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SYNTHESIS OF 19-HYDROXY DOCETAXEL FROM A NOVEL BACCATIN

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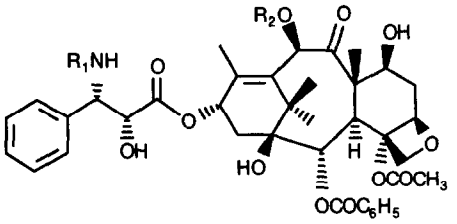
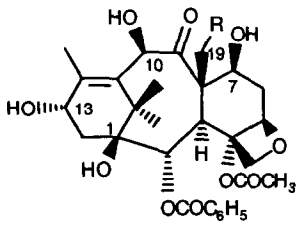
Abstract: The synthesis of 19-hydroxy docetaxel is described starting from a new baccatin derivative, **4**, which was extracted from the needles of *Taxus Baccata*. This analog exhibits a high level of cytotoxicity in *in vitro* experimental models.

Docetaxel (Taxotere®), **1**, is a promising anticancer agent currently under phase II clinical trials[1]. Docetaxel is prepared from 10-deacetyl baccatin III, **2**, which is extracted from the needles of *Taxus baccata*. This renewable source of **2** makes docetaxel more available than natural paclitaxel (Taxol®), **3**, which must be harvested from the bark.



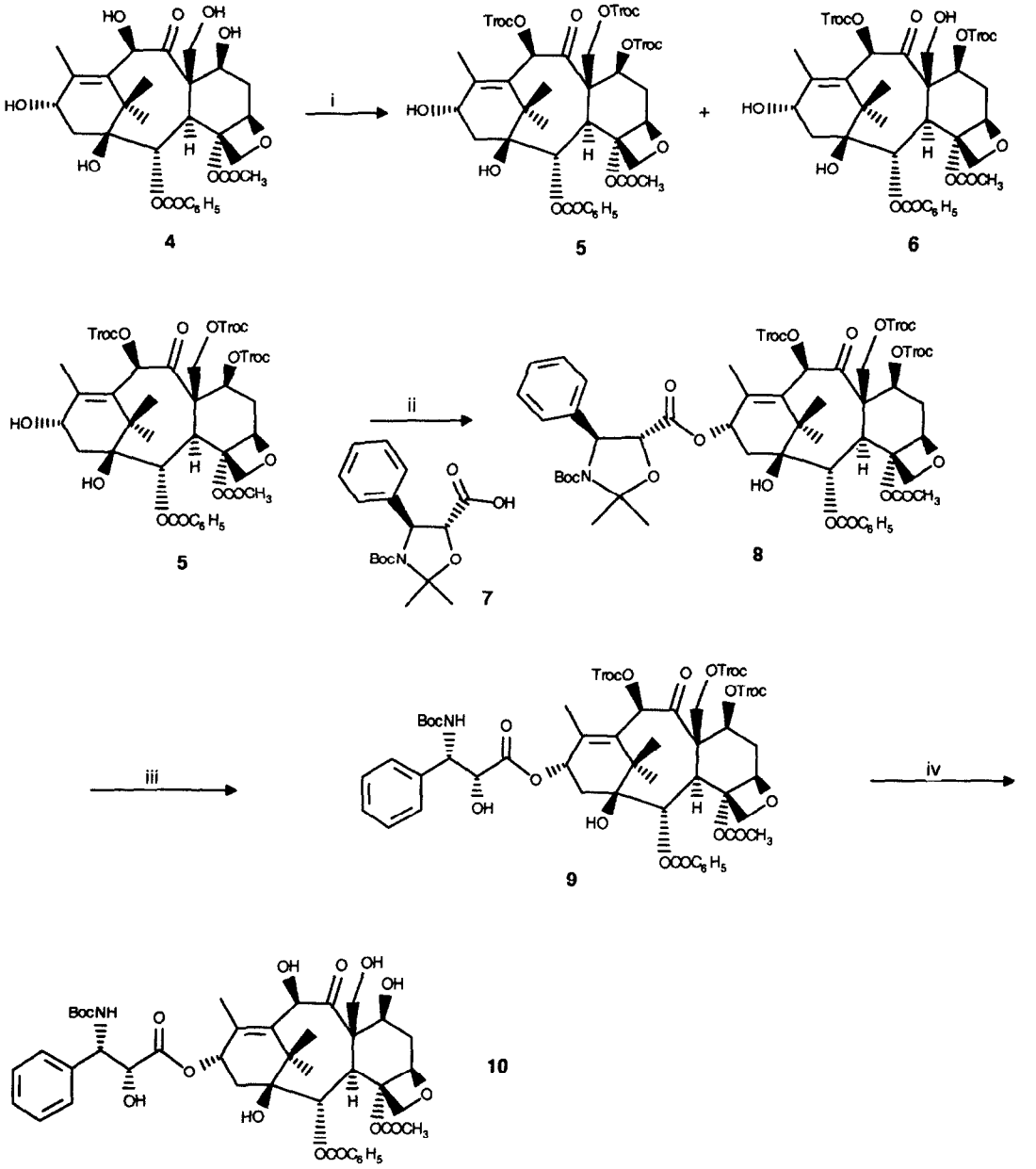
1, R_1 = tBuOCO, R_2 = H (docetaxel)
3, R_1 = C_6H_5CO, R_2 = Ac (paclitaxel)



