Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 1551–1556

BIOORGANIC & MEDICINAL CHEMISTRY

Semi-Synthesis of an *O*-Glycosylated Docetaxel Analogue

Anastasia Nikolakakis,[a] Khadidja Haidara,[a] Françoise Sauriol,[b] Orval Mamer[c] and Lolita O. Zamir[a,*]

[a]*Human Health Research Center, INRS-Institut Armand-Frappier, Université du Québec, 531 Boulevard des Prairies, Laval, Québec, Canada H7V 1B7*

[b]*Department of Chemistry, Queen's University, Kingston, Ontario, Canada K7L 3N6*

[c]*Biomedical Mass Spectrometry Unit, McGill University, 1130 Pine Avenue west, Montreal, Québec, Canada H3A 1A3*

Received 21 June 2002; accepted 15 October 2002

Abstract—A 7β-*O*-glycosylated docetaxel analogue was semi-synthesized from 9-dihydro-13-acetylbaccatin III, the most abundant taxane isolated from the needles of *Taxus canadensis*. It was shown to be more bioactive than paclitaxel according to the tubulin assay. It had a reduced potency in the MCF7 cell line cytotoxicity assay compared to paclitaxel, but it demonstrated better activity against the drug resistant cell line MCF7-ADR. In addition, the presence of one sugar moiety on C-7 doubled the water solubility versus that of paclitaxel.

Introduction

Paclitaxel (Taxol®) **1** can be considered as the anti-cancer wonder drug of the 1990s. It was approved by the Food and Drug Administration for the treatment of drug resistant ovarian cancer in 1992 and for the treatment of breast cancer in 1994. It is presently used also in the treatment of non-small cell lung cancer, cancer of the head and neck region, malignant melanoma and lymphomas.[1] One of the major drawbacks of this drug is its extremely low aqueous solubility due to its hydrophobic backbone as well as its side chain.[2] It is presently introduced intravenously in a solution containing cremophor. There is data from several sources that indicate that the cremophor may be responsible for the anaphylactic reactions observed in the early days of the development of paclitaxel.[3,4] A soluble paclitaxel analogue facilitating its bioavailability would therefore be a noticeable improvement. The Canadian yew is a small rampant shrub which contains specific taxanes in its needles.[5–14] Its most abundant taxane 9-dihydro-13-acetylbaccatin III **2** (5–7 times the amount of paclitaxel)[5,6,15] is only found as traces in one other yew *Taxus chinensis*.[16] This metabolite has been successfully converted to paclitaxel thereby increasing the yield of paclitaxel obtained from the needles.[17] Several taxane glycoconjugates have been isolated as natural products albeit in very small yields.[18] These compounds are paclitaxel analogues which contain a xylose moiety predominantly on the C7-OH. Sénilh et al. have isolated 7β-xylosyl-10-deacetyltaxol **3**, 7β-xylosyl-10-deacetyltaxol C **4** and 7β-xylosyl-10-deacetylcephalomannine **5** from the bark of *T. baccata* (Fig. 1).[19] We recently found in the rooted cuttings of *Taxus cuspidata* an *O*-glycosylated bioactive taxane.[20]

In this work, we are reporting for the first time the semi-synthesis of an *O*-glycosylated taxane: 7-(β-D-glucopyranosyloxy)-9-dihydro-10-acetyldocetaxel. This taxane was more soluble than paclitaxel. Its bioactivities towards both the tubulin and cytotoxicity assays against breast cancer cell lines MCF7 and MCF7-ADR (adriamycin resistant) will be discussed.

Results and Discussion

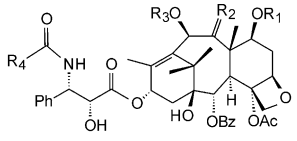
Semi-synthesis of 7-(β-D-glucopyranosyloxy)-9-dihydro-10-acetyldocetaxel, 15 (Scheme 1)

Our objective was to synthesize taxane glycoconjugates, similar to those found in nature, which maintained antitumor efficacy but which would be more soluble in water than paclitaxel. Mandai et al. have successfully

*Corresponding author. Tel.: +1-450-687-5010 (4260); fax: (office) +1-450-686-5301, (residence) +1-514-481-2797; e-mail: lolita.zamir@inrs-iaf.uquebec.ca

0968-0896/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
PII: S0968-0896(02)00607-7

attached a sugar moiety to a hydroxyl group at C-7 or C-10 of paclitaxel analogues **6** (Fig. 1) by means of an ester linkage.[21] Direct glycosylation was not attempted because they anticipated core skeletal rearrangements and oxirane cleavage brought on by Lewis acids necessary to achieve conventional glycosylation. Since 1992, we have been working with the Canadian yew. Consequently, we have access in our laboratory to a large amount of 9-dihydro-13-acetylbaccatin III **2**, its most abundant taxane. Since the discovery of **2** researchers have focussed their attention on this new 9-dihydro template. Klein and coworkers prepared 9-dihydrotaxol **7**, from **2**, which exhibited in vitro activity similar to that of Taxol®.[22] In the Taxol® series the replacement of the 3′-*N* benzoyl group with a *t*-butoxycarbonyl group substantially increased the tubulin activity and the in vitro cytotoxicity of docetaxel **8**, the 10-deacetyl-3′-*N*-*t*-butoxycarbonyl analogue of Taxol®.[23] Similarly, structure–activity relationship (SAR) studies of the 3′-*N*-acyl component in the 9-dihydro series have identified 9-dihydro-10-acetyldocetaxel **9** with the optimum activity.[24] Therefore, we used 9-dihydro-13-acetylbaccatin III **2** as the starting material for the semi-synthesis of a new substrate with a glucose molecule on the C7-OH and with a docetaxel side chain on the C13-OH.



