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Fluorescent and Biotinylated Analogues of Docetaxel: Synthesis and Biological Evaluation

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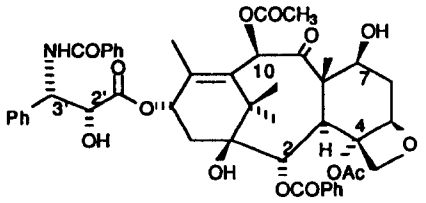
Abstract— Six novel docetaxel analogues that possess a *N*-(7-nitrobenz-2-oxa-1,3-diazo-4-yl)amido-6-caproyl chain in position 7 or 3' (**11** and **16a**), a *N*-(7-nitrobenz-2-oxa-1,3-diazo-4-yl)amido-3-propanoyl group at 3' (**16b**) and a 5'-biotinylamido-6-caproyl chain in position 7, 10 or 3', respectively, have been synthesized. These compounds exhibit activity against microtubule disassembly similar to that of docetaxel but show discrepant activities on living cells. Although addition of microtubules to **11**, **16a** and **b** enhance their fluorescence, no shift of the emission maxima was observed. The fluorescent docetaxel derivatives show a specific labeling of microtubules in living cells, demonstrating that the microtubule cytoskeleton constitutes their main subcellular localization.

Introduction

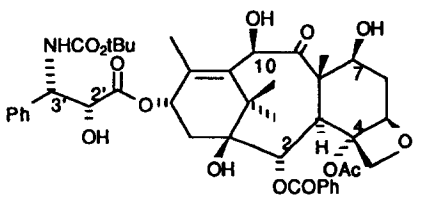
Paclitaxel (Taxol®:**1a**), a diterpene extracted from the bark of several species of the genus *Taxus*,[1,2] is one of the most promising drugs in cancer chemotherapy.[3] Recently, relationship studies between the structure of paclitaxel analogues and their antitubulin activities[4] led to the selection of docetaxel (Taxotere®:**1b**) as a potential anticancer agent.[5,6] Paclitaxel and structural analogues possess the unique ability to promote tubulin assembly and to stabilize microtubules both *in vitro* and *in vivo*.[7] In order to determine the location of the paclitaxel binding site on tubulin, some labeled derivatives have been prepared such as tritiated paclitaxel[8–10] or dansylpaclitaxel derivative.[11] Only very recently, photoaffinity labels have been synthesized[12–18] and used in the labeling of microtubules.[15,17,18] Photolabeling obtained with tritiated paclitaxel showed an interaction with the β-tubulin subunit.[10] Using photoaffinity derivatives, one group also reported a specific binding to the β-tubulin subunit[15] whereas two other groups described the involvement of both α and β subunits in the paclitaxel binding site.[17,18] Moreover, one study indicated the linkage of a photoaffinity analogue in the N-terminal part of the β-tubulin subunit.[19]

Using an approach different from photolabeling, we intended to design new analogues of docetaxel able to label microtubules both *in vitro* and *in vivo*, i.e. docetaxel derivatives bearing either a fluorescent or a biotin substituent. In addition to the importance of such biological probes in subcellular localization studies, fluorescent compounds could bring some information on the binding site due to their sensitivity toward environmental changes. Moreover, the biotinylated derivatives could be useful as ligands for affinity chromatography purification.[20]

As previously reported,[4,21] acylation of the C-7 and C-10 hydroxyl groups, and of the C-3' amino group does not decrease significantly the biological activity *in vitro*. In preliminary experiments we found that direct introduction of fluorescent groups such as dansyl, anthracene, rhodamine or 7-nitrobenz-2-oxa-1,3-diazol (NBD), led to unstable compounds with very low yield. Moreover, in the case of biotinylated derivatives it is well known that the affinity of biotin for avidin is maximal when the biotin moiety is far from the core of the molecule.[22] Therefore, we studied the coupling of biotin and fluorescent groups with docetaxel derivatives bearing a 6-aminocaproyl or a 3-aminopropanoyl side chain as spacer arm at carbon 7,10 or 3'. Among the



Paclitaxel (Taxol®) **1a**



Docetaxel (Taxotere®) **1b**

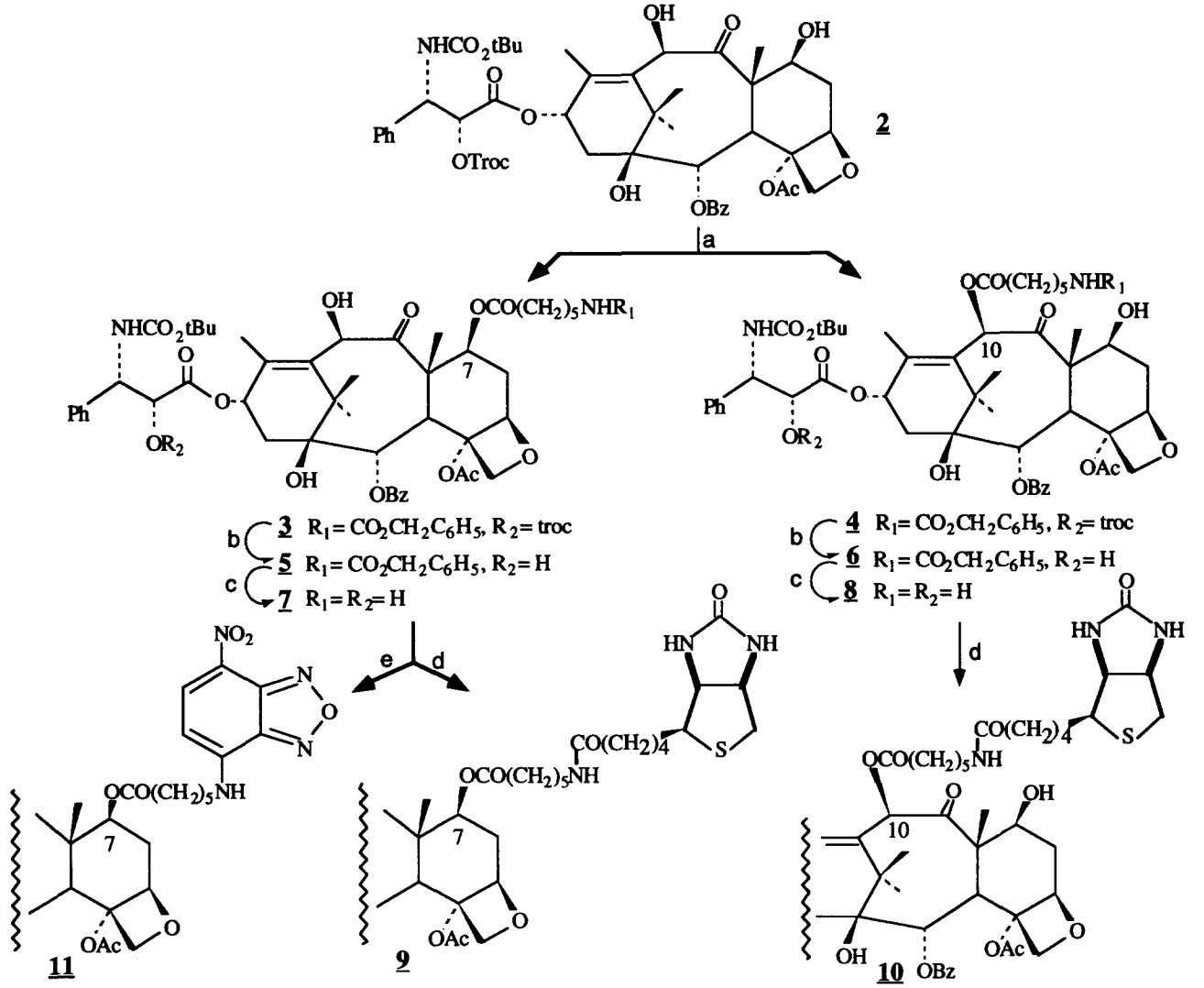
numerous fluorescent groups we used the NBD fluorophore which showed a good stability in our preliminary experiments and excitation and emission maxima (460–480 and 520–550 nm, respectively[23]) different from those of proteins.

We report here on the synthesis and biological evaluation of six novel docetaxel analogues: the 7 or 3'-[*N*-(7-nitrobenz-2-oxa-1,3-diazo-4-yl)amido-6-caproyl]-docetaxel (**11** and **16a**), the 3'-[*N*-(7-nitrobenz-2-oxa-1,3-diazo-4-yl)amido-3-propanoyl]-docetaxel (**16b**) and the 7, 10 or 3'-[5'-biotinylamido-6-caproyl]-docetaxel(**9**, **10** and **18**, respectively).