2, R = H
4, R = OH

In our search for new biologically active taxoids and crucial information on structure-activity relationships, we looked for other minor baccatins besides the most abundant compound, **2**, in *Taxus baccata* needles. The most profuse congener, which cocrystallized with **2** with a relative abundance of a few percent (usually 3 to 5% although concentrations as high as 20% were found in some samples) was identified as 10-deacetyl-19-hydroxybaccatin III (**4**)[2,3]. This new baccatin derivative which probably originates from 10-deacetylbaccatin III through a further biosynthetic oxidation step, was isolated by preparative HPLC on a 75X25 cm Amicon C_{18} bonded 20 microns 100 Å phase column. For injection, 6.6 g of a crude extraction baccatin mixture was absorbed by vacuum evaporation on 75 g C_{18} Bondesil 40 microns silica gel and loaded in a 5x7 cm precolumn. Isocratic elution with MeOH/H_2O : 47/53 (v/v) afforded 0.08 g of 10-acetyl-19-hydroxybaccatin III, **4**.

Related 19-hydroxylated baccatins have been previously described in the literature as constituents of *Taxus baccata*[4] and other Taxus species such as *Taxus chinensis*[5], *Taxus yunnanensis*[6] and *Taxus wallichiana*[2].



Reagents: (i) CCl_3CH_2OCOCl (5eq.), C_5H_5N, 20°C, 3h. (ii) 7 (1.5eq.), DCC (1.6eq.), DMAP (0.5eq.), toluene, 80°C, 2h. (iii) a) HCOOH, 20°C, 4h., b) $(Boc)_2O$, CH_2Cl_2, $NaHCO_3$, 20°C, iv) Zn, AcOH, MeOH, 60°C, 1h.

This original structure prompted us to prepare the corresponding docetaxel analog. Because of the uncommon position of the hydroxyl group at C-19, we might expect better water solubility for this analog with possible favorable effect on the antitumoral activity spectrum.