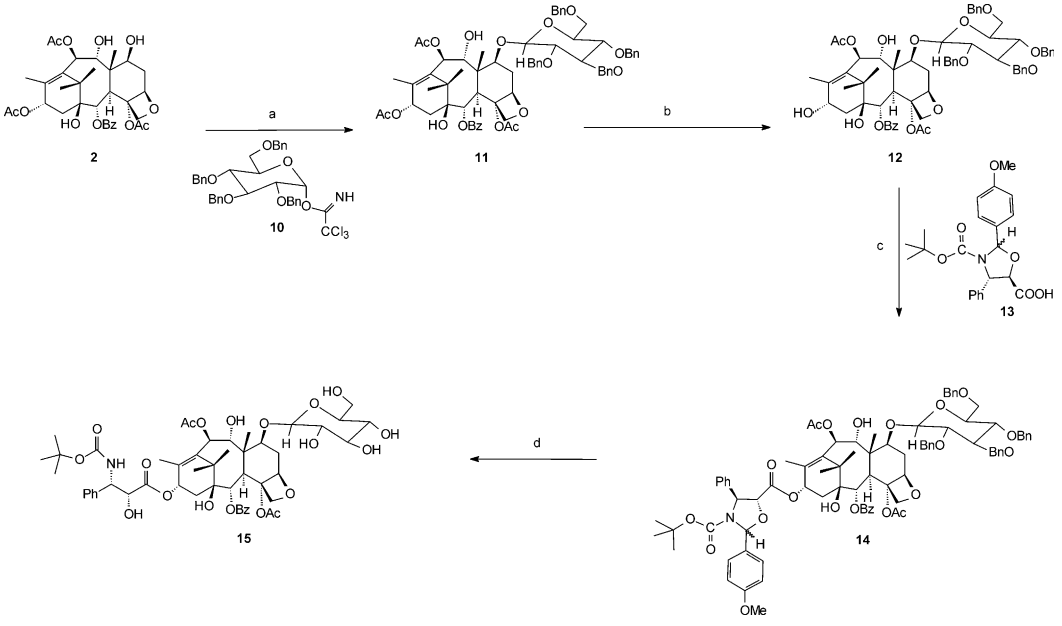
1 R_1 = H R_2 = O R_3 = Ac R_4 = C_6H_5
3 R_1 = xylosyl R_2 = O R_3 = H R_4 = C_6H_5
4 R_1 = xylosyl R_2 = O R_3 = H R_4 = C_5H_{11}
5 R_1 = xylosyl R_2 = O R_3 = H R_4 = $CH_3CH{=}CCH_3$
6 R_1 = $C(O)CH_2O$-glycosyl R_2 = O R_3 = Ac R_4 = C_6H_5
7 R_1 = H R_2 = α-OH R_3 = Ac R_4 = C_6H_5
8 R_1 = H R_2 = O R_3 = H R_4 = $OC(CH_3)_3$
9 R_1 = H R_2 = α-OH R_3 = Ac R_4 = $OC(CH_3)_3$

Figure 1. Structures of taxanes.

Spurred by the synthesis of glucoconjugates of anti-oxidative and anti-inflammatory drugs,[25] we attempted direct glycosylation on 9-dihydro-13-acetylbaccatin III **2**. The *O*-glycoside **11** was successfully synthesized using benzylated α-D-glucopyranosyl trichloracetimidate **10** as glycosyl donor.[26] This method employed catalysis by $BF_3{\cdot}OEt_2$, a Lewis acid, under mild conditions. None of the feared skeletal rearrangements was observed. Reductive cleavage of the 13-OAc of **11** with $NaBH_4$ gave intermediate **12**.[27] Esterification of the free C13-OH was realized with 2-(4-OMe)phenyl-1,3-oxazolidine of *N*-Boc phenylisoserine **13**,[28] dicyclohexylcarbodiimide and 4-pyrrolidinopyridine, in dichloromethane-toluene affording diastereomers **14**. Deprotection of the benzyl groups of the β-D-glucopyranosyl of **14** was performed by hydrogenation with palladium on charcoal. Without further purification the side-chain protecting group was removed by a catalytic amount of *p*-toluenesulfonic acid in methanol affording compound **15**. This first new docetaxel analogue **15** containing an *O*-β-glucosidic moiety was characterized by NMR and mass spectrometry.

Bioactivity of taxane 15 (Table 1, Fig. 2)

Paclitaxel and docetaxel are particularly known because of their unusual activity of promoting the assembly of tubulin to form super-stable microtubules. This unique mode of action was first discovered in the laboratories of Professor Horwitz.[29,30] This property was directly correlated to their anti-cancer activity.[31,32] Tubulin polymerization and cytotoxicity activities were therefore investigated for taxane **15** compared to the tubulin activity of Taxol® **1**, docetaxel **8** and 9-dihydro-10-acetyldocetaxel **9** (Fig. 2). Indeed, we can see that



Scheme 1. Reagents and conditions: (a) **10**, $BF_3{\cdot}OEt_2$, molecular sieves 4 Å, CH_2Cl_2, 4 °C, 1 h (43% based on recovered **2**); (b) $NaBH_4$, 2:1, THF/0.05 M KPO_4 buffer, pH 7, 23 °C, 6 h, 4 °C, 18 h (64%); (c) **13**, 4-PP, DCC, 2:1, toluene/CH_2Cl_2, 75 °C, 3.5 h (93%); (d) (i) 10% Pd/C, H_2, 1:2, acetone/methanol, 23 °C, 18 h; (ii) PTSA, MeOH, 23 °C, 5 h (38% overall yield for i and ii).