Results

Synthesis of the C-7 and C-10 docetaxel analogues

2'-(2,2,2-Trichloroethoxycarbonyl)-docetaxel[24] (2'-(troc)-docetaxel) **2** was esterified with *N*-benzyloxycarbonyl-6-aminocaproic acid in the presence of dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) to afford compounds **3** and **4**. Deprotection of the 2' hydroxyl group led to **5** and **6** and hydrogenolysis of the benzyl carbamate provided the C-7 and C-10 amino derivatives **7** and **8**. The 3-aminopropanoyl group was introduced in the same way but the esterification occurred almost exclusively at C-7 and deprotection of the amino group gave back docetaxel **1b**. The low stability of basic derivatives at this position has already been observed[25] and may be due to nucleophilic attack of the amino group at the carbonyl of the ester group. Biotin was coupled with **7** and **8** through its *N*-hydroxysuccinimide ester (biotin-ONSu) to afford the biotinylated derivatives **9** and **10**. Coupling of 7-nitrobenz-2-oxa-1,3-diazolyl chloride (NBD-Cl)[26] with **7** led to the C-7 fluorescent derivative **11**. However, the C-10-NBD-docetaxel derivative could not be isolated because of its instability (Scheme 1).



a) ZNH(CH2)5COOH, DCC, DMAP, 70°C, toluene; b) Zn, AcOH-MeOH (1:1), 60°C; c) H_2, Pd/C, MeOH; d) Biotin-ONSu, DMF-CH_2Cl_2; e) NBD-Cl, $NaHCO_3$, EtOH

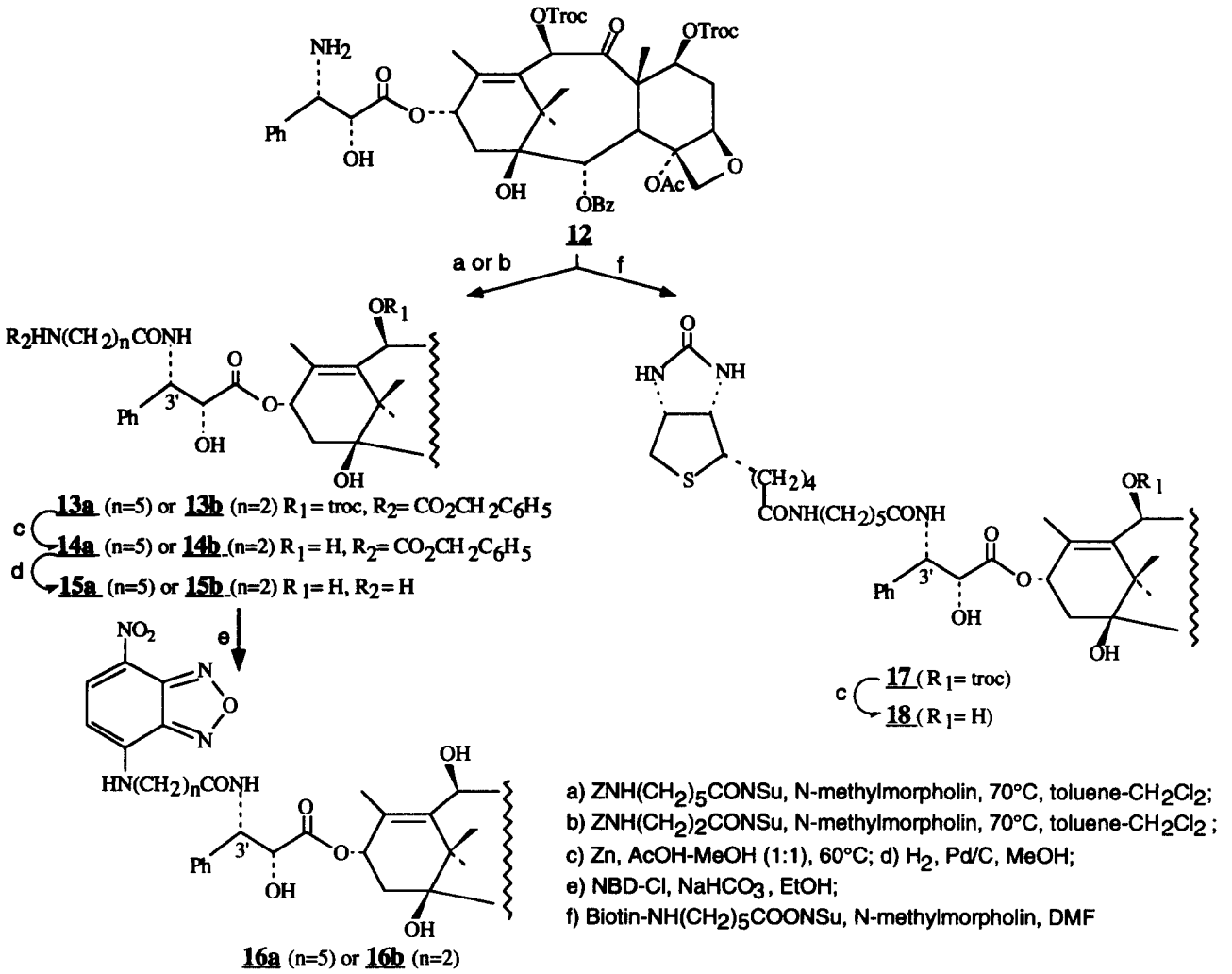
Scheme 1.

Synthesis of the C-3' amino derivatives of docetaxel

Compound **15a** with the 6-aminocaproyl chain on the C-3' amino group was obtained by coupling 7,10-di(2,2,2-trichloroethoxycarbonyl)-*N*-de-(*t*-butoxycarbonyl)-docetaxel[24] (**12**) with the *N*-hydroxysuccinimide ester of the *N*-benzyloxycarbonyl-6-aminocaproic acid (**13a**) followed by removal of the troc group (**14a**) and hydrogenolysis. The 3-aminopropanoyl chain was introduced in the same way to afford **15b** via the protected derivatives **13b** and **14b**. The fluorescent compounds **16a** and **b** were obtained by addition of NBD-Cl[26] to **15a** and **b**. The C-3' biotinylated derivative **17** was directly synthesized from **12** by coupling with the commercially available biotinamidocaproate *N*-hydroxysuccinnimide ester. Removal of the protective groups led to the 3'-biotinylated docetaxel derivative **18** (Scheme 2).

Biological evaluations

Microtubule disassembly inhibition. Compounds **9–11**, **16a, b** and **18** were tested for their ability to inhibit the *in vitro* disassembly of microtubular proteins at 0 °C;[27] their ID_{50} (concentration that inhibits 50% of microtubule disassembly) were compared to the ID_{50} of paclitaxel measured in the same experiment. The activity of all these compounds on microtubular proteins was similar to that of docetaxel (Table 1). It is worth noting that the 3'-biotinylated compound **18** showed the same activity as docetaxel itself, making it *a priori* quite suitable for biological studies.



Scheme 2.

Table 1.

Docetaxel analogue	Modified position	Spacer arm (n=)	Substituent	ID_{50}/ID_{50}(paclitaxel)
11	C-7	5	NBD	1.7
16a	NH-3'	5	NBD	2.1
16b	NH-3'	2	NBD	3.2
9	C-7	5	biotin	2.4
10	C-10	5	biotin	1.9
18	NH-3'	5	biotin	0.5

Avidin binding. The ability of compounds **9**, **10** and **18** to bind to avidin was investigated by the method described by Green:[28] the preformed 2-(4'-hydroxyazobenzene)benzoic acid (HABA)–avidin complex was displaced by additional amounts of biotin or biotin derivatives. The displacement curves monitored at 500 nm are shown in Figure 1.

Compounds **9**, **10** and **18** seemed to fulfill the conditions for biological probes, especially compound **18** which has the same affinity for tubulin as docetaxel and almost the same affinity for avidin as biotin itself. All three preformed avidin–biotinyl **9**, **10** or **18** complexes were able to prevent microtubule disassembly. However, they were less potent than compounds **9**, **10** or **18** and exhibited approximately the same activity as avidin alone. We then investigated the behaviour of microtubular proteins in the presence of avidin and found that avidin induced tubulin assembly and formed a complex with microtubular proteins; a similar activity has previously been observed with other basic proteins.[29] Because of the basic properties of avidin, these biotinyl derivatives of docetaxel would not be suitable for affinity chromatography, but might be useful for *in vivo* studies.

Activity in mammalian cells. Rat kangaroo cells (PtK2) were submitted to a 6 h treatment at 37 °C in the presence of various concentrations of paclitaxel, docetaxel and docetaxel derivatives dissolved in 1% dimethyl sulfoxide. Then, the cells were fixed and the microtubule cytoskeleton was observed by epifluorescence microscopy after immunolabeling with the α-tubulin antibodies YL1/2.[30] As previously reported,[31] paclitaxel induced both a reorganization of the interphase microtubule cytoskeleton characterized by the occurrence of numerous microtubule bundles, and abnormal metaphase figures exhibiting one or several microtubule asters. These effects, due to the stabilization of microtubules, could be observed also with unrelated chemicals which stabilize microtubules by another molecular mechanism.[32] No cells were observed with microtubule bundles in the presence of 0.25 μM paclitaxel; 11% of the cells contained microtubule bundles in the presence 0.5 μM paclitaxel, while the percentage of cells wtih microtubule bundles increased to 64 and 100% in the presence of 1.0 and 2.5 μM paclitaxel, respectively (Fig. 2). In no case, were microtubule bundles observed in the presence of dimethyl sulfoxide. Docetaxel (IC_{50}: 4.5 nM) was 180-fold more active than paclitaxel (IC_{50}: 0.8 μM). In contrast, all docetaxel derivatives were less active than the parent compound (Fig. 2). Compound **10**, the most active biotinylated docetaxel derivative (IC_{50}: 0.6 μM), was 133 times less active than docetaxel, although its activity was similar to that of paclitaxel. Among the two other biotinylated docetaxel derivatives, compound **9** (IC_{50}: 3.5 μM) was four-fold less active than paclitaxel, while compound **18** failed to induce any microtubule bundles at the highest concentration used (10 μM). Among the three fluorescent docetaxel derivatives, compound **11** (IC_{50}: 2.4 μM) was three-fold less active than paclitaxel, while compounds **16a** and **b** were devoid of activity (Fig. 2).

