their corresponding more polar analogues restored the activity of these molecules. It appears that the recognition of taxoids by tubulin depends on the location of the most important hydrophobic area.

Introduction

Paclitaxel **1**, a complex diterpene isolated from the yew tree,[1] *Taxus brevifolia*, and docetaxel **2**,[2] synthesized from 10-deacetyl baccatin III,[3] are currently used in the treatment of ovarian and breast cancers (see refs 4 and 5 for the latest reviews). These two molecules and their analogues block cell replication by promoting tubulin assembly and inhibiting microtubule disassembly.[6] These interesting properties have stimulated efforts for a better understanding of the mechanism of action. Thus, the synthesis of fluorescent (e.g. refs 7–9) and photoaffinity (e.g. refs 10 and 11) analogues of taxoids have provided useful tools for the study of the drug-binding site on tubulin and for the study of taxoid effects in the cells. Moreover, the three-dimensional structure of tubulin published recently[12] will certainly bring more information on the drug-binding site in the near future. Concerning the structure–activity relationships, a number of taxoids modified on the southern and northern parts of the taxane core have been prepared and evaluated as inhibitors of microtubule disassembly.[13] From these studies it has been shown that the northern part can support various modifications without great loss of activity (Scheme 1).

There are, however, some exceptions showing that large hydrophobic groups at carbons 7 and/or 10 can lead to a strong loss of activity. For example, the diprotected derivative of docetaxel possessing a trichloroethyl carbonate group at C-7 and C-10 (compound **3a**) has no effect on the cold disassembly of microtubules (unpublished results). 7-*O*-Acylpaclitaxel such as compound **3b** with a 3-isopropylbenzoyl group at C-7 is also inactive.[14] In contrast, 7-xylosylpaclitaxel **3c** is more active than paclitaxel on tubulin, showing that a large hydrophilic group at C-7 has no detrimental impact on the interaction.[15]

For the purpose of understanding the role of lipophilicity on the binding of taxoids to microtubules, we systematically evaluated the inhibition of microtubules disassembly, cytotoxicity and the hydrophobicity index φ_0 for a series of docetaxel analogues showing a continuous change in their lipophilicity. Thus, we prepared taxoids possessing alkyl side-chains of different lengths at C-7 and/or C-10. Within this group, we synthesized analogues having hydrophilic groups on the alkyl side-chains in order to compare the contribution of hydrophilicity/hydrophobicity on tubulin binding. Finally, taxoids with aromatic ester groups at C-7 or C-10 were synthesized to analyze, for another type of hydrophobic groups, the influence of the side-chain flexibility at these positions on the activity.

Results and Discussion

Chemistry

The new taxoids modified at C-7 and/or C-10 were prepared from 2′-(2,2,2-trichloroethoxycarbonyl)-docetaxel

Keywords: docetaxel; tubulin; hydrophobicity; structure–activity relationships.

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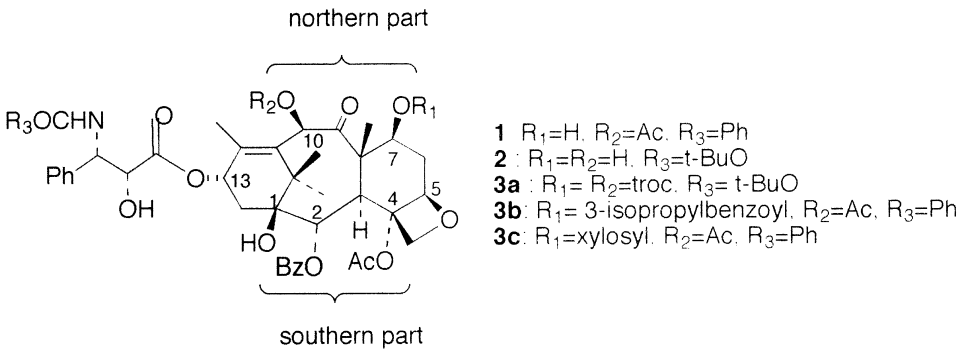


Scheme 1.

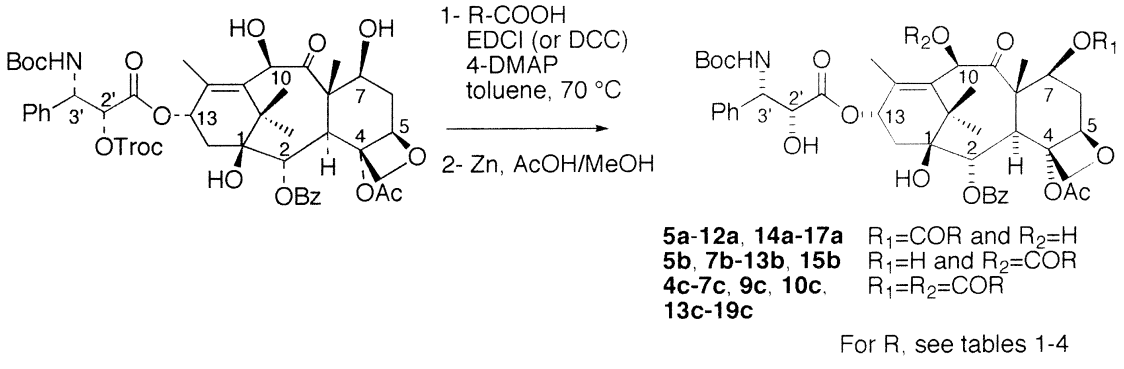
obtained from docetaxel **2**.[16] Esterification with various acids in the presence of DCC or EDCI and DMAP afforded the desired taxoids modified at C-7 or/and C-10 after removal of the 2′-protective group (Scheme 2).

First, linear chains were added at C-7 and/or C-10 and the length of the alkyl chains was increased from $n=1$ to $n=16$ (see Tables 1–3). Thus, the 7-monoesters (compounds **5a**–**12a**; Table 1), 10-monoesters (compounds **5b** and **7b**–**12b**; Table 2) and 7,10-diesters (compounds **4c**–**7c**, **9c** and **10c**; Table 3) were obtained. Under the conditions employed, the reactivity of the C-7 and C-10 hydroxyl groups depends on the bulkiness of the acid used in the reaction. Indeed, no selectivity occurred in the esterification of 2′-(2,2,2-trichloroethoxycarbonyl)-docetaxel with acids comprising fewer than 11 carbon atoms and the two C-7- and C-10-monoesters were obtained simultaneously. On the other hand, with more bulky acids such as myristic acid and stearic acid the 7,10-diesters could not be obtained. It should be noticed that no attempt was made to improve the selectivity of the esterification procedure despite the recently selective acylation by Holton at C-7 or C-10 of 10-deacetylbaccatin III.[17–19] Then, analogues bearing aromatic or conjugated aromatic carboxylic substituents at carbons 7/10 (Table 4; compounds **13**–**15**) were prepared to evaluate the effects on the biological activity of rigid linear side-chains situated on the northern part of the taxane core.

Four docetaxel analogues bearing hydrophilic substituents at carbons 7/10 (compounds **16**–**19**; see Scheme 3 and Experimental for their synthesis) were also synthesized for the purpose of comparing the effects of hydrophilicity versus hydrophobicity.

Biological activities

The biological activities were systematically evaluated for each compound (Tables 1–4). The drug concentration inhibiting 50% of microtubule disassembly (IC_{50}) induced by cold was evaluated and compared to that of paclitaxel **1**.[20] In vitro cytotoxicity assays were performed using the KB cell line.[21] The major concern in the evaluation of the biological activity of hydrophobic compounds is to know the real concentration of the free drug able to interact with microtubules. Thus, the absence of aggregates or precipitates in aqueous solution as checked for docetaxel was **2** and for 7,10-diundecanoyldocetaxel **10c** taken as a good example of an inactive hydrophobic compound (see Experimental). The data in Tables 1–3 show that there exists a good correlation between inhibition of microtubules disassembly and cytotoxicity. As illustrated in Tables 1 and 2, analogues monosubstituted at C-7 or C-10 with alkyl side-chains of less than 11 carbon atoms ($n=9$), exhibit good tubulin binding properties in comparison with the inactive ones. It should be noted that a number of 10-monoesters bearing a butyrate, pentanoate, hexanoate or octanoate function at C-10 have already been prepared in the paclitaxel series.[14,22,23] All these compounds also retain cytotoxicity, their potency decreasing with increasing of the chain length.



Scheme 2.

Table 1. Docetaxel analogues modified at C-7

Compd	R CH_3–$(CH_3)_n$– (n)	Microtubule disassembly assay IC_{50}/IC_{50} (paclitaxel)[a]	KB cytotoxicity IC_{50} (nM)[a]	φ_0[a]	S[a]
Docetaxel	—	0.5	0.5	64.30	0.00
5a	2	2.2	1.6	80.93	0.30
6a	3	3.0	2.1	84.80	0.41
7a	5	1.9	2.5	90.66	0.64
8a	6	1.9	3.0	94.57	0.76
9a	7	2.5	20.0	93.04	0.86
10a	9	Inactive	40.0	101.30	1.09
11a	12	Inactive	400.0	105.50	1.43
12a	16	Inactive	Inactive	106.70	1.88

[a]See Experimental.