Taxol® induced the initial rate of tubulin polymerization by three times compared to the control, whereas docetaxel induced this initial rate by close to 4 times which was consistent with the literature.[23] Taxanes **9** and **15** both showed a tubulin activity comparable to that of docetaxel. Taxane **15** differed from **9** by the sugar on the C7-OH, therefore we can conclude that the sugar moiety did not contribute to the increased tubulin activity but it was due to the presence of the *t*-butoxy group similar to the effect observed between Taxol® and docetaxel. We tested the in vitro cytotoxicity of taxane **15** against breast cancer cell lines MCF7 and MCF7-ADR and compared it to the cytotoxicities of Taxol® **1**, docetaxel **8** and 9-dihydro-10-acetyldocetaxel **9** (Table 1). The IC_{50} (concentration killing 50% of cells) of compounds **8**, **9**, and **15**, which all contained the *t*-butoxy side-chain, were comparable around the 50 nM range for the MCF7-wild type cell line. However, paclitaxel was a more potent compound as its IC_{50} was in the 2 nM range, whereas at this concentration taxanes **8**, **9**, and **15** did not initiate cell death. Despite these results our new glycosyl analogue may yet prove to be an interesting therapeutic agent because it presented a certain cytotoxicity against the resistant cell line MCF7-ADR. The cytotoxicities of the two 9-dihydro analogues, **9**, which contained no glycosyl group and **15**, our synthetic glycosyl analogue had comparable IC_{50}'s in the 12–15 nM range. Once again the sugar moiety did not contribute to any difference in the activity. On the other hand, these 9-dihydro compounds were more potent than docetaxel which had an IC_{50} of 40 nM and much more potent than Taxol® which had an IC_{50} greater than 5 μM. The 9-dihydro modification contributed to a decrease in the resistance of these compounds to the MCF7-ADR cell line. Compounds **9** and **15** had similar tubulin activity and cytotoxicities. Therefore the sugar moiety did not influence these activities neither positively or negatively. In addition, we tested qualitatively the water solubility of taxane **15** according to literature procedure[33] and were encouraged that it was at least twice as soluble as Taxol®.

Table 1. Cytotoxicity of 7-(β-D-glucopyranosyloxy)-9-dihydro-10-acetyldocetaxel **15**

Compd	Tumor cell cytotoxicity IC_{50} (nM)[a]	
	MCF7-wild type	MCF7-ADR
Taxol® **1**	2 ± 1.52	> 5[b]
Docetaxel **8**	50 ± 3.85	40 ± 3.64
9	50 ± 2.67	12 ± 2.60
15	70 ± 1.52	15 ± 3.64

[a]Cytotoxicity of taxanes on the breast cancer cell lines MCF7-wild type and MCF7-ADR (adriamycin resistant). Exponentially growing cells were continuously exposed to taxanes at varying concentrations, and cell viability was evaluated by the MTT metabolic assay. Data represents mean ±SEM of three separate experiments. IC_{50} is described as the concentration of agent required to inhibit cell proliferation to 50% versus untreated cells.
[b]Units in μM.

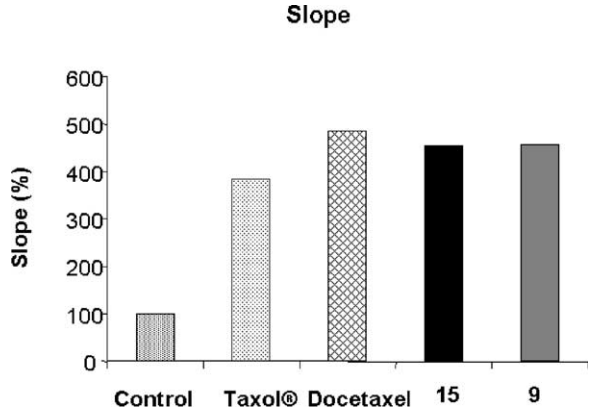


Figure 2. Effect of 7-(β-D-glucopyranosyloxy)-9-dihydro-10-acetyldocetaxel **15** on tubulin assembly. Microtubule turbidity was evaluated at 340 nm by incubating tubulin (1 mg/mL) at 37 °C in the presence or absence of taxanes. The slope percentage compared to control is depicted. The initial rate of tubulin polymerization was determined by initial slope detected during the first 3 min of tubulin assembly. This data represents mean ±SEM (standard error mean) for separate experiments.

Conclusion

In semi-synthesizing taxane **15** we showed that the glycosylation method using the benzylated α-D-glucopyranosyl trichloracetimidate **10** as glycosyl donor was indeed effective without the expected rearrangements due to the use of the acidic catalyst $BF_3{\cdot}OEt_2$. Our objective was to create a compound that contained more hydrophilic groups and at the same time which would not lose any biological activity. Taxane **15** is an interesting candidate since by the addition of a sugar moiety it proved to be twice as soluble as Taxol®. Although it was less potent in the MCF7 cytotoxicity assay compared to Taxol®, it exhibited similar activity as docetaxel and compound **9**. On the other hand, compound **15**, as well as docetaxel and compound **9** exhibited a higher activity against the adriamycin resistant MCF7-ADR cell line compared to Taxol®. We also showed that similarly to the Taxol® series, the increase in the rate of tubulin polymerization and cytotoxicity activity against the adriamycin resistant MCF7-ADR cell line was due to the *t*-butoxy side chain and not to the sugar moiety. These results are encouraging. The fact that many *O*-glycoconjugates of taxanes exist in nature leads us to believe that we are on the right path. The sugar moiety will be further investigated in our SAR study of taxanes.