In the conditions used, the same overall activity was observed when abnormal mitoses were recorded. All mitoses were abnormal in the presence of 1 nM docetaxel, 0.05 μM paclitaxel, 0.1 μM **9** and **10**, and 0.25 μM **11**. Compounds **18** and **16a** led to abnormal metaphases at higher concentrations (5 μM), while compound **16b** failed to perturb mitosis at the highest concentration used (10 μM). Docetaxel and all docetaxel derivatives inducing mitotic abnormalities failed to block more than 19% of the cells in the mitotic stage, while paclitaxel blocked 33% of the cells in mitosis in the same conditions. In contrast with *in vitro* results with microtubule proteins, all derivatives were less active than docetaxel in living cells. Among them, only compound **10** was as active as paclitaxel. The addition of biotinylated and fluorescent side chains at position 3' led to complete loss of activity, in contrast to compounds modified at positions 7 and 10 which showed only a decreased activity of more than

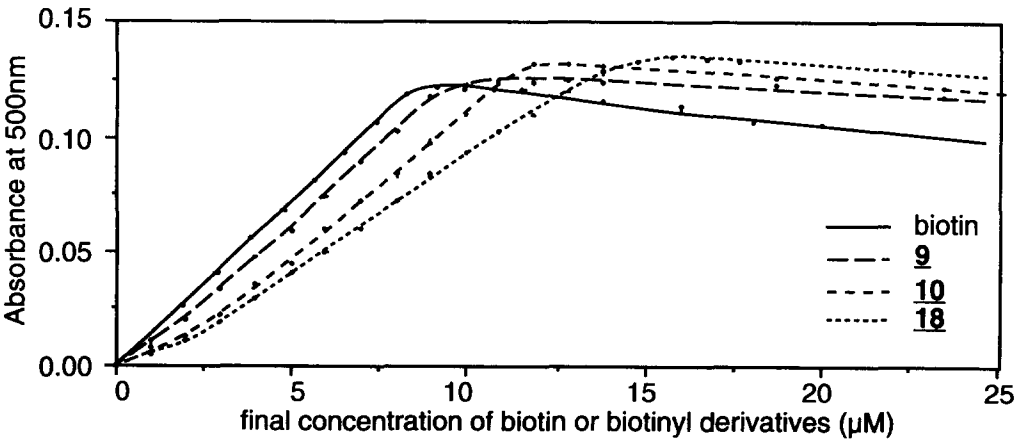


Figure 1. Displacement curves of the preformed avidin–HABA complex by successive additions of biotin and biotinyl derivatives of docetaxel; each value is the mean of two separate experiments.

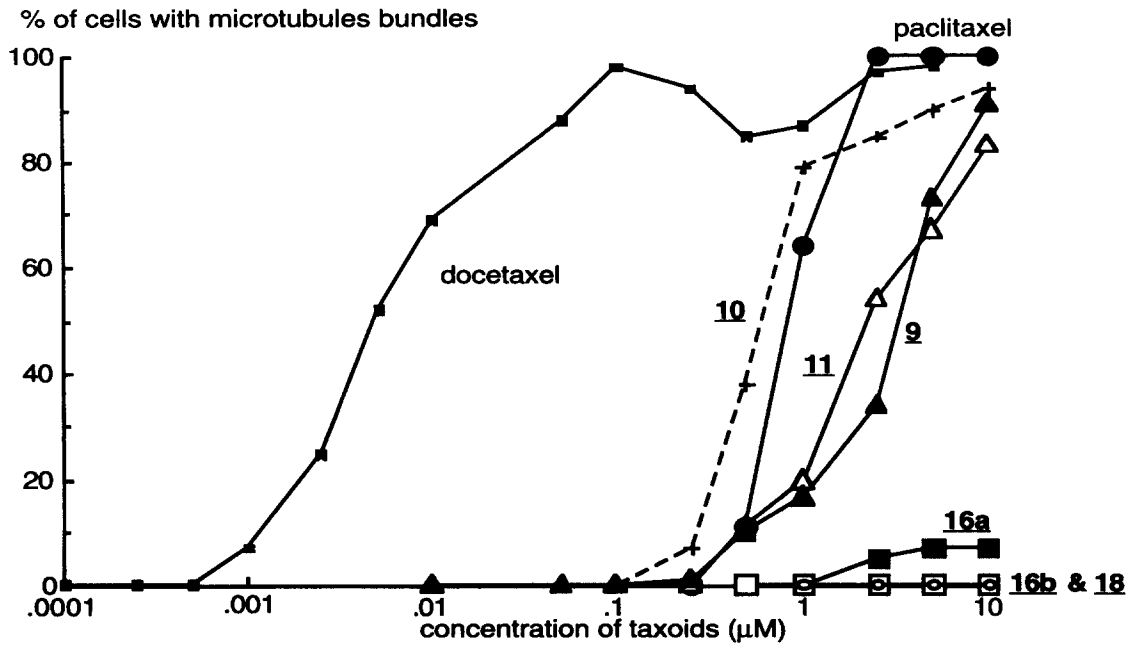


Figure 2. Appearance of microtubules bundles in PtK2 cells treated with the various docetaxel derivatives.

100-fold. The discrepancy between these results and the observations resulting from the *in vitro* assays with microtubule proteins are likely due to other biological factors such as permeation and subcellular distribution.

Fluorescent studies with tubulin

We examined the fluorescent properties of compounds **11**, **16a** and **b** in the presence or absence of tubulin. In ethanol, compounds **11** and **16a** showed the same UV spectra with the characteristic absorption maxima at 228, 332 and 467 nm. The excitation wavelengths were 467 (major) and 332 nm with emission at 520 nm. Compound **16b** showed only slight differences with **11** and **16a**: 228, 330 and 463 nm (the major excitation wavelength) for the UV maxima and emission at 525 nm. In the presence of microtubules, we observed an increase in fluorescence for all the compounds but no shift of the emission maxima was recorded. In order to specify the role of microtubules, we studied the fluorescent properties of compounds **11**, **16a** and **b** in the presence of bovine serum albumin: we observed the same enhancement as with microtubules for compounds **11** and **16a**. However, a maximum of fluorescence followed by quenching was observed with compound **16b** in the presence of increasing concentrations of microtubules; this result deserved further investigations. Moreover, addition of an excess of docetaxel had no effect on the intensity of fluorescence. These results suggested an increased solubility of the probes in a medium of lower polarity rather than a specific binding to a tubulin hydrophobic site.[23] In order to check the transfer of tubulin fluorescence to these derivatives, we excited microtubules at 290 nm and monitored the emission between 500 and 550 nm. For the three compounds, the intensity of fluorescence recorded was very low and was not modified by addition of an excess of docetaxel. Thus, under the conditions used, no transfer of fluorescence was detectable. This result might be due either to the low solubility of these compounds in hydrophylic media or to the high turbidity of microtubular solutions.

Intracellular microtubule labeling with fluorescent docetaxel derivatives. Living PtK2 cells were treated for 6 h at 37 °C with 10 μM **11** and **16a**. These living cells were then submitted to an excitation beam at 540–560 nm and the fluorescence was recorded over 590 nm. In the cytoplasm of interphasic cells, a red fluorescence was observed for compound **11** that corresponds to microtubule bundles (Fig. 3A) and to microtubule arrays (Fig. 3B). In mitotic cells, the red fluorescence was associated with the microtubules constituting the abnormal asters characteristic of cells blocked in mitosis (Fig. 3C). Compound **16a** was less effective than compound **11**, and no cytoplasmic microtubule bundles were induced. No microtubules could be detected in the cytoplasm. This observation suggests that the fluorescence raised by the binding of fluorescent docetaxel derivatives is insufficient to raise a detectable fluorescence at the level of isolated microtubules and microtubule arrays composed of a small number of microtubules. However, the microtubule asters induced by compound **16a** were observed in cells blocked in mitosis (Fig. 3E). Moreover, the region corresponding to the interdigitation region of the two microtubule arrays constituting the midbody between two dividing cells was visualized with compound **16a** (Fig. 3D, arrow). With both compounds, the fluorescence background was very low, suggesting that fluorescent docetaxel derivatives **11** and **16a** were not concentrated on other cytoplasmic structures and in the nucleus (Fig. 3A–E). In an alternative protocol, PtK2 cells were permeabilized and fixed, and then incubated in the presence of 10 μM fluorescent compounds **11** and **16a**. With both compounds, the

microtubule bundles, which constitute the midbody between the two dividing cells, were fluorescent (Fig. 4). In contrast to *in vivo* experiments, compound **16a** seemed to be more efficient than compound **11**. The fluorescent images of the midbody obtained in living cells and in fixed cells were similar. In both cases the interdigitated region of the two microtubule bundles forming the midbody was fluorescent. This result contrasts with the immunolabeling images obtained with tubulin antibodies which are unable to decorate these interdigitated microtubules. The permeabilization and fixation procedures led to an increase in the fluorescent background. The nuclei showed a homogeneous red fluorescence (Fig. 4B), while undetermined vesicular cytoplasmic structures were brightly stained by compound **16a** (Fig. 4B, v). It is likely that this high fluorescent background prevented the observation of the microtubule cytoskeleton in these