Table 2. Docetaxel analogues modified at C-10

Compd	R CH_3–$(CH_3)_n$– (n)	Microtubule disassembly assay IC_{50}/IC_{50} (paclitaxel)[a]	KB cytotoxicity IC_{50} (nM)[a]	φ_0[a]	S[a]
5b	2	1.8	1.0	74.60	0.43
7b	5	2.1	2.8	83.52	0.66
8b	6	2.1	4.0	88.61	0.77
9b	7	2.0	4.5	97.60	0.89
10b	9	Inactive	40.0	97.40	1.11
11b	12	Inactive	300.0	103.70	1.45
12b	16	Inactive	300.0	107.50	1.90

[a]See Experimental.

Table 3. Docetaxel analogues modified at C-7 and C10

Compd	R CH_3–$(CH_3)_n$– (n)	Microtubule disassembly assay IC_{50}/IC_{50} (paclitaxel)[a]	KB cytotoxicity IC_{50} (nM)[a]	φ_0[a]	S[a]
4c	1	0.7	6	86.40	0.38
5c	2	9.0	12	90.71	0.79
6c	3	14.0	20	96.16	0.96
7c	5	Inactive	500	102.90	1.14
9c	7	Inactive	5000	105.80	1.46
10c	9	Inactive	5000	118.10	1.88

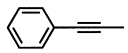
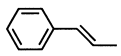
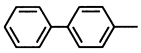
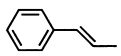
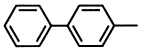
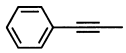
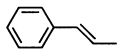
[a]See Experimental.

For 7,10-disubstituted derivatives, the inhibition of microtubules disassembly also decreases with the length of the carbon chain (Table 3), and the interaction with microtubules disappears from $n=9$ for monosubstituted analogues (Tables 1 and 2) and from $n=5$ for disubstituted derivatives (Table 3).

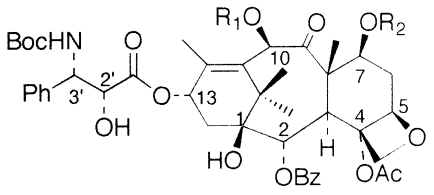
Aromatic analogues described in Table 4 were then prepared in order to check if this loss of activity could be due to a folding of the hydrophobic alkyl chains onto the taxane core. The position of the alkyl chains could indeed interfere with the interaction of these taxoids with microtubules. In this series of aromatic taxoids, 7-*O*-benzoylpaclitaxel,[14,24] 10-*O*-benzoyl-10-deacetylpaclitaxel,[22,23] 10-*O*-benzoyl-docetaxel[23] as well as 10-cinnamoyl-10-deacetylpaclitaxel[22] have been shown to display cytotoxic properties similar to that of paclitaxel and docetaxel. As illustrated in Table 4, monosubstituted analogues at C-7 (compounds **14a** and **15a**), and at C-10 (compounds **13b** and **15b**) interact with microtubules whereas the disubstituted derivatives **13c**–**15c** bearing the same substituents are inactive. There exists a good correlation between the inhibition of microtubules disassembly and cytotoxicity except for compound **13b** whose cytotoxicity value is less than expected. The results in Table 4 also indicate a behavior of the aromatic compounds similar to that of the alkylated analogues possessing a similar carbon chain length at C-7 and C-10. For example, 7-*O*-acyl derivatives **7a**, **14a** and **15a** as well as the 10-*O*-acyltaxoids **7b** and **15b** interact with microtubules in the same range of concentration. From these results, one can suggest that the loss of activity of C-7 and C-10 mono-alkylated analogues **10a**, **10b**, **11a**, **11b**, **12a** and **12b** is not due to a folding of the alkyl chains onto the taxane ring thereby preventing a direct interaction with the binding site.

When an acid function was added at the end of the carbon chain ($R=-(CH_2)_nCOOH$), the inhibition of microtubules disassembly was restored. Indeed, 7,10-disuccinyldocetaxel ($n=2$, **16**), 7,10-diglutaryldocetaxel[16] ($n=3$) and 7,10-diazelayldocetaxel ($n=7$, **17c**) possess an activity of, respectively, 1.6T, 2T, and 8T, whereas the corresponding hydrophobic compounds

Table 4. Aromatic substituted C-7 and/or C-10 analogues of docetaxel

Compd[a]	*R*	Microtubule disassembly assay IC_{50}/IC_{50} (paclitaxel)[b]	KB cytotoxicity IC_{50} (nM)[b]	φ_0[b]	*S*[b]
14a		3.3	7	83.55	0.68
15a		6.5	4	85.23	0.66
13b		1.0	20	84.43	0.91
15b		5.0	3	79.02	0.68
13c		Inactive	5000	97.80	1.70
14c		Inactive	500	91.20	1.28
15c		Inactive	60	91.59	1.12

[a]**a** for 7-mono substituted derivatives, **b** for 10-mono substituted derivatives and **c** for 7,10-disubstituted derivatives.
[b]See Experimental.



16 $R_1 = R_2 = -CO(CH_2)_2COOH$, **17** $R_1 = R_2 = -CO(CH_2)_7COOH$

18 $R_1 = H$, $R_2 = -CO(CH_2)_2CONH(CH_2)_3O(CH_2)_2O(CH_2)_2O(CH_2)_3NH_2$

19 $R_1 = H$, $R_2 = -CO(CH_2)_3CONH(CH_2)_4NH(CH_2)_3NH_2$

Scheme 3.

(**5c**, **6c**, and **9c**) were weakly active or inactive (9T, 14T and inactive, respectively). Likewise, derivatives bearing a long chain with heteroatom and hydrophilic functions such as compounds **18** and **19** were active on microtubule disassembly (2.2T and 1.4T, respectively) whereas the long chain ($n > 11$) hydrophobic analogues were totally inactive.

These results clearly show that the bulkiness of the functions in the northern part of the taxoid molecules is not a decisive parameter for good binding to tubulin: compounds **12a** (Table 1) and **18** or **19**, with similar volumes in this area, have opposite effects on the disassembly of microtubules. Thus, the lipophilicity of the molecules must be taken into account either for the recognition process or for the drug–tubulin complex stability.

Hydrophobicity

The chromatographic hydrophobicity index φ_0, similar to log *P*, have been evaluated using reverse-phase HPLC[25] (for details, see Experimental). As shown in an earlier conformational analysis, the predominant conformers of paclitaxel **1** and docetaxel **2**,[26] form a hydrophobic clustering of the substituents at C-3′ of the side-chain with the C-2 benzoate and the C-4 acetate moieties. In order to estimate the contribution of the northern area to the hydrophobicity of the compounds mentioned in Tables 1–4, we determined the water-accessible surface, a good parameter to evaluate water–solute interactions.[27] Applying a lipophilic potential to the water-accessible surface (see Experimental), two main hydrophobic areas could be distinguished. The hydrophobic southern part includes, as mentioned above, the 2-benzoate, 4-acetyl, 3′-phenyl and 3′-terbutyl moieties and the northern area is characterized by the newly added alkyl chains. We then determined the hydrophobic surface quotient *S* by dividing the hydrophobic surface of the northern area by that of the southern area. As illustrated in Figure 1, the chemical parameter of hydrophobicity φ_0 is proportional to the physical descriptor *S* of the molecules except for the very hydrophobic molecules **12a** and **12b**. This discrepancy is

explained by the fact that these compounds are not soluble in acetonitrile/water solutions containing more than 3% water, leading to a wrong evaluation of the hydrophobicity index φ_0 (see Experimental). For all the other compounds, we used *S* as a descriptor of the relative hydrophobicity of these molecules. As expected, *S* is proportional to the number of carbons of the added alkyl chains at C-7 and/or C-10 (Tables 1–3), but the hydrophobic surface of 7,10-diesters is less significant than that of the corresponding monoesters. This difference could be due to an overlapping of both alkyl chains in the *O*-diacyl derivatives.

As shown in Figure 1, the hydrophobic nature of 7-monoesters is greater than that of the 10-monoester analogues. Interestingly, docetaxel **2** belongs to the 10-monoester series. In the case of the 7-, 10-, and 7,10 aromatic esters, the hydrophobic index φ_0 is generally lower than that of the alkylated analogues with a similar *S* value (see, for example, compounds **7a** and **15**). This result is in agreement with the fact that aromatic groups are capable of undergoing a weak association with water molecules[28] and consequently possess a lower hydrophobic character which explains their better interaction with tubulin.