Experimental

General methods

The instrumentation and general procedures used were as previously described.[17]

7-(2′,3′,4′,6′-Tetra-*O*-benzyl-β-D-glucopyranosyloxy)-9-dihydro-13-acetylbaccatin III 11. A solution of 9-dihydro-13-acetylbaccatin III **2** (0.102 g, 0.162 mmol) and benzylated α-D-glucopyranosyl trichloracetimidate **10** (0.121 g, 0.177 mmol) in CH_2Cl_2 (5 mL) was cooled at 4 °C and treated with molecular sieves 4 Å for 15 min. A

solution of $BF_3{\cdot}OEt_2$ (4 μL, 0.032 mmol) in CH_2Cl_2 (40 μL) was added and the mixture was stirred at 4 °C for 1 h. The mixture was separated from the molecular sieves. After dilution with chloroform, the mixture was washed with aqueous $NaHCO_3$ to pH 8 and then with brine to neutrality. The organic phase was dried, filtered and evaporated. The residue was purified by chromatography on silica gel (gradient of EtOAc/hexane, 30:70 to 40:60) affording compound **11** (0.065 g, 43% based on recovered **2**). ^{1}H NMR of **11** (500 MHz, $CDCl_3$) δ 8.08 (d, J = 7.7 Hz, 2H, H-2,6 of Bz), 7.59 (t, J = 7.5 Hz, 1H, H-4 of Bz), 7.46 (t, J = 7.7 Hz, 2H, H-3,5 of Bz), 7.33–7.14 (m, 20H, Ph(Bn)), 6.15 (o.d, J = 11.0 Hz, 1H, H-10), 6.14 (o.m, 1H, H-13), 5.74 (d, J = 6.0 Hz, 1H, H-2), 4.91 (o.d, J = 10.0 Hz, 1H, H-5), 4.88 (d, J = 11.9 Hz, 1H, Bn-2a), 4.87 (o.d, J = 7.0 Hz, 1H, GL-H-1), 4.78 (o.d, 1H, Bn-2b), 4.75 (o.d, J = 11.2 Hz, 2H, Bn-3a/b), 4.75 (o.d, 1H, Bn-4a), 4.61 (d, J = 12.2 Hz, 1H, Bn-6a), 4.56 (o.d, J = 11.2 Hz, 1H, Bn-4b), 4.54 (o.d, J = 12.2 Hz, 1H, Bn-6b), 4.44 (d, J = 11.4 Hz, 1H, OH-9), 4.34 (o.dd, J = 9.5, 7.7 Hz, 1H, H-7), 4.31 (o.m, 1H, H-9), 4.29 (o.d, J = 8.5 Hz, 1H, H-20a), 4.15 (d, J = 8.3 Hz, 1H, H-20b), 3.84 (t, J = 8.5 Hz, 1H, GL-H-3), 3.73 (o.m, 2H, GL-H-6), 3.70 (o.t, J = 9.0 Hz, 1H, GL-H-4), 3.60 (d.t, J = 9.8, 2.8 Hz, 1H, GL-H-5), 3.46 (t, J = 7.6 Hz, 1H, GL-H-2), 3.07 (d, J = 5.8 Hz, 1H, H-3), 2.79 (ddd, J = 15.5, 9.0, 7.5 Hz, 1H, H-6a), 2.23 (s, 3H, Ac), 2.18 (d, J = 8.5 Hz, 1H, H-14a), 2.15 (s, 3H, Ac), 2.08 (dd, J = 15.4, 10.1 Hz, 1H, H-6b), 1.98 (s, 3H, Ac), 1.93 (s, 3H, H-18), 1.85 (s, 3H, H-19), 1.69 (s, 3H, H-17), 1.67 (s, 1H, OH-1), 1.24 (s, 3H, H-16); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2 (CO-Ac), 169.8 (CO-Ac), 168.8 (CO-Ac), 166.8 (CO-Bz), 138.0 (C-12), 138.0 (C-1 of Ph(Bn)), 135.7 (C-11), 133.6 (C-4 of Bz), 130.0 (C-2,6 of Bz), 128.5 (C-3,5 of Bz), 128.2 (C-2,6 of Ph(Bn)), 127.2 (C-4 of Ph(Bn)), 102.2 (GL-1), 84.7 (GL-3), 84.2 (C-5), 82.7 (C-7), 81.7 (C-4), 80.1 (GL-2), 78.6 (C-1), 77.9 (GL-4), 76.3 (C-20), 75.5 (C-9), 75.1 (GL-5), 74.9 (Bn-3), 74.6 (Bn-4), 74.1 (Bn-2), 73.3 (Bn-6), 73.3 (C-2), 72.0 (C-10), 69.6 (C-13), 68.6 (GL-6), 47.5 (C-3), 45.4 (C-8), 42.8 (C-15), 37.2 (C-6), 35.2 (C-14), 28.2 (C-16), 22.8 (Ac), 22.4 (C-17), 21.2 (Ac), 21.1 (Ac), 14.7 (C-18), 13.1 (C-19); FAB-HR-MS m/z: 1285.4141 (calculated for $C_{67}H_{76}O_{17}Cs$, 1285.4137).