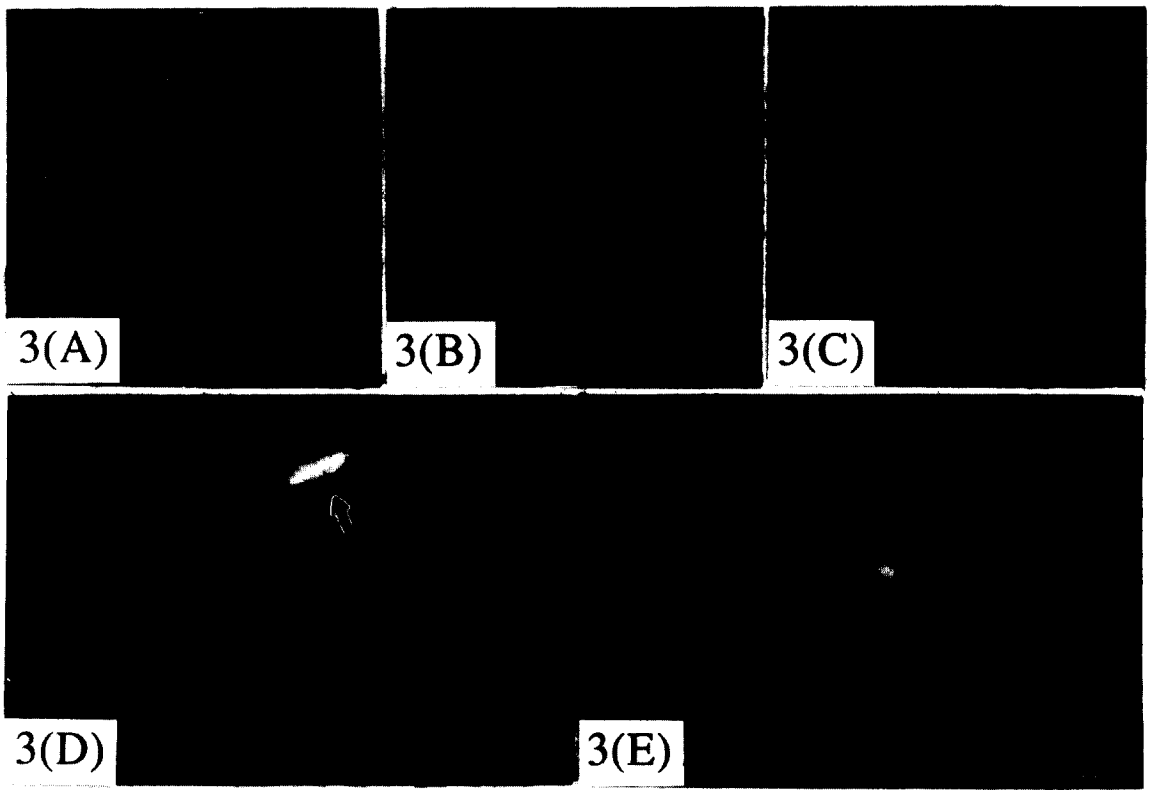


Figure 3. Fluorescent labeling of living PtK2 cells with compounds **11** and **16a**. (A–C) Compound **11** (10 μM for 6 h); (A) interphase cell showing fluorescent microtubule bundles (b); (B) interphase cells showing numerous fluorescent microtubule arrays (a); (C) mitotic cell blocked in abnormal metaphase and showing two fluorescent asters of microtubules. (D,E) Compound **16a** (10 μM for 6 h); (D) fluorescent staining of the midbody (arrow) localized between two daughter cells and of one of the two cytoplasmic microtubule arrays (a) which constitute the midbody (only one daughter cell is visible) ; the fluorescent spot (c) in the cytoplasm most likely corresponds to the centrosomal area; (E) mitotic cell blocked in abnormal metaphase and showing four fluorescent asters of microtubules. In all cases the nucleus was not fluorescent (A,B,D) and no fluorescent background was detected in the cytoplasm (C,D,E).

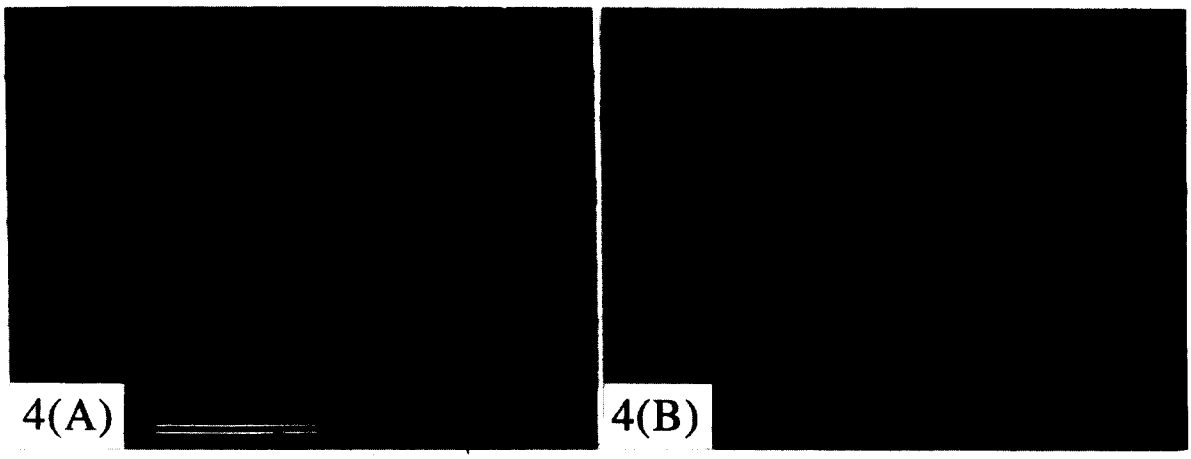


Figure 4. Fluorescent labeling of permeabilized and fixed PtK2 cells with compounds **11** and **16a**; (A) compound **11**; (B) compound **16a**. In both cases, the two microtubule arrays (a) interdigitating to constitute the midbody (arrow), localized between the two daughter cells (nucleus: n), showed a red fluorescence. In contrast, no microtubules could be detected in the cytoplasm, where the fluorescence was localized in undetermined vesicular structures (v).

cells. The highest background of fluorescence observed in cells submitted to a permeabilization/fixation procedure demonstrates the artefactual effects of these cytological techniques. Despite this limitation, fluorescent docetaxel derivatives bind to microtubules both in permeabilized/fixed cells and in living cells. These observations directly confirm the fixation of taxoids to cellular microtubules as previously suggested by the binding of radioactive paclitaxel in living cells.[33] Moreover, the fluorescence raised by docetaxel derivatives in living cells shows the specificity of the labeling and suggests that these compounds are concentrated at the level of the interphasic and mitotic microtubule cytoskeletons.

Conclusion

Six novel analogues of docetaxel bearing biotinyl or fluorescent probes at C-7, C-10 or NH-3' have been synthesized. The *in vitro* activities of all these compounds are strikingly different from the results on living cells. Although they were almost as active as docetaxel in the *in vitro* microtubular protein assay, their activity in living cells, followed by the appearance of microtubule bundles, was at least 100-fold lower than the activity of docetaxel (C-7 or C-10 derivatives) or even totally abolished (3'-NH deriv-atives). In PtK2 cell culture, docetaxel was much more potent than paclitaxel, as has already been observed in certain transplanted tumors (B16 melanoma).[6,34] Moreover, the fluorescence studies demonstrated that specific binding of taxoid analogues could only be observed in living cells. These findings emphasize how hazardous it can be to transpose *in vitro* results to *in vivo* mechanisms.

Experimental

Materials

^1H, ^{13}C NMR spectra were recorded on Bruker AC250, AM300 or AM400 spectrometers using tetramethylsilane as internal standard. Chemical shifts are expressed in parts per million (ppm). Coupling constants (*J*) are given in Hertz; *s*, *bs*, *d*, *bd*, *t*, *dd*, *q* and *m* indicate singlet, broad singlet, doublet, broad doublet, triplet, doublet of doublet, quadruplet and multiplet. Mass spectra were measured on a Kratos MS80. Absorption spectra were measured with a Perkin-Elmer lambda 5 spectrometer, fluorescent emission spectra were recorded with a Jobin Yvon JY3 fluorospectrometer. Epifluorescent images, obtained with a Zeiss Axiophot microscope equipped with a stable excitation beam and a Nocticon camera (LH 4015 form Lhesa), were digitalized (100 frame averaging) with an image processing system (Sapphire from Quantel) and recalculated using the histogram program.

Avidin, 2-(4'-hydroxyazobenzene)benzoic acid, biotin and biotin derivatives were from Sigma, 4-chloro-7-nitrobenz-2-oxa-1,3-diazole was from Fluka and docetaxel was a gift of Rhône-Poulenc Rorer. The synthesis of the docetaxel starting derivatives has been described previously.[4]

Bovine brain microtubular proteins were purified by two cycles of assembly/disassembly at 37 °C/0 °C. Pure tubulin was obtained after an additional step of phosphocellulose chromatography. The buffers used for biological studies were PIPES buffer: 100 mM PIPES (piperazin-*N*,*N*'-bis[2-ethanesulfonic acid], pH 6.9), 1 mM EGTA (ethyleneglycol-bis[β-aminoethyl ether]-*N*,*N*,*N*',*N*'-tetraacetic acid), 1 mM $MgCl_2$ and MES buffer: 100 mM MES (2-[*N*-morpholino]-ethanesulfonic acid, pH 6.6), 1 mM EGTA, 0.5 mM $MgCl_2$.

Synthesis

Derivatives with the biological probe at C-7 or C-10

7- or 10-(Benzyloxycarbonylaminocaproyl)-2'-(troc)docetaxel (3 and 4). 2'-(Troc)docetaxel (330 mg, 0.34 mmol) in 20 mL toluene was stirred at 70 °C with 141 mg dicyclohexylcarbodiimide (0.68 mmol), 42 mg 4-dimethylaminopyridine (0.34 mmol) and 181 mg of benzyloxycarbonylaminocaproic acid (0.68 mmol) for 2 h. After work-up, the crude products were purified by column chromatography on silica gel (eluent: CH_2Cl_2:MeOH, 98.5:1.5) to afford 202 mg 7-benzyloxycarbonylaminocaproyl-2'-(troc)docetaxel (**3**) and 144 mg 10-benzyloxycarbonylaminocaproyl-2'-(troc)docetaxel (**4**) (yield: 49 and 35% respectively).

7-(Benzyloxycarbonylaminocaproyl)-2'-(troc)docetaxel (3). ^1H NMR (250 MHz, $CDCl_3$): δ 8.16–7.31 (15H, *m*, aromatic); 6.28 (1H, *m*, H-13); 5.71 (1H, *d*, *J* = 7, H-2); 5.67–5.41 (3H, *m*, H-7, H-3' and H-2'); 5.38 (1H, *d*, *J* = 2, NH); 5.10 (3H, *s*, O-C$\underline{H_2}$-C_6H_5 and H-10); 4.96 (1H, *d*, *J* = 9, H-5); 4.77 (2H, qAB, *J* = 10, O-C$\underline{H_2}CCl_3$); 4.36 and 4.18 (2H, 2*d*, *J* = 8, H_2-20); 3.94 (1H, *d*, *J* = 7, H-3); 3.21 (2H, *m*, -NH-C$\underline{H_2}$-); 2.60 (1H, *m*, H-6); 2.49 (3H, *s*, OCOC$\underline{H_3}$); 2.31 (4H, *m*, H_2-14 and -C$\underline{H_2}$-CO-); 2.05 (3H, *s*, H_3-18); 1.92 (1H, *m*, H -6); 1.89 (3H, *s*, H_3-19); 1.77–1.42 (6H, *m*,-(C$\underline{H_2}$)$_3$-); 1.38 (9H, *s*, C(C$\underline{H_3}$)$_3$); 1.20 (3H, *s*, H_3-17); 1.07 (3H, *s*, H_3-16); MS-FAB$^+$ m/z 1253[MNa+2]$^+$, 1251[MNa]$^+$.