Figure 2 shows the correlation of cytotoxicity, represented by its logarithmic value, versus *S* values for all the compounds mentioned in Tables 1–3. The correlation of cytotoxicity values versus *S* values of the mono- and diesters bearing alkyl chains at C-7 and C-10 is not linear. Indeed, a dramatic decrease in cytotoxicity occurs when $S = 1$, that is to say, when the hydrophobicity of the northern area becomes higher than that of the southern area.

Molecular modeling studies were not easy to perform in this series of molecules because of the flexibility of the alkyl chains which can lead to a large number of different conformations of similar energy; this fact was in

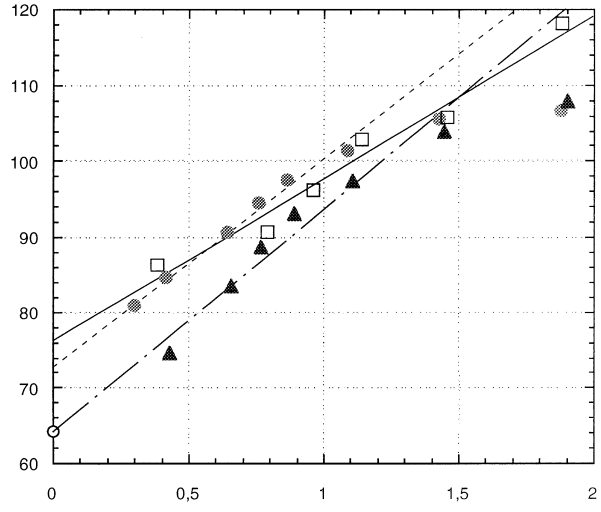


Figure 1. In abscissa, the hydrophobic surface quotient *S*, in ordinate the chromatographic hydrophobicity index φ_0; (—●—) represents the 7-mono alkyl ester series, (—▲—) the 10-mono alkyl ester series, (—□—) the 7,10-dialkyl ester series and (○) the docetaxel molecule.

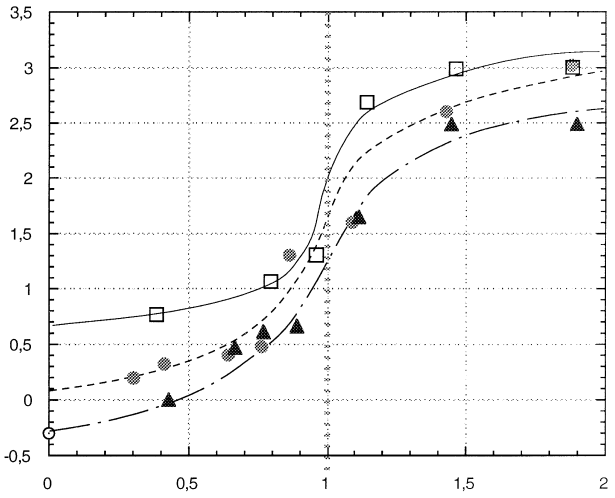


Figure 2. In abscissa, the hydrophobic surface quotient *S*, in ordinate the logarithm of the cytotoxicity; (—●—) represents the 7-mono alkyl ester series, (—▲—) the 10-mono alkyl ester series, (—□—) the 7,10-dialkyl ester series, and (○) the docetaxel molecule.

agreement with the NMR experiments (NOESY) where it was impossible to see any interaction of these alkyl chains with the other parts of the molecule. We thus focused our analysis on the conformational modifications of the C-13 side-chain brought by the substituents at C-7 and/or C-10. It appeared that compounds possessing a long alkyl chain ($n > 8$) at C-7 display, among the conformations of lowest energy, a different one from those already described for the taxoids.[29,30] This new conformation is characterized by a different position of the *tert*-butyloxy group which is no longer close to the hydrophobic south part of the molecule but lies in the vicinity of the methyl groups at C-16 and C-17. However, we were not able to observe this conformation by NMR spectroscopy of a DMSO solution of compound **11a**, taken as a good example of a hydrophobic analogue. Indeed, the NOEs between the hydrogen of the side-chain and those of the C-2 and C-4 acyl groups are similar to those described for docetaxel **2**[30] whereas no NOE effects occurred between the C-7 alkyl chain and the C-13 side-chain, showing no overlapping between these two groups.

Conclusion

This study allows us to suggest two hypotheses explaining the decreasing activity of apolar drugs with increasing hydrophobicity. The interaction of taxoids with microtubules may be divided, for a better understanding, into two parts not related to the kinetic aspect of the binding. An initial recognition step takes place, positioning the active taxoid at the right place and in a correct orientation in the tubulin site, that is to say the difference between an efficient and an unefficient impact. This allows, in the second part, specific bonds to occur in order to stabilize the drug–protein complex. The northern part of the molecule would thus be in a hydrophilic environment and probably outside the binding site. In the case of taxoids with increasing lipophilicity,

the decreasing activity could be the result of a destabilization of the drug–tubulin complex due to an unfavorable interaction between the hydrophobic chains and their hydrophilic environment or their aqueous vicinity.

It seems obvious in this hypothesis that the northern part of these highly hydrophobic molecules continues to be recognized for its own hydrophobic character despite the increasing contribution to the total hydrophobicity by the northern part as the alkyl side-chain increases. It would thus be expected that a linear increase in the hydrophobicity of the northern part of the molecule would lead to a correspondingly progressive loss of activity due to destabilization of the drug–protein complex. However, this is contrary to our observations.

An alternative hypothesis concerns the energies associated with the desolvating process occuring during the complexation of the hydrophobic portion of the molecule with the corresponding domain on tubulin. In this context, an increase in the hydrophobicity of the northern part would preferentially lead to its desolvation once this hydrophobicity exceeds that of the southern part, that is to say, when $S > 1$. The energy of entropic origin brought by water desolvation of the complexation would thus be a determining factor in drug–receptor binding. These two hypotheses are not exclusive, they are complementary. The probability of an inefficient recognition between taxoids and tubulin increases with the hydrophobicity of the northern part of taxoids, while, even if the drug–protein complex is obtained, its stability decreases proportionally with the hydrophobicity exposed on the north part of the molecule. This hypothesis may also explain the inactivity of aromatic derivatives **13c**–**15c** in which the strongest hydrophobic areas are located in the northern part. Inversely, the antitubulin activity of the aromatic monoester analogues **14a** and **15a**, is due to the superior hydrophobic character of the southern area. Thus, the observed activity or inactivity of these hydrophobic taxoids can be explained by the particular distribution of the hydrophobic groups in the molecules.

Experimental

The purity of the samples was checked by chromatographic methods (HPLC and TLC) and careful analysis of the NMR spectra. Absorption spectra were measured with a Perkin–Elmer lambda 5 spectrometer and mass spectra were recorded on a Kratos MS80 (FAB). ^{1}H and ^{13}C spectra were recorded on Bruker AC200, AC250 or AM300 spectrometers using tetramethylsilane as internal standard. Chemical shifts are expressed in part per million (ppm). Coupling constants (J) are given in Hertz; s, bs, t, d, dd, q and m indicate singlet, broad singlet, triplet, doublet, doublet of doublet, quadruplet and multiplet. All NMR spectra were very similar, the only modifications being the added signals corresponding to the acyl chains and the acylated positions at C-7/C-10. So only the first compound will be fully described. For the following ones, only the NMR characteristics of the chain(s) and of the acylated position(s) will be reported. To shorten this report, the NMR spectra of the troc-intermediate will not be reported herein. Docetaxel **2** was a gift from Rhône-Poulenc Rorer S.A. 2′-(2,2,2-trichloroethyloxy carbonyl) docetaxel was synthesized as previously described.[16]

General methods: esterification

Method A: 2′-(2,2,2-trichloroethyloxycarbonyl) docetaxel was heated in dry toluene at 70 °C with an organic acid (5 or 10 equiv), *N*′-(3-dimethylaminopropyl)-*N*-ethyl carbodiimide hydrochloride (EDCI, 10 equiv) and 4-(dimethylamino)pyridine (DMAP, 0.1 to 0.2 equiv). Method B: dicyclohexylcarbodiimide (DCC, 2 equiv) was used instead of EDCI with 2 equiv of acid and 0.5 equiv of DMAP. Work up: After a few h (the reaction time is specified for each compound), the solution was cooled and the solvent was removed under reduced pressure; the resulting residue was dissolved in CH_2Cl_2, the organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated. *Removal of the Troc group*: The compound was stirred vigorously in a mixture of HOAc and MeOH (1:1) with 0.5–1 weight equivalent of zinc powder for periods of time which are specified for each compound. The solution was filtered and the solvents were removed under reduced pressure. The residue was dissolved in CH_2Cl_2, the organic layer was washed with brine, dried over $MgSO_4$ and concentrated.

Synthesis of linear hydrophobic derivatives

Neither yields nor the ratio of mono-7, mono-10, and di-7,10 esterification were optimized.