The enantioselective access to the corresponding docetaxel analog was achieved by analogy to our previously published methodology[7]. The pentahydroxy compound **4**, was first O-tri-protected using 2,2,2-trichloroethoxycarbonyl chloride (TrocCl) in pyridine. Surprisingly, acylation of hydroxyl groups at C-7 and C-10 occurred spontaneously while acylation at C-19 was partial and difficult to complete even in the presence of a large excess of chloroformate in pyridine at reflux (64% of acylated products was obtained at room temperature for 3h. with a **5**:**6** ratio of 5:3). Although this O-tri-protection step has not yet been optimized, we were able to complete the synthesis without further difficulty. Esterification of the O-tri-protected baccatin derivative **5** using acid **7** gave under standard conditions ester **8** in excellent yield (98%). Deprotection of the oxazolidine-type protection under acidic conditions followed by N-acylation of the intermediate phenylisoserinate with di-tert-butyl dicarbonate afforded **9** in 60% overall yield. The final reductive deprotection was performed with zinc powder in acetic acid to give, after purification, 19-hydroxy-docetaxel, **10**, in 42% yield.

Compound **10** is less soluble than docetaxel in most of the organic solvents but, as expected, the additional hydroxyl function on the taxane skeleton improves the water solubility (measured 12 μg/ml for **10** versus 2 μg/ml for docetaxel after 1 h at room temperature).

This new taxoid was evaluated as an antitumor agent in experimental models. Different *in vitro* assays have been used to determine the activity of docetaxel and paclitaxel congeners on tubulin including inhibition of binding of labelled docetaxel or paclitaxel to microtubules[8], promotion of microtubule assembly in the absence of GTP[8] and inhibition of microtubule disassembly at 4°C[9]. The two last methods generally give the same results and the latter one was used here because of its rapidity. Compound **10** has been proven very active as an inhibitor of microtubule disassembly at 4°C [ID_{50}(**10**)/ID_{50}(paclitaxel) = 0.4, while ID_{50}(docetaxel)/ID_{50}(paclitaxel) = 0.6]; in addition this compound was cytotoxic against P388 leukemia cells (IC_{50} = 0.07 μg/ml).

These results demonstrate that chemical modifications at C-19 can be done without significant loss of biological activity. Compound **4** is currently used in our laboratory to obtain further information on structure activity-relationships.

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References and notes:

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2. Structural data for compound **4** can be compared to those reported for 19-hydroxybaccatin III, see: McLaughlin J.L., Miller R.W., Powell R.G., Smith C.R., Jr., *J. Nat. Prod.*, **1981**, *44*, 312.
3. All new compounds exhibited IR, ^1H and ^{13}C-NMR spectra, mass spectral and combustion data in agreement with the structures indicated.

4: mp: 194.7°C (AcOEt), $[\alpha]_D^{20}$ -58 (c 0.16, tetrahydrofuran), ^{1}H-NMR (400MHz; DMSO d6): δ 0.97 and 1.17 (two s, 6H, C-16H_3 and C-17H_3), 1.55 (bdd, 1H, C-6H), 1.90 (s, 3H, C-18H_3), 2.10 (m, 2H, C-14H_2), 2.25 (s and m, 4H, C-6H and $COCH_3$), 3.80 (d, 1H, J=7 Hz, C-3H), 4.08 (m, 2H, C-20H and C-7H), 4.35 (d, 1H, J=9 Hz, C-20H), 4.42 and 4,5 (ABX, 2H, C-19H_2OH), 4.65 (m, 2H, C-19H_2O$\underline{H}$ and C-13H), 4.95 (bd, 1H, C-5H), 5.07 (bs, 1H, C-10H), 6.5 (d, 1H, J=7 Hz, C-2H), 7.57, 7.68 and 8.08 (t, t and d, 2H, 1H and 2H, J=8 Hz, $OCOC_6H_5$).