10-(Benzyloxycarbonylaminocaproyl)-2'-(troc)docetaxel (4). ^1H NMR (250 MHz, $CDCl_3$): δ 8.13–7.28 (15H, *m*, aromatic); 6.37 (1H, *s*, H-10); 6.33 (1H, *m*, H-13); 5.63 (1H, *d*, *J* = 7, H-2); 5.48–5.31 (3H, *m*, NH, H-3' and H-2'); 5.10 (2H, *s*, O-C$\underline{H_2}$-C_6H_5); 4.91 (1H, *d*, *J* = 9, H-5); 4.75 (2H, qAB, *J* = 10, O-C$\underline{H_2}CCl_3$); 4.45 (1H, *m*, H-7); 4.33 and 4.18 (2H, 2*d*, *J* = 8, H_2-20); 3.84 (1H, *d*, *J* = 7, H-3); 3.21 (2H, *m*, -NH-C$\underline{H_2}$-); 2.55 (1H, *m*, H-6); 2.47 (3H, *s*, OCOCH_3); 2.31 (4H, *m*, H_2-14 and -C$\underline{H_2}$-CO-); 1.92 (3H, *s*, H_3-18); 1.85 (1H, *m*, H-6); 1.66 (3H, *s*, H_3-19); 1.55–1.37 (6H, *m*, -(C$\underline{H_2}$)$_3$-); 1.33 (9H, *s*, C(C$\underline{H_3}$)$_3$); 1.24 (3H, *s*, H_3-17); 1.15 (3H, *s*, H_3-16); MS-FAB$^+$ m/z 1253 [MNa+2] $^+$, 1251 [MNa]$^+$.

General procedure for the removal of the protecting groups

Removal of the Troc group

The compound was heated at 60 °C in a mixture of HOAc and MeOH (1:1) with 0.5–1 weight-equivalent of zinc powder for 2 h. After usual work-up, the crude compounds were purified by prep. TLC on silica gel (eluent: CH_2Cl_2:MeOH, 96:4).

7-(Benzyloxycarbonylaminocaproyl)docetaxel (5). Compound **3** (200 mg) yielded **5** (107 mg, 62%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.18–7.32 (15H, *m*, aromatic); 6.21 (1H, *m*, H-13); 5.68 (1H, *d*, *J* = 7, H-2); 5.55–5.40 (2H, *m*, H-7 and H-3'); 5.31 (1H, *s*, H-10); 5.12 (2H, *s*, -O-C$\underline{H}_2$-C_6H_5); 4.93 (1H, *d*, *J* = 9, H-5); 4.62 (1H, *bs*, H-2'); 4.34 and 4.21 (2H, 2*d*, *J* = 8, H_2-20); 4.08 (1H, *bs*, OH); 3.99 (1H, *d*, *J* = 7, H-3); 3.22 (2H, *m*, -NH-C$\underline{H}_2$-); 2.51 (1H, *m*, H-6); 2.41 (3H, *s*, OCOC$\underline{H}_3$) 2.38 (4H, *m*, H_2-14 and -C$\underline{H}_2$-CO-) 1.99 (1H, *m*, H-6) 1.91 (3H, *s*, H_3-18) 1.86 (3H, *s*, H_3-19); 1.70–1.44 (6H, *m*, -(C$\underline{H}_2$)$_3$-); 1.38 (9H, *s*, C(C$\underline{H}_3$)$_3$); 1.21 (3H, *s*, H_3-17); 1.09 (3H, *s*, H_3-16); MS-FAB$^+$ m/z 1061 [MLi]$^+$, 780 ([MH–C–13 side chain] $^+$,100%).

10-(Benzyloxycarbonylaminocaproyl)docetaxel(6). Compound **4** (144 mg) yielded **6** (71 mg, 57%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.13–7.28 (15H, *m*, aromatic); 6.28 (1H, *s*, H-10); 6.20 (1H, *m*, H-13); 5.63 (1H, *d*, *J* = 7, H-2); 5.30 and 5.22 (2H, 2*m*, H-3' and -N$\underline{H}$-); 5.07 (2H, *s*, OC$\underline{H}_2C_6H_5$); 4.90 (1H, *d*, *J* = 9, H -5); 4.58 (1H, *bs*, H-2'); 4.38 (1H, *m*, H-7); 4.25 and 4.12 (2H, 2*d*, *J* = 8, H_2-20); 3.75 (1H, *d*, *J* = 7, H-3); 3.20 (2H, *m*, -NH-C$\underline{H}_2$-); 2.47 (3H, *m*, H-6 and -C$\underline{H}_2$-CO-); 2.35 (3H, *s*, -OCOC$\underline{H}_3$); 2.25 (2H, *m*, H_2-14); 1.88–1.37 (7H, *m*, H-6 and -(C$\underline{H}_2$)$_3$-); 1.80 (3H, *s*, H_3-18); 1.57 (3H, *s*, H_3-19); 1.28 (9H, *s*, -C(C$\underline{H}_3$)$_3$); 1.19 (3H, *s*, H_3-17); 1.07 (3H, *s*, H_3-16); MS-FAB$^+$ m/z 1061 ([MLi]$^+$,100%), 780 [MH–C–13 side chain] $^+$.

Removal of the benzyloxycarbonyl (Z) group

The compounds were hydrogenolyzed in MeOH under H_2 with Pd on charcoal (10%) for 1–3 h. After filtration and extensive washing of the catalyst, the organic layers were concentrated and the compounds were purified by prep. TLC on silica gel (eluent: CH_2Cl_2:MeOH, 90:10 saturated with NH_3).

7-(Aminocaproyl)docetaxel (7). Compound **5** (85 mg) yielded **7** (71 mg, 95%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 811–7.34 (10H, *m*, aromatic); 6.22 (1H, *m*, H-13); 5.68 (1H, *d*, *J* = 7, H-2); 5.50 (1H, *m*, H-3'); 5.38 (1H, *s*, H-10); 5.27 (1H, *m*, H-7); 4.94 (1H, *d*, *J* = 9, H-5); 4.67 (1H, *bs*, H-2'); 4.30 and 4.18 (2H, 2*d*, *J* = 8, H_2-20); 3.97 (1H, *d*, *J* = 7, H-3); 3.13 (2H, *m*, NH_2-C$\underline{H}_2$-); 2.55 (1H, *m*, H-6); 2.39 (3H, *s*, OCOC$\underline{H}_3$); 2.30 (4H, *m*, H_2-14 and -C$\underline{H}_2$-CO-); 1.95 (3H, *s*, H_3-18); 1.87 (1H, *m*, H-6); 1.80 (3H, *s*, H_3-19); 1.70–1.38 (6H, *m*, -(C$\underline{H}_2$)$_3$-); 1.32 (9H, *s*, -C(C$\underline{H}_3$)$_3$); 1.25 (3H, *s*, H_3-17); 1.10 (3H, *s*, H_3-16); MS-FAB$^+$ m/z 943 [MNa]$^+$, 921 [MH]$^+$.

10-(Aminocaproyl)docetaxel (8). Compound **6** (60 mg) yielded **8** (48 mg, 91%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.07–7.27 (10H, *m*, aromatic); 6.31 (1H, *s*, H-10); 6.23 (1H, *m*, H-13); 5.66 (1H, *d*, *J* = 7, H-2); 5.47 (1H, *d*, *J* = 9, NH); 5.25 (1H, *m*, H-3'); 4.95 (1H, *d*, *J* = 9, H-5); 4.62 (1H, *bs*, H-2'); 4.39 (1H, *m*, H-7); 4.30 and 4.17 (2H, 2*d*, *J* = 8, H_2-20); 3.80 (1H, *d*, *J* = 7, H-3); 3.22 (2H, *m*, NH_2-C$\underline{H}_2$-); 2.53 (3H, *m*, H-6 and -C$\underline{H}_2$-CO-); 2.30 (3H, *s*, OCOC$\underline{H}_3$); 2.20 (2H, *m*, H_2-14); 1.86 (3H, *s*, H_3-18); 1.75 (1H, *m*, H-6); 1.65 (3H, *s*, H_3-19); 1.60–1.38 (6H, *m*, -(C$\underline{H}_2$)$_3$-); 1.34 (9H, *s*, -C(C$\underline{H}_3$)$_3$); 1.26 (3H, *s*, H_3-17); 1.15 (3H, *s*, H_3-16); MS-FAB$^+$ m/z 943 [MNa]$^+$, 921 [MH]$^+$.

Procedure for the coupling of the probes

Coupling of biotin

A solution of 1.5 equivalent of *N*-hydroxy-succinimidobiotin in DMF was added at 4 °C to a stirred solution of the docetaxel derivatives **7** and **8** in CH_2Cl_2 containing 1.3 equiv. of *N*-methylmorpholin. After 5 h at room temperature, the solvents were evaporated and the residue dissolved in EtOAc. After washing with a solution of 10% citric acid then with H_2O, the organic layers were dried and concentrated. The crude products were purified by prep. TLC on silica gel (eluent: CH_2Cl_2:MeOH, 96:4).