Preparation of compound 4c. 2′-(2,2,2-Trichloroethyloxycarbonyl) docetaxel (550 mg, 0.56 mmol) was esterified with propionic anhydride (2 mL) in pyridine (2 mL). The solution was stirred for 24 h at room temperature. The solvents were removed under reduced pressure and, after standard work up, the residue was purified on TLC (EtOAc:heptane, 1:1) to yield 7,10-dipropionyl 2′-troc docetaxel (325 mg, 53%). According to the general procedure, removal of the troc group of 7,10-dipropionyl 2′-troc docetaxel (200 mg, 4 h at room temperature then 1 h at 60 °C) and purification by preparative TLC (EtOAc:heptane, 1:1) yielded 7,10-dipropionyldocetaxel (**4c**) (159 mg, 79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.10 (t, $J = 7$, CH_3 propionyl), 1.15 (s, C-16H_3), 1.17 (t, $J = 7$ Hz, CH_3 propionyl), 1.24 (s, C-17H3), 1.35 (s, 3′-*t*Bu), 1.77 (s, C-19H_3), 1.85 (m, C-6H), 1.87 (s, C-18H_3), 2.28 (m, C-14H_2), 2.37 (s, 4-OAc), 2.46 (q, $J = 7$ Hz, 2 CH_2 propionyl), 2.58 (m, C-6H), 3.91 (d, $J = 7$ Hz, C-3H), 4.19 (d, $J = 8$ Hz, C-20 H), 4.32 (d, $J = 8$ Hz, C-20 H), 4.62 (sl, C-2′H), 4.94 (dl, $J = 9$ Hz, C-5H), 5.26 (dl, $J = 9$ Hz, C-3′H), 5.46 (d, $J = 9$ Hz, 3′NH), 5.56 (m, C-7H), 5.68 (d, $J = 7$ Hz, C-2H), 6.22 (t, $J = 9$ Hz, C-13H), 6.30 (s, C-10H), 7.38 (m, 3′-C_6H_5), 7.5, 7.6 and 8.12 (m, 2-OBz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 8.75 (CH_3 propionyl), 10.67 (C-19), 14.58 (C-18), 20.75 (C-16), 23.10 (CH_3-Ac), 26.55 (C-17), 27.05 (CH_2 propionyl), 28.32 (CH_3-Boc), 35.96 (C-14), 38.56 (C-6), 43.06 (C-15), 46.66 (C-3), 55.07 (C-3′),

56.20 (C-8), 57.91 (C-20), 61.27 (C-5), 72.48 (C-13 and C-2′), 73.88 (C-7), 74.69 (C-2), 75.24 (C-10), 79.00 (Cq-Boc), 80.05 (C-1 or C-4), 83.25 (C-4 or C-1), 126.88, 128.10, 128.79, 128.89, 129.57, 130.30, 135.94 (aromatic), 133.68 (C-11), 138.51 (C-12), 155.43 (CO-Boc), 167.17 (CO-Bz), 170.51 (CO-Ac), 172.00 (C-1′), 212.02 (C-9); MS-FAB$^+$ m/z 942 (M + Na$^+$), 661.

Preparation of compounds 5a,b,c. Esterification of 2′-(2,2,2-trichloroethyloxycarbonyl) docetaxel (282 mg, 0.29 mmol, 20 mL toluene) with butyric acid by method A (24 h) afforded, after work up and purification by column silica gel chromatography (EtOAc:heptane, 3:7), 7-butyryl 2′-troc docetaxel (103.2 mg, 34%), 10-butyryl 2′-troc docetaxel (59.5 mg, 20%) and 7,10-dibutyryl 2′-troc docetaxel (15.3 mg, 5%). 7,10-Dibutyryl 2′-troc docetaxel was obtained with a better yield by method B: 2′-(2,2,2-trichloroethyloxycarbonyl) docetaxel (120 mg) was esterified with DCC (4 equiv) and butyric acid (4 equiv) for 17 h at 60 °C. Purification by preparative TLC (EtOAc:heptane, 30:70) afforded 77 mg of 7,10-dibutyryl 2′-troc docetaxel (56%). The troc protective groups were removed for each compound as described in general methods. 7-Butyryl 2′-troc docetaxel (79 mg) afforded, after 4 h at 40 °C and purification by preparative TLC (EtOAc:heptane, 1:1) 7-butyryldocetaxel (**5a**) (21 mg, 32%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J = 7 Hz, CH_3), 1.61 (m, CH_2), 2.25 (m, CH_2–CO), 5.28 (s, C-10H), 5.50 (m, C-7H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.31 (CH_3), 19.08 (CH_2), 36.53 (CH_2), 172.81 (CO chain); MS-FAB$^+$ m/z 900 (M + Na$^+$), 619. 10-Butyryl 2′-troc docetaxel (41.4 mg) afforded, after 2 h at 50 °C and purification by preparative TLC (EtOAc:heptane, 1:1) 10-butyryldocetaxel (**5b**) (32.5 mg, 94%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (t, J = 7 Hz, CH_3), 1.72 (m, CH_2), 2.49 (CH_2–CO), 4.43 (m, C-7H), 6.31 (s, C-10H); ^{13}C NMR (75 MHz,CDCl3) δ 13.27 (CH_3), 22.23 (CH_2), 35.73 (CH_2), 172.5 (CO chain); MS-FAB$^+$ m/z 884 (M + Li$^+$), 603. 7,10-Dibutyryl 2′-troc docetaxel (43.2 mg) afforded, after 7 h at 40 °C and purification by preparative TLC (EtOAc:heptane, 1:1) compound 7,10-dibutyryldocetaxel (**5c**) (24 mg, 67%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93 and 1.02 (t, J = 7 Hz, CH_3), 1.61 and 1.71 (m, CH_2), 2.55 (m, CH_2–CO), 5.57 (m,C-7H), 6;32 (s,C-10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.17 (CH_3), 14.21 (CH_3), 18.47 (CH_2), 18.94 (CH_2), 33.96, 35.98 (CH_2), 171.88 (CO); MS-FAB$^+$ m/z 954 (M + Li$^+$), 673.

Preparation of compounds 6a. Esterification of 2′,10 ditroc docetaxel (500 mg, 0.43 mmol, 25 mL toluene) with valeric acid by method A (6 h) afforded, after work up and purification by column silica gel chromatography (EtOAc:heptane, 20:80) 7-valeryl 2′,10-ditroc docetaxel (223 mg, 42%). The troc protective groups were removed as described in general methods. 7-Valeryl 2′,10-ditroc docetaxel (138 mg) afforded, after 4 h at room temperature and purification by preparative TLC (EtOAc:heptane, 40:60) 7-pentanoyldocetaxel (**6a**) (48 mg, 54%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 7 Hz, CH_3), 1.21 (m, CH_2), 1.50 (m, CH_2), 2.22 (CH_2–CO), 5.29 (s, C-10H), 5.43 (m, C-7H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.6 (CH_3), 22.92 (CH_2), 26.79 (CH_2), 33.90 (CH_2), 172.93 (CO chain); MS-FAB$^+$ m/z 914 (M + Na$^+$), 632.

Preparation of compounds 6c. Esterification of 2′-(2,2,2-trichloroethyloxycarbonyl) docetaxel (1 g, 1.02 mmol, 60 mL toluene) with valeric acid by method A (2 h) afforded, after work up and purification by column silica gel chromatography (EtOAc:heptane, 2:8) 7,10-Divaleryl 2′-troc docetaxel (1.01 g, 87%). The troc protective group was removed as described in general methods. 7,10-Divaleryl 2′-troc docetaxel (205 mg) afforded, after 4 h and purification by preparative TLC (EtOAc:heptane, 40:60) 7,10-dipentanoyldocetaxel (**6c**) (80 mg, 46%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, J = 7 Hz, CH_3), 0.96 (t, J = 7 Hz, CH_3), 1.20 (m, CH_2), 1.41 (m, CH_2), 1.56 (m, CH_2), 2.30 (m, CH_2–CO), 2.42 (m, CH_2–CO), 5.54 (m, C-7H), 6.31 (s, C-10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.70 (CH_3), 22.17 (CH_2), 26.45 (CH_2), 33.41 (CH_2), 33.69 (CH_2), 170.06 (CO chain), 172.70 (CO chain); MS-FAB$^+$ m/z 998 (M + Li$^+$), 717.