7-(2′,3′,4′,6′-Tetra-*O*-benzyl-β-D-glucopyranosyloxy)-9-dihydrobaccatin III 12. A solution of glucoside **11** (0.065 g, 0.056 mmol) in THF/0.05 M KPO_4, pH 7.0 buffer (2.1 mL/1.05 mL) was treated with $NaBH_4$ (3 × 8.6 mg, 0.23 mmol) at 23 °C over a period of 6 h and at 4 °C for 18 h. The reaction was quenched with acetone and brine. The mixture was extracted with EtOAc and the combined organic layers were dried, filtered and evaporated. The residue was purified by chromatography on silica gel (EtOAc/hexane, 40:60) affording compound **12** (0.040 g, 64%). ^{1}H NMR of **12** (500 MHz, $CDCl_3$) δ 8.10 (d, J = 7.9 Hz, 2H, H-2,6 of Bz), 7.61 (t, J = 7.3 Hz, 1H, H-4 of Bz), 7.59 (t, J = 7.7 Hz, 2H, H-3,5 of Bz), 7.33–7.14 (m, 20H, Ph(Bn)), 6.12 (d, J = 11.1 Hz, 1H, H-10), 5.72 (d, J = 6.0 Hz, 1H, H-2), 4.89 (o.d, 1H, Bn-2a), 4.88 (o.d, 1H, H-5), 4.89 (o.d, 1H, GL-H-1), 4.80 (o.d, 1H, Bn-3a), 4.78 (o.d, 1H, Bn-4a), 4.78 (o.m, 1H, H-13), 4.78 (o.d, J = 11.4 Hz, 1H, Bn-2b), 4.74 (d, J = 11.3 Hz, 1H, Bn-3b), 4.61 (d, J = 12.4 Hz, 1H, Bn-6a), 4.56 (d, J = 11.2 Hz, 1H, Bn-4b), 4.53 (d, J = 12.4 Hz, 1H, Bn-6b), 4.39 (d, J = 11.4 Hz, 1H, OH-9), 4.34 (dd, J = 9.7, 7.6 Hz, 1H, H-7), 4.30 (o.m, 1H, H-9), 4.30 (o.d, 1H, H-20a), 4.16 (o.d, J = 8.5 Hz, 1H, H-20b), 3.85 (t, J = 8.6 Hz, 1H, GL-H-3), 3.73 (o.m, 2H, GL-H-6), 3.70 (o.t, J = 9.0 Hz, 1H, GL-H-4), 3.59 (d.t, J = 10.0, 3.0 Hz, 1H, GL-H-5), 3.45 (t, J = 8.1 Hz, 1H, GL-H-2), 3.12 (d, J = 6.0 Hz, 1H, H-3), 2.76 (ddd, J = 15.5, 9.0, 7.8 Hz, 1H, H-6a), 2.26 (o.m, 1H, H-14a), 2.23 (s, 3H, Ac), 2.08 (br.s, 3H, H-18), 2.07 (o.m, 1H, H-14b), 2.07 (o.m, 1H, H-6b), 1.99 (s, 3H, Ac), 1.84 (s, 3H, H-19), 1.65 (s, 3H, H-17), 1.64 (s, 1H, OH-1), 1.09 (s, 3H, H-16); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.5 (CO-Ac), 169.9 (CO-Ac), 166.9 (CO-Bz), 141.1(C-12), 138.0 (C-1 of Ph(Bn)), 135.3 (C-11), 133.5 (C-4 of Bz), 130.0 (C-2,6 of Bz), 128.5 (C-3,5 of Bz), 128.2 (C-2,6 of Ph(Bn)), 127.2 (C-4 of Ph(Bn)), 102.1 (GL-1), 84.7 (GL-3), 84.4 (C-5), 82.3 (C-7), 82.1 (C-4), 80.3 (GL-2), 78.6 (C-1), 77.9 (GL-4), 76.3 (C-20), 75.8 (C-9), 75.1 (GL-5), 74.8 (Bn-3), 74.5 (Bn-4), 74.1 (Bn-2), 73.2 (Bn-6), 73.0 (C-2), 72.5 (C-10), 68.5 (C-13), 68.5 (GL-6), 47.4 (C-3), 45.4 (C-8), 42.6 (C-15), 38.6 (C-14), 37.2 (C-6), 28.2 (C-16), 22.9 (Ac), 21.1 (Ac), 22.0 (C-17), 14.9 (C-18), 13.0 (C-19); FAB-HR-MS m/z: 1243.4033 (calculated for $C_{65}H_{74}O_{16}Cs$, 1243.4031).