5: foam, $[\alpha]_D^{20}$ -34 (c 0.47, MeOH), ^{1}H-NMR (300 MHz; $CDCl_3$); δ 1.15 and 1.29 (two s, 6H, C-16H_3 and C-17H_3), 1.87 (m, 1H, C-6H), 2.15-2.40 (m, 2H, C-14H_2), 2.19 and 2.35 (two s, 6H, C-18H_3 and $OCOCH_3$), 2.70 (m, 1H, C-6H), 4.10 (d, 1H, J=7 Hz, C-3H), 4.20 (d, 1H, J=8 Hz, C-20H), 4.42 (d, 1H, J=8 Hz, C-20H), 4.62 and 4.95 (2d, 1H each, J=12 Hz, OCOO-CH_2-CCl_3 at C-7), 4.75 and 5.03 (2d, 1H each, J=12 Hz, OCOO-CH_2-CCl_3 at C-19), 4.78 (limit ab, 2H, J=11 Hz, OCOO-CH_2-CCl_3 at C-10), 4.90 (m, 1H, C-13H), 5.03 (bd, 1H, J=10 Hz, C-5H), 5.46 (limit ab, 2H, C-19H_2), 5.66 (dd, 1H, J=11 and 8.5 Hz, C-7H), 6.28 (s, 1H, C-10H), 6.41 (d, 1H, J=7 Hz, C-2H), 7.49, 7.63 and 8.13 (t, t and d, 2H, 1H and 2H, J=7.5 Hz, $OCOC_6H_5$).

6: foam, $[\alpha]_D^{20}$ -29 (c 0.54, MeOH), ^{1}H-NMR (300 MHz; $CDCl_3$); δ 1.15 and 1.27 (two s, 6H, C-16H_3 and C-17H_3), 1.87 (m, 1H, C-6H), 2.15-2.35 (m, 2H, C-14H_2), 2.20 and 2.35 (two s, 6H, C-18H_3 and $OCOCH_3$), 2.63 (m, 1H, C-6H), 4.00 (d, 1H, J=7 Hz, C-3H), 4.38 (d, 1H, J=8 Hz, C-20H), 4.45 (d, 1H, J=8 Hz, C-20H), 4.65 and 4.95 (2d, 1H each, J=12 Hz, OCOO-CH_2-CCl_3 at C-7), 4.70-4.95 (m, 2H, C-19H_2), 4.82 (limit ab, 2H, OCOO-CH_2-CCl_3 at C-10), 4.90 (m, 1H, C-13H), 5.03 (bd, 1H, J=10 Hz, C-5H), 5.62 (dd, 1H, J=11 and 8.5 Hz, C-7H), 6.30 (s, 1H, C-10H), 6.64 (d, 1H, J=7 Hz, C-2H), 7.49, 7.63 and 8.15 (t, t and d, 2H, 1H and 2H, J=7.5 Hz, $OCOC_6H_5$).

10: foam, $[\alpha]_D^{20}$ -39 (c 0.52, MeOH), ^{1}H-NMR (400MHz; DMSO d6): δ 0.98 and 1.17 (two s, 6H, C-16H_3 and C-17H_3), 1.33 (s, 9H, tBu), 1.51 (dd, 1H, J=13 and 12 Hz, C-6H), 1.59 and 1.83 (two dd, 1H each, J=15 and 9 Hz, C-14H_2), 1.7 (s, 3H, C-18H_3), 2.22 (mt, 1H, C-6H), 2.23 (s, 3H, $OCOCH_3$), 3.61 (d, 1H, J=7 Hz, C-3H), 3.98 (mt, 1H, C-7H), 4.01 (d, 1H, J=8 Hz, C-20H), 4,11 (d, 1H, J=8 Hz, C-20H), 4.32 (mt, 1H, C-2'H), 4.38 and 4.48 (two dd, 1H each, J=12 and 5 Hz, C-19H_2), 4.87 (mt, 1H, C-3'H), 4.92 (d, 1H, J=10 Hz, C-5H), 5.0 (d, 1H, J=1.5 Hz, C-10H), 5.83 (t, 1H, J=9 Hz, C-13H), 6.5 (d, 1H, J=7 Hz, C-2H), 7.13, 7.27 and 7.34 (t, d and t, 1H, 2H and 2H, J=7.5 Hz, C_6H_5), 7.42 (d, 1H, J=9 Hz, NHCO), 7.6, 7.68 and 7.98 (t, t and d, 2H, 1H and 2H, J=7.5 Hz, $OCOC_6H_5$).

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