Preparation of compounds 7a,b,c. Esterification of 2′-(2,2,2-trichloroethyloxycarbonyl) docetaxel (259 mg, 0.26 mmol, 20 mL toluene) with heptanoic acid by method A (24 h) afforded, after work up and purification by column silica gel chromatography (EtOAc:heptane, 3:7), 7-heptanoyl 2′-troc docetaxel (107 mg, 38%), 10-heptanoyl 2′-troc docetaxel (75 mg, 26%) and 7,10-diheptanoyl 2′-troc docetaxel (65 mg, 20%). The troc protective groups were removed for each compound as described in general methods. 7-Heptanoyl 2′-troc docetaxel (70 mg) afforded after 8 h at room temperature and purification by preparative TLC (EtOAc:heptane, 1:1) 7-heptanoyldocetaxel (**7a**) (39 mg, 66%) along with starting material (4 mg, 6%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J = 7 Hz, CH_3), 1.21 (m, CH_2), 1.48 (m, CH_2), 2.25 (m, CH_2–CO), 5.5 (m, C-7H), 5.33 (s, C-10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.66 (CH_3), 23.14 (CH_2), 29.35 (CH_2), 36.53 (CH_2), 173.18 (CO chain); MS-FAB$^+$ m/z 926 (M + Li$^+$), 645. 10-Heptanoyl 2′-troc docetaxel (51 mg) afforded, after 2 h at 50 °C and purification by preparative TLC (EtOAc:heptane, 1:1) 7-heptanoyldocetaxel (**7b**) (31 mg, 74%) along with starting material (6 mg, 12%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, J = 7 Hz, CH_3), 1.24 (m, CH_2), 1.66 (m, CH_2), 2.43 (m, CH_2–CO), 4.35 (m, C-7H), 6.22 (s, C-10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.59 (CH_3), 22.25 (CH_2), 22.40 (CH_2), 28.52 (CH_2), 31.21 (CH_2), 172.73 (CO); MS-FAB$^+$ m/z 926 (M + Li$^+$), 646. 7,10-Diheptanoyl 2′-troc docetaxel (46 mg) afforded, after 6 h at room temperature and 1 h at 50 °C, and purification by preparative TLC (EtOAc:heptane, 1:1) 7,10-diheptanoyldocetaxel (**7c**) (19 mg, 48%) along with starting material (6 mg, 13%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (m, CH_3), 1.23 (CH_2), 1.63 (m, CH_2), 2.24 (CH_2–CO), 5.46 (m, C-7H), 6.21 (s, C-10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.95 (CH_3), 21.35 (CH_2), 29.14 (CH_2), 34.41 (CH_2), 34.56 (CH_2), 171.66 (CO), 173.03 (CO); MS-FAB$^+$ m/z 1038 (M + Li$^+$), 757.

Preparation of compounds 8a,b. Esterification of 2′-(2,2,2-trichloroethyloxycarbonyl) docetaxel (102 mg,

0.1 mmol, 8 mL toluene) with octanoic acid by method B (2 h) afforded, after work up and purification by preparative TLC (CH_2Cl_2:MeOH, 96:4), 7-octanoyl 2′-troc docetaxel (60 mg, 52%) and 10-heptanoyl 2′-troc docetaxel (41 mg, 35%) along with starting material (9.5 mg, 9%). The troc protective groups were removed for each compound as described in general methods. 7-octanoyl 2′-troc docetaxel (50 mg) afforded after 1 h at room temperature and purification by preparative TLC (CH_2Cl_2:MeOH, 96:4) 7-octanoyldocetaxel **8a** (25 mg, 59%) along with starting material (19 mg, 38%), 7-octanoyldocetaxel (**8a**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83 (t, $J=7$ Hz, CH_3), 1.29 (m, CH_2), 1.48 (m, CH_2), 2.15 (m, CH_2–CO), 5.23 (s, C-10H), 5.40 (m, C-7H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.43 (CH_3), 21.72 (CH_2), 24.06 (CH_2), 28.10 (CH_2), 28.22 (CH_2), 30.86 (CH_2), 35.02 (CH_2), 171.75 (CO chain); MS-FAB$^+$ m/z 956 ($M+Na^+$), 675. 10-Heptanoyl 2′-troc docetaxel (40 mg) afforded, after 45 min at room temperature and purification by preparative TLC (CH_2Cl_2:MeOH, 95:5) 7,10-dioctanoyldocetaxel **8b** (16 mg, 47%) along with starting material (5 mg, 12%). 7,10-Dioctanoyldocetaxel (**8b**). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, $J=7$ Hz, CH_3), 1.24 (m, CH_2), 2.43 (m, CH_2–CO), 4.35 (m, C-7H), 6.22 (s, C-10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.47 (CH_3), 23.03 (CH_2), 25.27 (CH_2), 29.31 (CH_2), 29.46 (CH_2), 32.07 (CH_2), 34.62 (CH_2), 173.50 (CO); MS-FAB$^+$ m/z 956 ($M+Na^+$), 675.

Preparation of compounds 9a,b,c. Esterification of 2′-(2,2,2-trichloroethyloxycarbonyl) docetaxel (265 mg, 0.27 mmol, 20 mL toluene) with nonanoic acid by method A (1 h) afforded, after work up and purification by column silica gel chromatography (CH_2Cl_2:MeOH, 99.5:0.5), 7-nonanoyl 2′-troc docetaxel (63 mg, 21%), 10-nonanoyl 2′-troc docetaxel (45 mg, 15%) and 7,10-dinonanoyl 2′-troc docetaxel (179 mg, 53%). The troc protective groups were removed for each compound as described in general methods. 7-Nonanoyl 2′-troc docetaxel (70 mg) afforded after 3 h at room temperature and purification by preparative TLC (EtOAc:heptane, 1:1) 7-nonanoyldocetaxel (**9a**) (32.4 mg, 55%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, $J=7$ Hz, CH_3), 1.28 (m, CH_2), 1.53 (m, CH_2), 2.25 (m, CH_2–CO), 5.28 (s, C-10H), 5.46 (m, C-7H); MS-FAB$^+$ m/z 970 ($M+Na^+$), 948 ($M+H^+$), 689, 667. 10-Nonanoyl 2′-troc docetaxel (56 mg) afforded after 2 h at 60 °C and purification by preparative TLC (EtOAc:heptane, 1:1) 10-nonanoyldocetaxel (**9b**) (37 mg, 82%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, $J=7$ Hz, CH_3), 1.28 (m, CH_2), 1.72 (m, CH_2), 2.29 (m, CH_2–CO), 4.43 (m,C-7H), 6.29 (s, C-10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.02 (CH_3), 23.12 (CH_2), 25.33 (CH_2), 29.59 (CH_2), 32.30 (CH_2), 36.06 (CH_2), 170.72 (CO); MS-FAB$^+$ m/z 970 ($M+Na^+$), 948 ($M+H^+$), 689, 667. 7,10-Dinonanoyl 2′-troc docetaxel (316 mg) afforded after 5 h at room temperature and purification by preparative TLC (EtOAc:heptane, 1:1) 7,10-dinonanoyldocetaxel (**9c**) (77 mg, 28%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (m, CH_3), 1.29 (m, CH_2), 1.65 (m, CH_2), 2.32 (m, CH_2–CO), 5.56 (m, C-7H), 6.31 (s, C-10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.62 (CH_3), 23.19 (CH_2), 24.98 (CH_2), 29.66 (CH_2), 29.80 (CH_2), 32.39 (CH_2), 34.72 (CH_2), 172.06 (CO), 173.64 (CO); MS-FAB$^+$ m/z 1110 ($M+Na^+$), 1088 ($M+H^+$), 830, 807.

Preparation of compounds 10a,b,c. Esterification of 2′-(2,2,2-trichloroethyloxycarbonyl) docetaxel (255 mg, 0.25 mmol, 15 mL toluene) with undecanoic acid by method A (24 h) afforded, after work up and purification by column silica gel chromatography (EtOAc:heptane, 1:1), then preparative TLC (EtOAc:heptane, 1:1), 7-undecanoyl 2′-troc docetaxel (91 mg, 32%), 10-undecanoyl 2′-troc docetaxel (65 mg, 23%) and 7,10-diundecanoyl 2′-troc docetaxel (10.4 mg, 3%). The troc protective groups were removed for each compound as described in general methods). 7-Undecanoyl 2′-troc docetaxel (65 mg) afforded after 4 h at 40 °C and purification by preparative TLC (EtOAc:heptane, 1:1), 7-undecanoyldocetaxel (**10a**) (45.7 mg, 84%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.81 (t, $J=7$ Hz, CH_3), 1.20 (m, CH_2), 1.48 (m, CH_2), 2.16 (m, CH_2–CO), 5.23 (s, C-10H), 5.40 (m, C-7H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.66 (CH_3), 21.96 (CH_2), 24.29 (CH_2), 28.67 (CH_2), 28.90 (CH_2), 29.01 (CH_2), 31.35 (CH_2), 35.28 (CH_2), 171.99 (CO); MS-FAB$^+$ m/z 982 ($M+Li^+$), 701. 10-Undecanoyl 2′-troc docetaxel (45 mg) afforded after 4 h at 40 °C and purification by preparative TLC (EtOAc:heptane, 1:1) 10-undecanoyldocetaxel (**10b**) (24.5 mg, 74%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, $J=7$ Hz, CH_3), 1.35 (m, CH_2), 1.78 (m, CH_2), 2.56 (m, CH_2–CO), 4.47 (m, C-7H), 6.35 (s, C-10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.55 (CH_3), 23.12 (CH_2), 25.29 (CH_2), 29.54 (CH_2), 29.69 (CH_2), 29.90 (CH_2), 32.35 (CH_2), 36.01 (CH_2), 173.57 (CO); MS-FAB$^+$ m/z 982 ($M+Li^+$), 701. 7,10-Diundecanoyl 2′-troc docetaxel (9 mg) afforded after 5 h at 40 °C and overnight at room temperature and purification by preparative TLC (EtOAc:heptane, 1:1) 7,10-diundecanoyldocetaxel (**10c**) (5.6 mg, 72%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (m, CH_3), 1.33 (m, CH_2), 1.63 (m, CH_2), 2.35 (m, CH_2–CO), 5.63 (m, C-7H), 6.37 (s, C-10H); MS-FAB$^+$ m/z 1150 ($M+Li^+$), 869.