7-(5'Biotinylamidocaproyl)docetaxel 9. Compound **7** (60 mg) yielded **9** (57 mg, 76%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.14–7.31 (10H, *m*, aromatic); 6.72, 6.37 and 6.18 (3H, 3*m*, 3x-NH-); 6.13 (1H, *m*, H-13); 5.68 (1H, *d*, *J* = 7, H-2); 5.58 (1H, *m*, H-3'); 5.27 (1H, *s*, H-10); 5.21 (1H, *m*, H-7); 4.98 (1H, *d*, *J* = 9, H-5); 4.62 (1H, *bs*, H-2'); 4.52 (1H, *m*, biotin H-4); 4.42 (1H, *m*, biotin H-3); 4.36 and 4.20 (2H, 2*d*, *J* = 8, H_2-20); 3.99 (1H, *d*, *J* = 7, H-3); 3.45 (1H, *m*, biotin H-2); 3.24 (2H, *m*, -NH-C$\underline{H}_2$-); 2.93 and 2.72 (2H, *m*, biotin H_2-5); 2.53 (1H, *m*, H-6); 2.42 (3H, *s*, OCOC$\underline{H}_3$); 2.38–2.10 (6H, *m*, H_2-14 and 2x-C$\underline{H}_2$-CO-); 1.98 (3H, *s*, H_3-18); 1.93 (1H, *m*, H-6); 1.88 (3H, *s*, H_3-19); 1.83–1.40 (12H, *m*, 2x-(C$\underline{H}_2$)$_3$-); 1.37 (9H, *s*, -C(C$\underline{H}_3$)$_3$); 1.26 (3H, *s*, H_3-17); 1.14 (3H, *s*, H_3-16); ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.01 (C-19); 14.16 (C-18); 20.69 (C-17); 22.57 (4-OAc); 25.50 (CH_2); 25.36 (CH_2); 26.50 (C-16); 26.63 (biotin C-2'); 28.03 (biotin C-1'); 28.37 (-C(C$\underline{H}_3$)$_3$); 29.13 (CH_2); 33.45 (CH_2); 34.00 (CH_2); 35.74 (C-14); 36.0 (C-6); 39.17 (CH_2); 40.68 (CH_2); 43.31 (C-15); 46.78 (C-3); 50.10 (C-3'); 56.45 (C-8 and biotin C-5); 60.37 (biotin C-4); 62.02 (biotin C-3); 71.90 (C-7); 72.61 (C-13); 74.35 (C-2'); 74.45 (C-10); 74.72 (C-2); 77.32 (C-20); 79.10 (C-1); 79.97 (C(CH$_3$)$_3$); 80.86 (C-4); 83.92 (C-5); 126.96, 127.85, 128.78, 128.85, 130.28 (C aromatic); 129.26 (OBz-C-1); 133.86 (C-11 and C_6H_5-C-1); 135.45 (OBz-C-4); 140.34 (C-12); 155.90 (CO Boc); 164.12 (biotin CO); 166.97 (CO-OBz); 170.22 (CO-OAc); 172.61 (7-OCOR and biotin C-5') ;173.05 (C-1'); 211.18 (C-9); MS-FAB$^+$ m/z 1169 [MNa]$^+$, 1147 [MH]$^+$.

10-(5'Biotinylamidocaproyl)docetaxel (10). Compound **8** (60 mg) yielded **10** (41 mg, 54%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.14–7.33 (10H, *m*, aromatic); 6.54 (1H, *m*, -NH-); 6.40 (1H, *s*, H-10); 6.25 (1H, *m*, H-13); 5.70 (2H, *d*, *J* = 7, H-2 and -NH-); 5.29 (1H, *m*, H-3'); 4.98 (1H, *d*, *J* = 9, H-5); 4.66 (1H, *bs*, H-2'); 4.55 (1H, *m*, biotin H-4); 4.42 (2H, *m*, H-7 and biotin H-3); 4.33 and 4.20 (2H, 2*d*, *J* = 8, H_2-20); 3.83 (1H, *d*, *J* = 7, H-3); 3.33 (2H, *m*, -NH-C$\underline{H}_2$-); 3.20 (1H, *m*, biotin H-2); 2.95 and 2.80 (2H, *m*, biotin H_2-5); 2.58–2.33 (7H, *m*, H-6, H_2-14 and 2x-C$\underline{H}_2$-CO-); 2.42 (3H, *s*, OCOC$\underline{H}_3$); 1.97 (3H, *s*, H_3-18); 1.90–1.48 (13H, *m*, H-6 and 2x-(C$\underline{H}_2$)$_3$-); 1.77 (3H, *s*, H_3-19); 1.43 (9H, *s*, -C(C$\underline{H}_3$)$_3$); 1.35 (3H, *s*, H_3-16); 1.26 (3H, *s*, H_3-17); ^{13}C NMR (75 MHz, $CDCl_3$): δ 9.91 (C-19); 14.92 (C-18); 22.03 (C-17); 22.70 (4-OAc); 24.46 (CH_2); 25.93 (CH_2); 26.90 (C-16); 28.34 (biotin C-2' and biotin C-1'); 28.46 (-C(C$\underline{H}_3$)$_3$); 28.89 (CH_2); 34.19 (CH_2); 35.72 (CH_2); 36.21 (C-14 and C-6); 39.07 (CH_2); 40.56 (CH_2); 43.39 (C-15); 46.14 (C-3); 55.71 (C-3'); 58.55 (C-8 and biotin C-5); 60.40 (biotin C-4); 62.03 (biotin C-3); 71.94 (C-7); 72.13 (C-13); 73.99 (C-2'); 75.20 (C-10); 75.55 (C-2); 76.63 (C-20); 79.08 (C-1); 80.18 (C(CH$_3$)$_3$); 81.30; (C-4); 84.58 (C-5); 126.95, 128.01, 128.82, 130.30 (C aromatic); 129.41 (OBz-C-1); 133.24 (C-11); 133.77 (OBz-C-4); 138.86 (C_6H_5-C-1); 142.16 (C-12); 155.66 (COBoc); 164.18 (biotin CO); 167.10 (CO-OBz); 170.34 (CO-OAc); 173.10 (biotin C-5'); 173.56 (7-OCOR); 173.65 (C-1'); 204.17(C-9); MS-FAB$^+$ m/z 1169 [MNa]$^+$.

Coupling of the 7-nitrobenz-2-oxa-1,3-diazol-4-yl group (NBD)

4-Chloro-7-nitrobenz-2-oxa-1,3-diazole (8.4 mg in 1 mL of EtOH) was added to a stirred solution of **7** (39 mg in 2 mL of EtOH) containing 1.5 equiv. of sodium bicarbonate. After 1.5 h at 40 °C, the solution was cooled, filtered and extracted with EtOAc. The organic layers were washed with water, dried and concentrated. The crude product was purified by prep. TLC on silica gel (eluent: CH_2Cl_2:MeOH, 96:4) to yield **11** (23 mg, 53%).

7-(N-[4-NBD]-aminocaproyl)docetaxel (11). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.50 (1H, *d*, *J* = 9, NBD); 8.12–7.31 (10H, *m*, aromatic); 6.78 (1H, *m*, NH); 6.22 (1H, *m*, H-13); 6.19 (1H, *d*, *J* = 9, NBD); 5.68 (1H, *d*, *J* = 7, H-2); 5.51 (2H, *m*, 3'NH and H-7); 5.31 (2H, *m*, H-10 and H-3'); 4.93 (1H, *d*, *J* = 9, H-5); 4.65 (1H, *bs*, H-2'); 4.34 and 4.21 (2H, 2*d*, *J* = 8, H_2-20); 4.33 (1H, *bs*, 10-OH); 3.99 (1H, *d*, *J* = 7, H-3); 3.55 (2H, *m*, -NH-C$\underline{H}_2$-); 2.51 (1H, *m*, H-6); 2.40 (3H, *s*, OCOC$\underline{H}_3$); 2.31 (4H, *m*, H_2-14 and -C$\underline{H}_2$-CO-); 1.91 (1H, *m*, H-6); 1.86 (6H, *s*, H_3-18 and H_3-19); 1.78–1.38 (6H, *m*, -(C$\underline{H}_2$)$_3$-); 1.35 (9H, *s*, -C(C$\underline{H}_3$)$_3$); 1.25 (3H, *s*, H_3-17); 1.10 (3H, *s*, H_3-16); ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.05 (C-19); 14.32 (C-18); 20.67 (C-17); 22.62 (4-OAc); 24.30 (CH_2); 26.42 (C-16 and CH_2); 28.33 (-C(C$\underline{H}_3$)$_3$); 30.02 (CH_2); 33.56 (CH_2); 33.96 (C-14); 35.93 (C-6); 43.16 (CH_2); 43.89 (C-15); 46.41 (C-3); 56.56 (C-8 and C-3'); 71.95 (C-7); 72.48 (C-13); 73.74 (C-2'); 74.67 (C-10 and C-2); 77.55 (C-20); 78.84 (C-1); 80.18 (C(CH$_3$)$_3$); 80.78 (C-4); 83.78 (C-5); 98.79 (C-NBD); 126.89, 128.15, 128.95, 130.30 (C aromatic); 129.06 (OBz-C-1); 133.90 (C-11 and C_6H_5-C-1); 135.76 (OBz-C-4); 138.48, 139.39 and 144.24 (C-NBD); 144.85 (C-12); 155.61 (CO Boc); 167.32 (CO-OBz); 170.04 (CO-OAc;) 172.20 (7-OCOR); 173.05 (C-1'); 211.24 (C9); MS-FAB$^+$ m/z 1106 [MNa]$^+$; UV: 467(20320), 332.5(7970), 228(28420).

Derivatives with the biological probe at C-3'

Coupling of biotin

Biotinamidocaproate-*N*-hydroxysuccinimide ester (53 mg in 1.5 mL DMF) was added at 0 °C to a solution of 7,10-ditroc-3'-*N*-de(tert-butoxycarbonyl) docetaxel **12** (61 mg in 1.5 mL DMF) containing 1.1 equiv. of *N*-methylmorpholine. The solution was stirred for 5.5 h at 60 °C. After usual work-up the crude product was purified by prep. TLC on silica gel (eluent: CH_2Cl_2:MeOH, 90:10) to yield compound **17** (66 mg, 81%).

^{1}H NMR (250 MHz, $CDCl_3$): δ 8.25–7.32 (10H, *m*, aromatic); 6.51 (1H, *m*, -N$\underline{H}$-); 6.37 (1H, *m*, H-13); 6.23 (1H, *s*, H-10); 5.91 (1H, *m*, -N$\underline{H}$-); 5.76 (2H, *m*, H-2 and H-7); 5.61 (1H, *m*, H-3'); 4.96 (1H, *d*, *J* = 9, H-5); 4.91 and 4.61 (2H, 2*d*, *J* = 12, -OC$\underline{H}_2$CCl$_3$); 4.80 (2H, *s*, -OC$\underline{H}_2$CCl$_3$); 4.72 (1H, *d*, *J* = 3, H-2'); 4.54 (1H, *m*, biotin H-4); 4.39 (1H, *m*, biotin H-3); 4.32 and 4.26 (2H, 2*d*, *J* = 8, H_2-20); 3.94 (1H, *d*, *J* = 7, H-3); 3.18 (3H, *m*, -NH-C$\underline{H}_2$- and biotin H-2); 2.91 (1H, *m*, biotin H-5); 2.68 (2H, *m*, H-6 and biotin H-5); 2.52 (3H, *s*, -OCOC$\underline{H}_3$); 2.48–2.21 (6H, *m*, H_2-14 and 2x-C$\underline{H}_2$-CO-); 2.07 (1H, *m*, H-6); 2.00 (3H, *s*, H_3-18); 1.88 (3H, *s*, H_3-19); 1.83–1.37 (12H, *m*, 2x-(C$\underline{H}_2$)$_3$-); 1.27 (3H, *s*, H_3-17); 1.18 (3H, *s*, H_3-16); MS-FAB$^+$ m/z 1395 [MH]$^+$.