7-(2′,3′,4′,6′-Tetra-*O*-benzyl-β-D-glucopyranosyloxy)-9-dihydrobaccatin III-13-(4S,5R)-*N*-(*t*-butyloxycarbonyl)-2-(4-methoxyphenyl)-4-phenyl-5-oxazolidinecarboxylic acid ester 14. Glucoside **12** (0.037 g, 0.033 mmol) in toluene (0.6 mL) was treated with (4*S*,5*R*)-*N*-(*t*-butyloxycarbonyl)-2-(4-methoxyphenyl)-4-phenyl-5-oxazolidinecarboxylic acid (**13**), (0.025 g, 0.063 mmol) in 0.3 mL CH_2Cl_2, 4-pyrrolidinopyridine (3 mg, 0.020 mmol) and DCC (0.014 g, 0.068 mmol) at 75 °C for 3.5 h. The reaction mixture was diluted with ethyl acetate, washed with 5% $NaHSO_4$ to pH 1, 5% $NaHCO_3$ to pH 8 and then with brine to neutrality. The organic layer was dried, filtered and evaporated. The residue was purified by flash chromatography on silica gel with EtOAc/hexane, 40:60 as eluent to give a mixture of diastereomers **14** (0.046 g, 93%). ^{1}H NMR of **14** (500 MHz, $CDCl_3$) δ 8.06 (d, J = 7.5 Hz, 2H, H-2,6 of Bz), 7.61 (t, J = 7.5 Hz, 1H, H-4 of Bz), 7.47 (t, J = 7.8 Hz, 2H, H-3,5 of Bz), 7.38 (o.m, 2H, H-2,6 of *p*-(OMe)Ph), 7.33–7.14 (m, 20H, Ph(Bn)), 6.88 (d, J = 8.8 Hz, 2H, H-3,5 of *p*-(OMe)Ph), 6.36 (v.br, 1H, H-5′), 6.06 (d, J = 11.3 Hz, 1H, H-10), 6.00 (br, 1H, H-13), 5.68 (d, J = 6.0 Hz, 1H, H-2), 5.40 (br, 1H, H-3′), 4.86 (o.d, 1H, Bn-2a), 4.81 (br.d, 1H, GL-H-1), 4.77 (o.m, 1H, H-5), 4.77 (o.d, 1H, Bn-3a), 4.76 (o.d, 1H, Bn-2b), 4.75 (o.d, 1H, Bn-4a), 4.72 (o.d, 1H, Bn-3b), 4.60 (o.d, 1H, Bn-6a), 4.56 (o.d, 1H, Bn-4b), 4.55 (o.m, 1H, H-2′), 4.51 (o.d, 1H, Bn-6b), 4.42 (d, J = 11.3 Hz, 1H, OH-9), 4.26 (o.m, 1H, H-7), 4.26 (o.m, 1H, H-9), 4.20 (d, J = 8.5 Hz, 1H, H-20a), 4.08 (d, J = 8.5 Hz, 1H, H-20b), 3.81 (o.m, 1H, GL-H-3), 3.75 (o.m, 2H, GL-H-6), 3.75 (o.br.s, 3H, CH_3 of *p*-(OMe)Ph), 3.72 (o.m, 1H, GL-H-4), 3.56 (br.d, J = 9.7 Hz, 1H, GL-H-5), 3.43 (t, J = 8.0 Hz, 1H, GL-H-2), 2.95 (d, J = 6.0 Hz, 1H, H-3), 2.71 (m, 1H, H-6a), 2.17 (o.m, 1H, H-14a), 2.03 (o.m, 1H, H-14b), 2.02 (o.m, 1H, H-6b), 1.96 (s, 3H, Ac), 1.79 (s, 3H, H-19),

1.67 (s, 3H, H-17), 1.66 (br.s, 3H, Ac), 1.66 (s, 1H, OH-1), 1.63 (br.s, 3H, H-18), 1.22 (s, 3H, H-16), 1.04 (s, 9H, *t*-Bu); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.6 (CO-Ac), 166.7 (CO-Bz), 160.1 (C-4 of *p*-(OMe)Ph), 137.6 (C-12), 135.4 (C-11), 133.7 (C-4 of Bz), 129.9 (C-2,6 of Bz), 128.5 (C-3,5 of Bz), 126.3 (C-2,6 of *p*-(OMe)Ph), 113.7 (C-3,5 of *p*-(OMe)Ph), 102.1 (GL-1), 92.6 (C-5′), 84.7 (GL-3), 84.3 (C-5), 83.4 (C-2′), 82.7 (C-7), 81.3 (C-4), 80.5 (C-*t*-Bu), 80.3 (GL-2), 78.9 (C-1), 77.9 (GL-4), 76.2 (C-20), 75.6 (C-9), 75.2 (GL-5), 74.8 (Bn-3), 74.7 (Bn-4), 74.1 (Bn-2), 73.4 (Bn-6), 73.4 (C-2), 72.0 (C-13), 71.8 (C-10), 68.6 (GL-6), 63.4 (C-3′), 55.3 (CH_3 of *p*-(OMe)Ph), 47.5 (C-3), 45.2 (C-8), 42.9 (C-15), 37.1 (C-6), 35.2 (C-14), 28.3 (C-16), 27.8 (CH_3-*t*-Bu), 22.8 (C-17), 21.8 (Ac), 21.0 (Ac), 14.1 (C-18), 12.9 (C-19); FAB-HR-MS *m*/*z*: 1243.4033 (calculated for $C_{65}H_{74}O_{16}Cs$, 1243.4031). Electron Spray-MS (FAB-HR-MS could not be run since compound **14** was too high in mass): $C_{87}H_{97}N_1O_{21}$ at *m*/*z* 1491.7; $[M+H]^+$ at *m*/*z* 1492.8; $[M+Na]^+$ at *m*/*z* 1548.8 and $[M+Cs]^+$ at *m*/*z* 1624.8.