Preparation of compounds 11a,b. Esterification of 2′-(2,2,2-trichloroethyloxycarbonyl) docetaxel (474 mg, 0.48 mmol, 10 mL toluene) with myristic acid by method A (6.5 h) afforded, after work up and purification by column silica gel chromatography (EtOAc:heptane, 30:70), 7-myristyl 2′-troc docetaxel (152 mg, 26%), 10-myristyl 2′-troc docetaxel (187 mg, 32%).The troc protective groups were removed for each compound as described in general methods. 7-Myristyl 2′-troc docetaxel (110 mg) afforded after 4 h at room temperature and 1 h at 60 °C and purification by preparative TLC (EtOAc:heptane, 30:70) 7-myristyldocetaxel (**11a**) (63.4 mg, 68%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80 (t, $J=7$ Hz, CH_3), 1.18 (m, CH_2), 1.55 (m, CH_2), 2.23 (m, CH_2–CO), 5.22 (s, C-10H), 5.68 (m, C-7H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.44 (CH_3), 23.04 (CH_2), 25.19 (CH_2), 29.42 (CH2), 29.59 (CH_2), 29.69 (CH_2), 29.80 (CH_2), 32.27 (CH_2), 36.19 (CH_2), 172.87 (CO); MS-FAB$^+$ m/z 1024 ($M+Li^+$), 743. 10-Myristyl 2′-troc docetaxel (157 mg) afforded after 1.5 h at 60 °C and purification by preparative TLC (EtOAc:heptane, 1:1) 10-myristyldocetaxel (**11b**) (121 mg, 91%): ^{1}H NMR

(300 MHz, $CDCl_3$) δ 0.77 (t, $J=7$ Hz, CH_3), 1.17 (m, CH_2), 1.55 (m, CH_2), 2.19 (m, CH_2–CO), 4.31 (m, C-7H), 6.19 (s, C-10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.63 (CH_3), 22.50 (CH_2), 24.67 (CH_2), 28.92 (CH_2), 29.07 (CH_2), 29.17 (CH_2), 29.27 (CH_2), 31.74 (CH_2), 35.39 (CH_2), 172.73 (CO); MS-FAB$^+$ m/z 1024 ($M+Li^+$), 743.

Preparation of compounds 12a,b. Esterification of 2′-(2,2,2-trichloroethyloxycarbonyl) docetaxel (100 mg, 0.1 mmol, 8 mL toluene) with stearic acid by method B (2 h) afforded, after work up and purification by preparative TLC (CH_2Cl_2:MeOH, 96:4), 7-stearyl 2′-troc docetaxel (65.5 mg, 51%) and 10-stearyl 2′-troc docetaxel (39 mg, 31%). The troc protective groups were removed for each compound as described in general methods. 7-Stearyl 2′-troc docetaxel (56 mg) afforded after 1.25 h at 60 °C and purification by preparative TLC (CH_2Cl_2:MeOH, 96:4) 7-stearyl docetaxel **12a** (31.5 mg, 66%) along with starting material (13 mg, 23%), 7-stearyl docetaxel (**12a**): ^1H NMR (300 MHz, $CDCl_3$) δ 0.81 (t, $J=7$ Hz, CH_3), 1.19 (m, CH_2), 1.48 (m, CH_2), 2.15 (m, CH_2–CO), 5.22 (s, C-10H), 5.40 (m, C-7H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.89 (CH_3), 23.46 (CH_2), 25.59 (CH_2), 29.80 (CH_2), 29.98 (CH_2), 30.14 (CH_2), 30.22 (CH_2), 30.39 (CH_2), 30.46 (CH_2), 32.67 (CH_2), 36.54 (CH_2), 170.98 (CO); MS-FAB$^+$ m/z 1096 ($M+Na^+$), 815. 10-Stearyl 2′-troc docetaxel (32 mg) afforded after 1.3 h at 60 °C and purification on TLC (CH_2Cl_2:MeOH, 95:5) 10-stearyl docetaxel **12b** (14 mg, 51%) along with starting material (8.5 mg, 26%), 10-Stearyl docetaxel (**12b**): ^1H NMR (300 MHz, $CDCl_3$) δ 0.81 (t, $J=7$ Hz, CH_3), 1.19 (m, CH_2), 1.62 (m, CH_2), 2.42 (m, CH_2–CO), 4.33 (m, C-7H), 6.21 (s, C-10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.61 (CH_3), 22.14 (CH_2), 24.38 (CH_2), 28.64 (CH_2), 28.79 (CH_2), 28.99 (CH_2), 29.21 (CH_2), 31.45 (CH_2), 35.10 (CH_2), 173.33 (CO); MS-FAB$^+$ m/z 1096 ($M+Na^+$).

Synthesis of aromatic hydrophobic derivatives

Neither yields nor the ratio of the aromatic mono-7, mono-10, and di-7,10 esters were optimized.

Preparation of compounds 13b,c. Esterification of 2′-(2,2,2-trichloroethyloxycarbonyl) docetaxel (500 mg, 0.51 mmol, 30 mL toluene) with 4-biphenyl carboxylic acid by method A (19 h) afforded, after work up and purification by column silica gel chromatography (EtOAc:heptane, 40:60), 10-(4-biphenyl)carboxyl 2′-troc docetaxel (67 mg, 11%) and 7,10-di(4-biphenyl)carboxyl 2′-troc docetaxel (292 mg, 43%). The troc protective groups were removed for each compound as described in general methods. 10-(4-Biphenyl)carboxyl 2′-troc docetaxel (53 mg) afforded after 6 h at room temperature and purification by preparative TLC (EtOAc:heptane, 1:1) 10-(4-biphenyl)carboxyldocetaxel (**13b**) (22.5 mg, 50%): ^1H NMR (300 MHz, $CDCl_3$) δ 4.45 (m, C-7H), 6.50 (s, C-10H), 7.42 and 8.10 (m, biphenyl); MS-FAB$^+$ m/z 994 ($M+Li^+$), 713. 7,10-Di(4-biphenyl)carboxyl 2′-troc docetaxel (54 mg) afforded after 6 h at room temperature and purification by preparative TLC (EtOAc:heptane, 1:1) 7,10-bis(4-biphenyl)carboxyl-docetaxel (**13c**) (19 mg, 40%): ^1H NMR (300 MHz, $CDCl_3$) δ 5.74 (m, C-7H), 6.65 (s, C-10H), 7.47, 7.54, 7.77, and 7.94 (m, biphenyl); MS-FAB$^+$ m/z 1174 ($M+Li^+$), 893.

Preparation of compounds 14a,c. Esterification of 2′-(2,2,2-trichloroethyloxycarbonyl) docetaxel (375 mg, 0.38 mmol, 16 mL toluene) with phenylpropiolic acid by method A (DCC instead of EDCI, 4 h) afforded, after work up and purification by column silica gel chromatography (CH_2Cl_2:MeOH, 99:1), then preparative TLC (CH_2Cl_2:MeOH, 98:2), 7-phenylpropionyl 2′-troc docetaxel (84.5 mg, 20%) and 7,10-diphenylpropiolyl 2′-troc docetaxel (49 mg, 12%). The troc protective groups were removed for each compound as described in general methods. 7-Phenylpropionyl 2′-troc docetaxel (42 mg) afforded after 3 h at 60 °C and purification by preparative TLC (CH_2Cl_2:MeOH. 95:5) 7-phenyl-propionyldocetaxel (**14a**) (19.4 mg, 56%): ^1H NMR (300 MHz, $CDCl_3$) δ 5.26 (s, C-10H), 5.54 (m, C-7H), 7.30 (m, C_6H_5); MS-FAB$^+$ m/z 958 ($M+Na^+$), 936 ($M+H^+$), 677, 655. 7,10-Diphenylpropiolyl 2′-troc docetaxel (16 mg) afforded after 2 h at 60 °C and purification by preparative TLC (CH_2Cl_2:MeOH, 98:2) 7,10-diphenyl-propionyldocetaxel (**14c**) (10.2 mg, 50%): ^1H NMR (300 MHz, $CDCl_3$) δ 5.76 (m, C-7H), 6.56 (s, C-10H), 7.40 (m, C_6H_5); MS-FAB$^+$ m/z 1071 ($M+Li^+$), 794.