Removal of the Troc groups

The previous compound **17** (60 mg) was deprotected as described above to yield, after purification by prep. TLC on silica gel (eluent: CH_2Cl_2:MeOH, 90:10) compound **18** (25 mg, 56%).

3'-N-(5'Biotinylamidocaproyl)-3'-N-de(tertbutoxycarbonyl)docetaxel (18). ^{1}H NMR (250 MHz, CD_3OD): δ 8.24–7.30 (10H, *m*, aromatic); 6.24 (1H, *m*, H-13); 5.72 (1H, *d*, *J* = 7, H-2); 5.54 (1H, *m*, H-3'); 5.34 (1H, *s*, H-10); 5.06 (1H, *d*, *J* = 9, H-5); 4.65 (1H, *d*, *J* = 3, H-2'); 4.53 (1H, *m*, biotin H-4); 4.31 (2H, *m*, H-7 and biotin H-3); 4.24 (2H, *s*, H_2-20); 3.92 (1H, *d*, *J* = 7, H-3); 3.16 (3H, *m*, -NH-C$\underline{H}_2$- and biotin H-2); 2.95 and 2.73 (2H, 2m, biotin H_2-5); 2.43 (1H, *m*, H-6); 2.36 (3H, *s*, -OCOC$\underline{H}_3$); 2.32–2.11 (6H, *m*, H_2-14 and 2x-C$\underline{H}_2$-CO-); 2.03 (1H, *m*, H-6); 1.92 (3H, *s*, H_3-18); 1.70 (3H, *s*, H_3-19); 1.65–1.24 (12H, *m*, 2x-(C$\underline{H}_2$)$_3$-); 1.19 (3H, *s*, H_3-17); 1.12 (3H, *s*, H_3-16); MS-FAB$^+$ m/z 1069 [MNa]$^+$.

Coupling of ZHN(CH_2)$_n$COOH (n=2 or 5)

7,10-Ditroc-3'-*N*-de-(*t*-butoxycarbonyl)docetaxel (**12**) in toluene:CH_2Cl_2(2:1; v:v) was added to a stirred solution of 2 equiv. of the *N*-hydroxysuccinimide ester of the *Z*-protected chain with 1 equiv. of *N*-methylmorpholine and heated at 60 °C for 3 h. After work-up, the crude products were purified by prep. TLC on silica gel (eluent: CH_2Cl_2:MeOH, 95:5).

*7,10-Ditroc-3'-N-(Z-aminocaproyl)-3'-N-de(tert-butoxycarbonyl)docetaxel (**13a**).* Compound **12** (350 mg) yielded the expected compound **13a** (409 mg, 94%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.11–7.28 (15H, *m*, aromatic); 6.48 (1H, *m*, -NH-); 6.21 (2H, *m*, H-13 and H-10); 5.68 (1H, *d*, *J* = 7, H-2); 5.54 (2H, *m*, H-7 and H3'-); 4.97 (2H, *s*, CH_2-C_6H_5); 4.91 (1H, *d*, *J* = 9, H-5); 4.87 and 4.56 (2H, 2*d*, *J* = 12, -OCH_2CCl$_3$); 4.74 (2H, *s*, -OCH_2CCl$_3$); 4.68 (1H, *d*, *J* = 3, H-2'); 4.29 and 4.17 (2H, 2*d*, *J* = 8, H_2-20); 3.87 (1H, *d*, *J* = 7, H-3); 2.98 (2H, *m*, -NH-CH_2-); 2.56 (1H, *m*, H-6); 2.35 (3H, *s*, -OCOCH_3); 2.31 (2H, *m*, H_2-14); 2.11 (2H, *m*, -CH_2-CO-); 2.02 (1H, *m*, H-6); 1.90 (3H, *s*, H_3-18); 1.84 (3H, *s*, H_3-19); 1.72–1.20 (6H, *m*, -(CH_2)$_3$-); 1.24 (3H, *s*, H_3-17); 1.15 (3H, *s*, H_3-16); MS-FAB$^+$ m/z 1329 [MNa+4]$^+$, 1327 [MNa+2]$^+$.

*7,10-Ditroc-3'-N-(Z-aminopropanoyl)-3'-N-de(tert-butoxycarbonyl)docetaxel (**13b**).* Compound **12** (190 mg) yielded the expected compound **13b** (214 mg, 94%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.20–7.22 (15H, *m*, aromatic); 6.91 (1H, *m*, -NH-); 6.20 (2H, *m*, H-13 and H-10); 5.69 (1H, *d*, *J* = 7, H-2); 5.57 (2H, *m*, H-7 and -NH-); 5.41 (1H, *m*, H-3'); 4.98 (2H, *s*, CH_2-C_6H_5); 4.94 (1H, *d*, *J* = 9, H-5); 4.92 and 4.61 (2H, 2*d*, *J* = 12, -OCH_2CCl$_3$); 4.80 (2H, qAB, *J* = 12, -OCH_2CCl$_3$); 4.68 (1H, *bs*, H-2'); 4.33 and 4.20 (2H, 2d, *J* = 8, H_2-20); 3.78 (1H, *d*, *J* = 7, H-3); 3.29 (2H, *m*, -NH-CH_2-); 2.60 (1H, *m*, H-6); 2.38 (3H, *s*, -OCOCH_3); 2.41–2.26 (4H, *m*, H_2-14 and -CH_2-CO-); 2.06 (1H, *m*, H-6); 1.93 (3H, *s*, H_3-18); 1.86 (3H, *s*, H_3-19); 1.18 (6H, *s*, H_3-17 and H_3-16); MS-FAB$^+$ m/z 1287 [MNa+4]$^+$, 1285 [MNa+2]$^+$, 1265 [MH+4]$^+$, 1263 [MH+2]$^+$.

Removal of the protecting groups

This was carried out as described above.

Removal of the Troc groups

*3'-N-(Z-Aminocaproyl)-3'-N-de(tert-butoxycarbonyl)docetaxel (**14a**).* The aminocaproyl derivative **13a** (270 mg) yielded the desired compound **14a** (181 mg, 92%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.18–7.31 (15H, *m*, aromatic); 6.78 (1H, *d*, *J* = 8, -NH-); 6.25 (1H, *m*, H-13); 5.73 (1H, *d*, *J* = 7, H-2); 5.62 (1H, *dd*, *J* = 8 and 2.5, H-3'); 5.30 (1H, *s*, H-10); 5.08 (2H, *s*, CH_2-C_6H_5); 4.96 (1H, *d*, *J* = 9, H-5); 4.78 (1H, *d*, *J* = 2.5, H-2'); 4.35 and 4.24 (2H, 2*d*, *J* = 8, H_2-20); 4.30 (1H, *m*, H-7); 3.90 (1H, *d*, *J* = 7, H-3); 3.10 (2H, *m*, -NH-CH_2-); 2.58 (1H, *m*, H-6); 2.40 (3H, *s*, -OCOCH_3); 2.40–2.22 (2H, *m*, H_2-14); 2.19 (2H, *m*, -CH_2-CO-); 1.90 (4H, *m*, H_3-18 and H-6); 1.85 (3H, *s*, H_3-19); 1.68–1.30 (6H, *m*, -(CH_2)$_3$-); 1.26 (3H, *s*, H_3-17); 1.12 (3H, *s*, H_3-16); MS-FAB$^+$ m/z 977 [MNa]$^+$, 955 [MH]$^+$.

*3'-N-(Z-Aminopropanoyl)-3'-N-de(tert-butoxycarbonyl)docetaxel (**14b**).* The aminopropanoyl derivative **13b** (210 mg) yielded the desired compound **14b** (80 mg, 53%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.10–7.25 (15H, *m*, aromatic); 7.13 (1H, *m*, -NH-); 6.20 (1H, *m*, H-13); 5.66 (1H, *d*, *J* = 7, H-2); 5.54 (2H, *m*, H-3' and -NH-); 5.23 (1H, *s*, H-10); 5.01 (2H, *s*, CH_2-C_6H_5); 4.90 (1H, *d*, *J* = 9, H-5); 4.67 (1H, *bs*, H-2'); 4.30 and 4.21 (2H, 2*d*, *J* = 8, H_2-20); 4.25 (1H, *m*, H-7); 3.87 (1H, *d*, *J* = 7, H-3); 3.34 (2H, *m*, -NH-CH_2-); 2.47 (1H, *m*, H-6); 2.36 (3H, *s*, -OCOCH_3); 2.35–2.13 (4H, *m*, H_2-14 and -CH_2-CO-); 1.90 (1H, *m*, H-6); 1.86 (3H, *s*, H_3-18); 1.75 (3H, *s*, H_3-19); 1.20 (3H, *s*, H_3-17); 1.11 (1H, *s*, H_3-16); MS-FAB$^+$ m/z 935 [MNa]$^+$.

Removal of the Z group

*3'-N-(Aminocaproyl)-3'-N-des-t-butoxycarbonyl-docetaxel (**15a**).* Deprotection of **14a** (51 mg) yielded **15a** (45 mg, 90%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.09–7.22 (10H, *m*, aromatic); 6.14 (1H, *m*, H-13); 5.65 (1H, *d*, *J* = 7, H-2); 5.46 (1H, *d*, *J* = 4.5, H-3'); 5.27 (1H, *s*, H-10); 4.96 (1H, *d*, *J* = 9, H-5); 4.58 (1H, *d*, *J* = 4.5, H-2'); 4.23 (1H, *m*, H-7); 4.19 (2H, *s*, H_2-20); 3.87 (1H, *d*, *J* = 7, H-3); 2.71 (2H, *m*, -NH-CH_2-); 2.44 (1H, *m*, H-6); 2.33 (3H, *s*, -OCOCH_3); 2.31–2.08 (4H, *m*, H_2-14 and -CH_2-CO-); 2.05 (1H, *m*, H-6); 1.88 (3H, *s*, H_3-18); 1.68 (3H, *s*, H_3-19); 1.65–1.26 (6H, *m*, -(CH_2)$_3$-); 1.18 (3H, *s*, H_3-17); 1.12 (3H, *s*, H_3-16); MS-FAB$^+$ m/z 843 [MNa]$^+$, 821 [MH]$^+$.