7-(β-D-Glucopyranosyloxy)-9-dihydro-10-acetyldocetaxel 15. The N-protected phenylisoserinate **14** (0.023 g, 0.015 mmol) in MeOH/acetone, 2:1, (3 mL) was treated with 10% Pd/C (3 mg) and the solution was stirred under a hydrogen atmosphere at 23 °C for 18 h. The mixture was filtered through kimwipes, rinsed with acetone and evaporated to dryness. The crude residue was diluted in MeOH (3.0 mL) and was treated with *p*-toluenesulfonic acid monohydrate (3 mg, 0.016 mmol) at 23 °C for 5 h. The methanol was evaporated and the residue was diluted with EtOAc and washed with brine to neutrality. The organic layer was dried, filtered and evaporated. The residue was purified by flash chromatography on silica gel with $MeOH/CH_2Cl_2$, 10:90 as eluent to give compound **15** (6 mg, 38% overall two steps). ^{1}H NMR of **15** (500 MHz, $CDCl_3$) δ 8.06 (d, $J=7.5$ Hz, 2H, H-2,6 of Bz), 7.61 (t, $J=7.5$ Hz, 1H, H-4 of Bz), 7.47 (t, $J=7.8$ Hz, 2H, H-3,5 of Bz), 7.40 (d, $J=7.5$ Hz, 2H, H-2,6 of Ph-3′), 7.36 (t, $J=7.8$ Hz, 2H, H-3,5 of Ph-3′), 7.30 (t, $J=7.2$ Hz, 1H, H-4 of Ph-3′), 6.28 (d, $J=10.5$ Hz, 1H, H-10), 6.08 (br.t, $J=8.8$ Hz, 1H, H-13), 5.75 (d, $J=6.0$ Hz, 1H, H-2), 5.57 (br.d, $J=9.8$ Hz, 1H, NH-4′), 5.26 (br.d, $J=9.8$ Hz, 1H, H-3′), 4.92 (d, $J=8.9$ Hz, 1H, H-5), 4.61 (br.s, 1H, H-2′), 4.45 (br.d, $J=7.9$ Hz, 1H, GL-H-1), 4.37 (br.t, $J=8.3$ Hz, 1H, H-7), 4.30 (o.d, $J=8.5$ Hz, 1H, H-20a), 4.28 (o.m, 1H, H-9), 4.19 (o.d, $J=8.5$ Hz, 1H, H-20b), 3.96 (br.s, 1H, OH-2′), 3.88 (br.d, $J=12.2$ Hz, 1H, GL-H-6a), 3.77 (br.d.m, 1H, GL-H-6b), 3.57 (o.m, 1H, GL-H-3), 3.56 (o.m, 1H, GL-H-4), 3.41 (br.s, 1H, GL-H-5), 3.33 (br.q, 1H, GL-H-2), 3.03 (d, $J=6.0$ Hz, 1H, H-3), 2.58 (br.m, 1H, H-6a), 2.38 (br.dd, $J=15.0$, 10.2 Hz, 1H, H-14a), 2.25 (br.s, 3H, Ac), 2.19 (o.m, 1H, H-6b), 2.17 (s, 3H, Ac), 2.08 (o.m, 1H, H-14b), 1.87 (s, 3H, H-19), 1.79 (br.s, 3H, H-18), 1.77 (s, 1H, OH-1), 1.70 (s, 3H, H-17), 1.39 (br.s, 9H, *t*-Bu), 1.26 (s, 3H, H-16); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4 (CO-Ac), 170.8 (CO-Ac), 166.7 (CO-Bz), 138.1 (C-12), 135.4 (C-11), 133.7 (C-4 of Bz), 129.9 (C-2,6 of Bz), 128.5 (C-3,5 of Ph-3′), 128.5 (C-3,5 of Bz), 127.8 (C-4 of Ph-3′), 126.8 (C-2,6 of Ph-3′), 102.8 (GL-1), 85.1 (C-7), 83.5 (C-5), 82.2 (C-4), 79.6 (C-*t*-Bu), 78.2 (C-1), 76.7 (GL-4), 76.6 (C-9), 76.6 (C-20), 75.6 (GL-5), 73.7 (C-2′), 73.6 (GL-2), 73.1 (C-10), 72.7 (C-2), 71.9 (C-13), 70.1 (GL-3), 62.5 (GL-6), 56.0 (C-3′), 47.8 (C-3), 45.6 (C-8), 43.0 (C-15), 35.4 (C-14), 35.3 (C-6), 28.2 (C-16), 28.2 (CH_3-*t*-Bu), 22.6 (Ac), 22.0 (C-17), 21.5 (Ac), 15.2 (C-18), 13.3 (C-19); FAB-HR-MS *m*/*z*: 1146.3308 (calculated for $C_{51}H_{67}N_1O_{20}Cs$, 1146.3311).

Tubulin assay

Tubulin was extracted from cow brain according to the method of Williams and Lee.[34] In vitro tubulin assembly was then evaluated by measuring microtubule turbidity at 340 nm. Briefly, tubulin at a final concentration of 1 mg/mL was incubated in PM-4M buffer (100 mM PIPES–NaOH, 2 mM EGTA, 1 mM $MgSO_4$, 4 M glycerol and 2 mM dithioerythritol (DTE), pH 6.9) at 37 °C, with or without the various taxanes at a concentration of 10 μM.

Bioactivity assay

In vitro cytotoxicity of taxane **15** was determined on breast adenocarcinoma cell line MCF7, using 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium (MTT) (Sigma, St. Louis, MO, USA) metabolic assay.[35] Briefly, exponentially growing cells were seeded in 96-well micro-plates (1×10^3 cells per 200 μL per well). Following 18 h, cells were continuously exposed to varying concentrations of taxane **15**. Cell survival was evaluated 96 h later by replacing the culture media with 50 μL of 2.5 mg/mL MTT, in phosphate buffer solution pH 7.5. After 4 h of incubation at 37 °C in the dark, MTT was removed and 100 μL of solubilization solution (10% Triton 100X, and 1 N HCl in anhydrous isopropanol) were added. The absorbance was determined at 570 nm with a microplate reader (Biorad).

Acknowledgements

We thank the Natural Science and Engineering Research Council of Canada and the Canadian Breast Cancer Research Initiative grant for support via operating grants to L.O.Z. We thank the pharmaceutical company Aventis Pharma for a generous gift of Taxotere® (docetaxel).