Preparation of compounds 15a. Esterification of 2′,10 ditroc docetaxel (240 mg, 0.21 mmol, 15 mL toluene) with *trans*-cinnamic acid by method B (4 h) afforded, after work up and purification by column silica gel chromatography (EtOAc:heptane, 30:70) 7-*trans*-cinnamoyl 2′,10-ditroc docetaxel (268 mg, 76%). The troc protective group was removed as described in general methods. 7-*trans*-cinnamyl 2′,10-ditroc docetaxel (100 mg) afforded, after 4 h at room temperature and purification by preparative TLC (CH_2Cl_2:MeOH, 98:2) 7-cinnamoyldocetaxel (**15a**) (53 mg, 74%): ^1H NMR (300 MHz, $CDCl_3$) δ 5.31 (s, C-10H), 5.55 (m, C-7H), 6.30 (d, $J=16$ Hz, CH=CH), 7.35 (m, C_6H_5), 7.57 (d, $J=16$ Hz, CH=CH); MS-FAB$^+$ m/z 944 ($M+Li^+$), 663.

Preparation of compounds 15b,c. Esterification of 2′-(2,2,2-trichloroethyloxycarbonyl) docetaxel (214 mg, 0.22 mmol, 12 mL toluene) with *trans*-cinnamic acid by method A (3 h) afforded, after work up and purification by column silica gel chromatography (EtOAc:heptane, 40:60), 10-*trans*-cinnamoyl 2′-troc docetaxel (50 mg, 20%) and 7,10-di*trans*-cinnamoyl 2′-troc docetaxel (101 mg, 44%). The troc protective groups were removed for each compound as described in general methods. 10-Cinnamoyl 2′-troc docetaxel (24 mg) afforded after 4 h at 40 °C and purification by preparative TLC (EtOAc:heptane, 1:1) then (CH_2Cl_2:MeOH, 95:5) 10-cinnamoyldocetaxel (**15b**) (13.2 mg, 66%): ^1H NMR (300 MHz, $CDCl_3$) δ 4.56 (m, C-7H), 6.50 (s, C-10H), 6.65 (d, $J=16$ Hz, CH=CH), 7.44 (m, C_6H_5), 7.65 (d, $J=16$ Hz, CH=CH); MS-FAB$^+$ m/z 944 ($M+Li^+$), 663. 7,10-Dicinnamoyl 2′-troc docetaxel (59 mg) afforded after 3 h at room temperature and purification by preparative TLC (EtOAc:heptane, 40:60) 7,10-dicinnamoyldocetaxel (**15c**) (39 mg, 78%): ^1H NMR (300 MHz,

$CDCl_3$) δ 5.65 (m, C-7H), 6.42 (2d, $J = 16$ Hz, 2 CH=CH), 6.48 (s, C-10H), 7.30 (m, C_6H_5), 7.55 and 7.58 (2d, $J = 16$ Hz, 2 CH=CH); MS-FAB$^+$ m/z 1074 ($M + Li^+$), 793.

Synthesis of linear hydrophilic derivatives

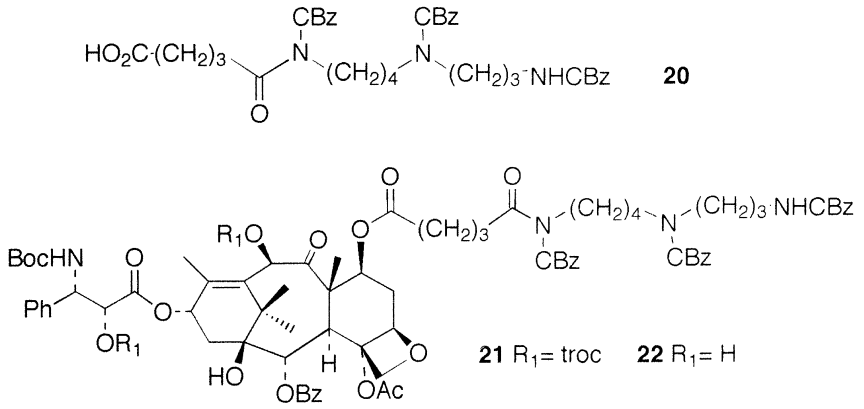
Preparation of compound 16c. Succinic anhydride (1 g, 10 mmol) in dry toluene (20 mL) was stirred for 6 h at 60 °C with 2,2,2-trichloroethanol (1.16 mL, 12 mmol). The solution was cooled, the solvent was removed under reduced pressure; the resulting residue was dissolved in CH_2Cl_2 and extracted with $NaHCO_3$ 1 M. The aqueous layer was acidified to pH 1 and extracted with CH_2Cl_2. The organic layers were washed with brine, dried over $MgSO_4$ and concentrated. The residue was crystallized in CH_2Cl_2-pentane to yield mono 2,2,2-trichloroethylsuccinate (1.89 g, 76%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.80 (s, CH_2–CH_2), 4.80 (s, CH_2CCl_3); mp 88–89 °C (lit. 86–88 °C). Esterification of 2′-(2,2,2-trichloroethyloxycarbonyl) docetaxel (147 mg, 0.15 mmol, 15 mL toluene) with mono 2,2,2-trichloroethylsuccinate by method B (2 h) afforded, after work up and purification by column silica gel chromatography (EtOAc:cyclohexane, 3:7), 7,10-di(2,2,2trichloroethyl) succinyl 2′-troc docetaxel (165 mg, 76%). The troc and trichloroethyl ester were removed as described in general procedure. 7,10-Di(2,2,2trichloroethyl) succinyl 2′-troc docetaxel (160 mg) afforded after 3.5 h at 60 °C and purification by preparative TLC (CH_2Cl_2:MeOH:AcOH, 94.5:5:0.5) 7,10-disuccinyldocetaxel (**16c**) (89 mg, 80%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.67 (m, CH_2–CO), 5.57 (m, C-7H), 6.30 (s, C-10H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 29.30 (CH_2), 29.9 (CH_2), 171.2 and 172.04 (CO); MS-FAB$^+$ m/z 1030 ($M + Na^+$), 749.

Preparation of compound 17c. To a solution of azelaic acid (2.5 g, 13.2 mmol) and pyridine (1 mL, 13.2 mmol) in CH_2Cl_2 (70 mL) was added at 0 °C a solution of 2,2,2-trichloroethanol (6.3 mL, 66 mmol) and DCC (2.7 g, 13.2 mmol) in CH_2Cl_2 (20 mL). The mixture was stirred overnight at room temperature and filtered. The solid was washed with CH_2Cl_2 and the combined filtrates were concentrated. The resulting residue was dissolved in CH_2Cl_2 and the organic layer was washed with a solution of 5% citric acid, with brine, dried with $MgSO_4$ and concentrated. The residue was purified by column silica gel chromatography (EtOAc:heptane, 40:60) to yield pure mono(2,2,2-trichloroethyl) ester of the azelaic acid (2.86 g, 67%): ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD) δ 1.18 (m, CH_2), 1.47 (m, CH_2), 2.12 (m, CH_2), 4.80 (s, CH_2CCl_3); MS-IC m/z 323, 321, 319 ($M + H^+$). Esterification of 2′-(2,2,2-trichloroethyloxycarbonyl) docetaxel (215 mg, 0.2 mmol) with the mono(2,2,2-trichloroethyl) ester of the azelaic acid (10 equiv) by method B (10 equiv of DCC, 17 h) afforded 7,10-di((mono 2,2,2-trichloroethyl) azelayl) 2′ troc docetaxel (190 mg, 43%). The troc and trichloroethyl ester were removed as described in general procedure. 7,10-Di((mono 2,2,2-trichloroethyl) azelayl) 2′ troc docetaxel (134.5 mg) afforded after 3 h at room temperature and purification by preparative TLC (EtOAc:heptane: AcOH, 50:50:0.25) 7,10-diazelayldocetaxel **17c** (50 mg, 52%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (m, CH_2), 1.61 (m, CH_2), 2.35 (m, CH_2–CO), 5.52 (m, C-7H), 6.26 (s, C-10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.98, 28.09 and 33.85 (CH_2), 172.72 (CO), 179.39 (COOH); MS-FAB$^+$ m/z 1030 ($M + Li^+$), 873.