*3'-N-(Aminopropanoyl)-3'-N-de(tert-butoxycarbonyl)docetaxel (**15b**).* Deprotection of **14b** (58 mg) yielded **15b** (44 mg, 88%). ^{1}H NMR (250 MHz, CD_3OD): δ 8.07–7.21 (10H, *m*, aromatic); 6.13 (1H, *m*, H-13); 5.63 (1H, *d*, *J* = 7, H-2); 5.43 (1H, *d*, *J* = 4, H-3'); 5.19 (1H, *s*, H-10); 4.93 (1H, *d*, *J* = 9, H-5); 4.54 (1H, *d*, *J* = 4, H-2'); 4.22 (2H, *s*, H_2-20); 4.18 (1H, *m*, H-7); 3.82 (1H, *d*, *J* = 7, H-3); 2.94 (2H, *m*, -NH-CH_2-); 2.43 (3H, *m*, H-6 and -CH_2-CO-); 2.28 (3H, *s*, -OCOCH_3); 2.25–1.97 (2H, *m*, H_2-14); 1.88 (1H, *m*, H-6); 1.85 (3H, *s*, H_3-18); 1.69 (3H, *s*, H_3-19); 1.17 (3H, *s*, H_3-17); 1.09 (3H, *s*, H_3-16); MS-FAB$^+$ m/z 793 [MNa]$^+$, 779 [MH]$^+$.

Coupling of the 7-nitrobenz-2-oxa-1,3-diazol-4-yl group (NBD)

Compound **15a** or **b** (40 mg in 2 mL of EtOH) was heated at 40 °C with sodium bicarbonate (5 mg) and 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (7 mg) for 1.5 h. After work-up, the crude products were purified by prep. TLC on silica gel (eluent: CH_2Cl_2:MeOH, 90:10) to afford **16a** and **b** in 39% (19 mg) and 42% (17.8 mg) yields, respectively.

3'-N-*(*N-*[4-NBD]-aminocaproyl)-3'*-N-*de(tert-butoxycarbonyl)docetaxel (16a).* ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.41 (1H, *d*, *J* = 9, NBD) 8.08–7.25 (10H, *m*, aromatic); 6.12 (1H, *m*, H-13); 6.07 (1H, *d*, *J* = 9, NBD); 5.68 (1H, *d*, *J* = 7, H-2); 5.51 (1H, *d*, *J* = 3, H-3'); 5.18 (1H, *s*, H-10); 4.92 (1H, *d*, *J* = 9, H-5); 4.63 (1H, *d*, *J* = 3, H-2'); 4.29 and 4.18 (2H, 2*d*, *J* = 8, H_2-20); 4.16 (1H, *m*, H-7); 3.83 (1H, *d*, *J* = 7, H-3); 3.38 (2H, *m*, -NH-CH_2-); 2.48 (1H, *m*, H-6); 2.31 (3H, *s*, -OCOCH_3); 2.26 (4H, *m*, H_2-14 and -CH_2-CO-); 1.90 (1H, *m*, H-6); 1.88 (3H, *s*, H_3-18); 1.68 (3H, *s*, H_3-19); 1.65–1.32 (6H, *m*, -(CH_2)$_3$-); 1.18 (3H, *s*, H_3-17); 1.10 (3H, *s*, H_3-16);^{13}C NMR (62.5 MHz, $CDCl_3$): δ 9.82 (C-19); 13.91 (C-18); 20.88 (C-17); 22.49 (4-OAc); 25.00 (CH_2); 26.22 (C-16); 26.41 (CH_2); 29.67 (CH_2); 35.64 (CH_2); 35.80 (C-14); 36.40 (C-6); 43.27 (CH_2); 43.43 (C-15); 46.42 (C-3); 54.70 (C-8); 57.75 (C-3'); 71.47 (C-7); 72.24 (C-13); 73.04 (C-2'); 74.30 (C-10); 75.01 (C-2); 77.25 (C-20); 78.10 (C-1); 81.34 (C-4); 84.54 (C-5); 98.53 (C-NBD); 126.85, 128.02, 128.61, 128.78, 130.11 (C aromatic); 129.52 (OBz-C1); 133.54 (C11 and C_6H_5-C-1); 136.27 (OBz-C-4); 136.96 and 138.16 (C-NBD and C-12); 166.29 (CO-OBz); 170.36 (CO-OAc); 173.04 (3'NH-COR); 173.59 (C-1'); 210.91 (C-9): MS-FAB$^+$ m/z 1006 [MNa]$^+$; UV: 467(16,400), 332(6600), 228(23,630).

3'-N-*(*N-*[4-NBD]-aminopropanoyl)-3'*-N-*de(tert-butoxycarbonyl)docetaxel (16b).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (1H, *d*, *J* = 9, NBD); 8.03–7.16 (10H, *m*, aromatic); 6.08 (1H, *m*, H-13); 6.04 (1H, *d*, *J* = 9, NBD); 5.58 (1H, *d*, *J* = 7, H-2); 5.43 (1H, *d*, *J* = 3, H-3'); 5.13 (1H, *s*, H-10) 4.88 (1H, *d*, *J* = 9, H-5); 4.51 (1H, *d*, *J* = 3, H-2'); 4.22 and 4.15 (2H, 2*d*, *J* = 8, H_2-20); 4.00 (1H, *m*, H-7); 3.78 (1H, *d*, *J* = 7, H-3); 3.66 (2H, *m*, -NH-CH_2-); 2.64 (2H, *t*, *J* = 7, -CH_2-CO-); 2.43 (1H, *m*, H-6); 2.27 (3H, *s*, -OCOCH_3); 2.22–1.96 (2H, *m*, H_2-14); 1.85 (1H, *m*, H-6); 1.81 (3H, *s*, H_3-18); 1.67 (3H, *s*, H_3-19); 1.12 (3H, *s*, H_3-17); 1.06 (3H, *s*, H_3-16); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 9.88 (C-19); 14.01 (C-18); 20.85 (C-17); 22.52 (4-OAc); 26.41 (C-16); 34.74 (CH_2); 35.93 (C-14); 36.45 (C-6); 43.18 (CH_2); 43.18 (C-15); 46.53 (C-3); 55.10 (C-8); 57.76 (C-3'); 71.73 (C-7); 72.07 (C-13); 73.14 (C-2'); 74.61 (C-10); 75.06 (C-2); 77.57 (C-20); 78.44 (C-1); 81.35 (C-4); 84.49 (C-5); 99.33 (C-NBD); 126.96, 128.19, 128.68, 128.80, 130.16 (C aromatic); 129.49 (OBz-C-1); 133.70 (OBz-C-4); 136.25 (C-11); 136.78, 138.28, 143.95 (C-NBD); 137.86 (C_6H_5-C-1); 144.73 (C-12); 166.80 (CO-OBz); 170.58 (CO-OAc); 173.05 (3'NH-COR and C-1'); 211.15 (C-9); MS-FAB$^+$ m/z 964 [MNa]$^+$; UV: 463(24,050), 330(9800), 228(37,450).

Fluorescence study in vitro

Concentrations of **11**, **16a** and **b** varying from 0.2 to 0.5 μM were added to solutions of MES buffer, bovine serum albumin (2–5 mg mL^{-1}) or pure tubulin(1.5–5 mg mL^{-1}) previously stabilized at 37 °C. Excitation–emission spectra were obtained in 1 cm light-path cells thermostated at 37 °C. For compounds **11** and **16a**, after excitation at 467 nm, emission was recorded at 520 nm and for compound **16b**, the excitation wavelength was 463 nm and the emission recorded at 525 nm. For the experiments on fluorescence transfer, 290 nm was chosen for the excitation wavelength of microtubular proteins.

Biological studies

Microtubular proteins assay

The measure of the concentration that inhibited the *in vitro* disassembly of microtubular proteins (ID_{50}) was determined as previously described.[27] The values were reported to that of paclitaxel measured the same day under the same conditions.

HABA titration curves

The titrations were performed at 500 nm, by the successive additions of 10 μL of biotin (100 μM in H_2O) or 1 μL of biotinyl derivative of docetaxel (1 mM in EtOH) to 1 mL of avidin (3.03 μM) and HABA (2-(4'-hydroxyazobenzene)benzoic acid, 206 μM) solution in 0.2 M NH_4OAc, 0.2% NaN_3(w/v), pH 7.0. The absorbancies, corrected for dilution, were plotted versus the amount of the added compound.

Activity on mammalian cells

PtK2 cells (*Potorous tridactylis* kidney cells) were treated at 37 °C for 6 h in presence of various concentrations of paclitaxel, docetaxel and derivatives dissolved in DMSO (final concentration 1%). The cells were fixed for 0.5 h by 3% v/v formaldehyde and processed for immunolabeling: microtubules were stained with anti-tubulin antibody YL 1/2 (specific for tyrosinated α-tubulin). Then the cells were observed by epifluorescence microscopy.

Intracellular microtubule labeling

PtK2 cells were treated with 10 μM **11** and **16a** for 6 h. Epifluorescence images of living cells were digitalized (100 frame averaging) with an image processing system (Sapphire from Quantel) and recalculated using the histogram program. Alternatively, the cells were permeabilized in PIPES buffer containing 4% polyethylene glycol 6000 and 0.5% Triton X-100. After several washes in the same medium containing polyethylene glycol, cells were fixed in 3% formaldehyde in the same buffer containing 1% DMSO, washed and incubated for 1 h in the presence of 10 μM **11** and **16a**. Images were obtained as described above.

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