References and Notes

1. Cragg, G. M. *Med. Res. Rev.* **1998**, *18*, 315.
2. Suffness, M. *Ann. Rep. Med. Chem.* **1993**, *28*, 305.
3. Scialli, A. R.; DeSesso, J. M.; Rahman, A.; Husain, S. R.; Goeringer, G. C. *Pharmacology* **1995**, *51*, 145.
4. Mackie, F. E.; Umetsu, D.; Salvatierra, O.; Sarwal, M. M. *Pediatr. Transplant* **2000**, *4*, 35.
5. Zamir, L. O.; Nedea, M. E.; Belair, S.; Sauriol, F.; Mamer, O.; Jacqmain, E.; Jean, F. I.; Garneau, F.-X. *Tetrahedron Lett.* **1992**, *33*, 5173.
6. Zamir, L. O.; Nedea, M. E.; Zhou, Z.-H.; Belair, S.; Caron, G.; Sauriol, F.; Jacqmain, E.; Jean, F. I.; Garneau, F.-X.; Mamer, O. *Can. J. Chem.* **1995**, *73*, 655.
7. Zamir, L. O.; Zhou, Z.-H.; Caron, G.; Nedea, M. E.; Sauriol, F.; Mamer, O. *J. Chem. Soc., Chem. Comm.* **1995**, 529.

8. Zamir, L. O.; Nedea, M. E.; Zhou, Z.-H.; Caron, G.; Sauriol, F.; Mamer, O. *Phytochemistry* **1996**, *41*, 803.
9. Zamir, L. O.; Zhang, J.; Kutterer, K.; Sauriol, F.; Mamer, O.; Khiat, A.; Boulanger, Y. *Tetrahedron* **1998**, *54*, 15845.
10. Zamir, L. O.; Zhang, J.; Wu, J.-H.; Sauriol, F.; Mamer, O. *J. Nat. Prod.* **1999**, *62*, 1268.
11. Zamir, L. O.; Zhang, J.; Wu, J.-H.; Sauriol, F.; Mamer, O. *Tetrahedron* **1999**, *55*, 14323.
12. Zhang, J.; Sauriol, F.; Mamer, O.; Zamir, L. O. *Phytochemistry* **2000**, *54*, 221.
13. Zhang, J.; Sauriol, F.; Mamer, O.; Zamir, L. O. *J. Nat. Prod.* **2000**, *63*, 929.
14. Zhang, J.; Sauriol, F.; Mamer, O.; You, X-L.; Alaoui-Jamali, M. A.; Batist, G.; Zamir, L. O. *J. Nat. Prod.* **2001**, *64*, 450.
15. Gunawardana, G. P.; Premachandran, U.; Burres, N. S.; Whittern, D. N.; Henry, R.; Spanton, S.; McAlpine, J. B. *J. Nat. Prod.* **1992**, *55*, 1686.
16. Zhang, S.; Chen, W. M.; Chen, Y. H. *Yaoxue Xuebao* **1992**, *27*, 268.
17. Nikolakakis, A.; Caron, G.; Cherestes, A.; Sauriol, F.; Mamer, O.; Zamir, L. O. *Bioorg. Med. Chem.* **2000**, *8*, 1269.
18. Parmar, V. S.; Jha, A.; Bisht, K. S.; Taneja, P.; Singh, S. K.; Kumar, A.; Poonam; Jain, R.; Olsen, C. E. *Phytochemistry* **1999**, *50*, 1267.
19. Sénilh, V.; Blechert, S.; Colin, M.; Guénard, D.; Picot, F.; Potier, P.; Varenne, P. *J. Nat. Prod.* **1984**, *47*, 131.
20. Shi, Q.-W.; Sauriol, F.; Mamer, O.; Zamir, L. O. Unpublished results.
21. Mandai, T.; Okumoto, H.; Oshitari, T.; Nakanishi, K.; Mikuni, K.; Hara, K.; Hara, K.; Iwatani, W.; Amano, T.; Nakamura, K.; Tsuchiya, Y. *Heterocycles* **2001**, *54*, 561.
22. Klein, L. L. *Tetrahedron Lett.* **1993**, *34*, 2047.
23. Lavelle, F.; Guéritte-Voegelein, F.; Guénard, D. *Bull Cancer* **1993**, *80*, 326.
24. Maring, C. J.; Grampovnik, D. J.; Yeung, C. M.; Klein, L. L.; Li, L.; Thomas, S. A.; Plattner, J. *J. Bioorg. Med. Chem. Lett.* **1994**, *4*, 1429.
25. Uhrig, R. K.; Picard, M. A.; Beyreuther, K.; Wiessler, M. *Carbohydrate Res.* **2000**, *325*, 72.
26. Schmidt, R. R.; Stumpp, M. *Liebigs Ann. Chem.* **1983**, 1249.
27. Zamir, L. O.; Cherestes, A. D.; Nikolakakis, A.; Sauriol, F.; Mamer, O. *Tetrahedron Lett.* **1999**, *40*, 7917.
28. Didier, E.; Fouque, E.; Taillepied, I.; Commerçon, A. *Tetrahedron Lett.* **1994**, *35*, 2349.
29. Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665.
30. Schiff, P. B.; Horwitz, S. B. *Biochem.* **1981**, *20*, 3247.
31. Manfredi, J. J.; Horwitz, S. B. *Pharm. Therapeut.* **1984**, *25*, 83.
32. Ringel, I.; Horwitz, S. B. *J. Nat. Cancer Inst.* **1991**, *83*, 288.
33. Ali, S. M.; Hoemann, M. Z.; Aubé, J.; Mitscher, L. A.; Georg, G. I. *J. Med. Chem.* **1995**, *38*, 3821.
34. Williams, R. C., Jr.; Lee, J. C. *Methods in Enzymology* **1982**, *85*, 376.
35. Yen, L.; Nie, Z. R.; You, X. L.; Richard, S.; Langton-Webster, B. C.; Alaoui-Jamali, M. A. *Oncogene* **1997**, *14*, 1827.