Preparation of compound 18. Esterification of 2′-(2,2,2-trichloroethyloxycarbonyl) docetaxel (1 g, 1.02 mmol) with *O*-[3-(*N*-monomethoxytrityl)aminopropyl]-*O*′-[3-(*N*-succinamide)aminopropyl]diethyleneglycol (MMT TODAS)[31] (1.5 equiv) by method B (1.5 equiv of DCC, 2 h) afforded 7-MMTTODAS 2′-troc docetaxel (778 mg, 49%). The troc and monomethoxytrityl groups were removed simultaneously as described in general procedure. 7-MMTTODAS 2′-troc docetaxel (98 mg) was stirred 6 h at room temperature under the standard conditions. After filtration and removal of the solvent, the residue was dissolved in EtOAc and the organic layer was extracted with water. The aqueous layers were alkalinized, extracted with CH_2Cl_2. The organic layers were then extracted with acidic water (pH 2–3) and the aqueous layers were lyophilized to afford compound **18** (42 mg, 60%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.77 (m, CH_2), 2.42 (m, CH_2–CO), 2.88 (m, CH_2–NH_2), 3.30 (CH_2–NH–CO), 3.57 (CH_2–O), 5.29 (s, C-10H), 5.52 (m, C-7H), 6.97 (NH–CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.21, 29.93, 30.85 and 31.48 (CH_2–C), 38.18 and 40.20 (CH_2–N), 70.21, 70.37, 70.49, 70.71 and 70.91 (CH_2–O), 157.91 (CO–N), 172.43 (CO–O); MS-FAB$^+$ m/z 1116 ($M + Li^+$).

Preparation of compound 19. To a stirred solution of *N*-(3-[(4-aminobutyl)-phenyl-acetylamino]propyl)2-phenyl acetamide[32] (750 mg, 1.82 mmol) and DMAP (266 mg, 2.18 mmol) in dichloromethane (50 mL) was added glutaric acid (250 mg, 2.18 mmol). The reaction mixture was stirred at room temperature for 1.5 h, diluted with dichloromethane, washed with 1 M HCl and brine, respectively, dried and evaporated. The residue was purified by column chromatography on silica gel with CH_2Cl_2:MeOH (10:1) to afford 5-(4-[phenylacetyl-(3-phenylacetylaminopropyl)-aminobutylcarbamoyl)pentanoic acid **20** (860 mg): ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.46 (m, 4H), 1.73–1.68 (m, 2H), 1.94–1.89 (m, 2H), 2.40–2.20 (m, 4H), 3.30–3.10 (m, 8H, CH_2N), 5.11 and 5.08 (ds, 4H, $ArCH_2$), 5.30, 5.9, 6.4 (br s, NH), 7.33 (m, 10H, Ar-H), 9.1 (br s, 1H, COOH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.92 (CH_2), 25.33 (CH_2), 25.71 (CH_2), 26.53 (CH_2), 28.09 (CH_2), 28.80 (CH_2), 33.16 (CH_2), 35.19 (CH_2), 37.94 (CH_2), 38.20 (CH_2), 38.93 (CH_2), 44.38 (CH_2), 46.47 (CH_2), 47.00 (CH_2), 66.48 ($ArCH_2$), 67.17 ($ArCH_2$), 127.75, 127.89, 127.99, 128.41, 128.48, 136.53, 156.63, 173.08, 176.29; MS-FAB$^+$ m/z 534 ($M + Li^+$) (Scheme 4).

To a stirred solution of 2′-(2,2,2-trichloroethyloxycarbonyl) docetaxel (570 mg, 0.87 mmol) and acid **20** (460 mg, 0.87 mmol) in chloroform (30 mL) was added DMAP (71 mg, 0.58 mmol) and EDCI (223 mg, 1.16 mmol). The reaction mixture was stirred at room temperature for 2 days, diluted with water, extracted with dichloromethane. The combined extracts were washed with 1 M HCl, water, diluted sodium bicarbonate and brine, respectively, dried and evaporated. Column



Scheme 4.

chromatography on silica gel eluted with dichloromethane:acetone (10:1) afforded the corresponding 7-ester **21** (600 mg). The troc group of this compound (600 mg, 0.40 mmol) was removed as described in general procedure after stirring 18 h at room temperature. After filtration and removal of the solvent, the residue was dissolved in dichloromethane and the organic layer was extracted with water, brine, dried and evaporated. The residue was purified by column chromatography on silica gel with dichloromethane:acetone (3:1) to give the corresponding 7-ester-docetaxel **22** (460 mg, 87%). Compound **22** (100 mg, 0.074 mmol) was dissolved in acetic acid and hydrogenolyzed (1 atm, rt) in the presence of Pd-C (10%). After 4 h, the catalyst was filtered off. The colorless filtrate was taken to dryness to give compound **19** in quantitative yield: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.60 (m, CH_2), 2.08 (m, CH_2), 2.29 (m, CH_2CO), 3.44 (m, CH_2NH), 5.60 (s, C-10H), 5.52 (m, C-7H); ^{13}C 3NMR (75 MHz, $CDCl_3$) δ 22.90, 26.99, 27.66 and 28.47 (CH_2–C), 39.16, 40.36, 45.21 and 49.83 (CH_2–N), 34.97 and 36.75 (CH_2–CO), 158.57 (CO–N), 172.81 (CO–O); MS-FAB$^+$ m/z 1071 ($M+Na^+$).

HPLC experiments

The HPLC system consisted of a Waters 616 pump, Waters 717 with autoinjector and Waters 996 photodiode array detector (PDA) with a NEC Image 466es computer (Millenium software system) for controlling the analytical system and data processing. The column used was a Waters Symetry® C18, 5 μm, 4.6×250 mm. Each compound dissolved in DMF (1 mg/mL) was injected (1 μL) and was eluted at a flow rate of 1 mL/min with acetonitrile-TFA (0.05%) and increasing amounts of water. The compounds were detected at 280 nM. The retention time was measured for 5 concentrations from 0 to 30–35% of water. φ_0 was calculated by interpolation of the different measures, for a retention time equal to 2 times the void volume.

For very hydrophobic analogues ($n > 10$), only acetonitrile with less than 3% water could be used to elute these molecules. As a result, not enough values with different water concentrations were available, and consequently the determinations of φ_0 were not very accurate. This observation explained that the curve of φ_0 as a function of S, proportional to the number of carbons added—was not strictly straight for $n > 10$ (see Figure 1).

Biological assays

Evaluation of solubility: first, the UV spectrum of a solution of **10c** and docetaxel **2** in the buffer used for the tubulin assay were run at 37 and 0 °C (at a concentration of 10^{-6} and 10^{-5} with a final ethanol concentration of 1%); no difference was observed between the two compounds in the spectra showing that their behavior was identical under the test conditions. Then, in a second experiment, electronic microscopy of these same solutions also did not show any difference and only the presence of large precipitates, due to water evaporation on the grid, could be observed in both cases. These two results were not a demonstration of the absence of such aggregates, but they were in favor of a similar physical behavior between docetaxel and a more hydrophobic and inactive analogue. Preincubation, in the two cases, did not change the observed behavior. Furthermore, it was not possible to measure the diffusion coefficients of docetaxel and an analogue like **11b** by NMR[33] because the signals, even of the terbutyl group, were too weak at the concentration used in the biological experiments (10^{-5} M in D_2O and 1% DMSO) to carry out an evaluation of the difference of diffusion for the two compounds, they had the same appearance on the spectrum.

Tubulin test: bovine brain microtubule proteins were purified by two cycles of assembly/disassembly at 37 °C/0 °C in MES buffer: 100 mM MES (2-[*N*-morpholino]-ethanesulfonic acid, pH 6.6), 1 mM EGTA (ethyleneglycol-bis[β-aminoethyl ether]-*N,N,N′,N′*-tetraacetic acid), 0.5 mM $MgCl_2$. Each compound dissolved in DMSO was added at 37 °C at different concentrations to the solution of microtubules; the temperature was lowered to 0 °C and the optical density at 350 nM was recorded; the IC_{50} was calculated and compared to taxol according to the previous procedure.[34]

Cytotoxicity

IC_{50} measures the drug concentration required for the inhibition of 50% KB cell proliferation after 72 h incubation.

Molecular modeling studies

Sybyl software from Tripos was used in these studies with the MMFF94 force field (minimization for 1000 steps using the Powell algorithm), MOLCAD for the surface and lipophillic potential determination and the Grid Search procedure (rotation by 30° of C13-O, 1′-2′, 2′-3′ torsional angles) for the conformer generation of docetaxel analogues. The partial surfaces of ester alkyl analogues were determined by removing the surface of a 7-10 diformyl docetaxel from the whole surface. An identical procedure was used for the south part (i.e. the 2-benzoate, the 4-acetyl and the 3′ substituents) and the quotient *S* could then be evaluated. The surfaces retained in this work were a mean surface between the extended and folded conformations of lowest energy; these differences were not very important.

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