



Synthesis, tubulin assembly, and antiproliferative activity against MCF7 and NCI/ADR-RES cancer cells of 10-*O*-acetyl-5'-hydroxybutitaxel

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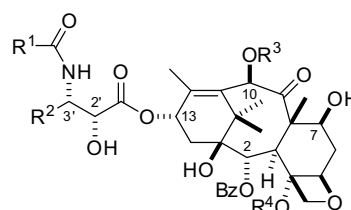
ABSTRACT

A highly efficient kinetic resolution of racemic *cis*-4-(2-*tert*-butyldimethylsilyloxy-1,1-dimethyl)ethyl-3-*tert*-butyldimethylsilyloxy-azetidin-2-one with 7-*O*-triethylsilylbaccatin III was carried out to furnish 10-*O*-acetyl-5'-hydroxybutitaxel after removal of the silyl protecting groups. The compound was 50% as active as paclitaxel in a tubulin assembly assay and showed significantly decreased activity against MCF7 cell proliferation compared to paclitaxel.

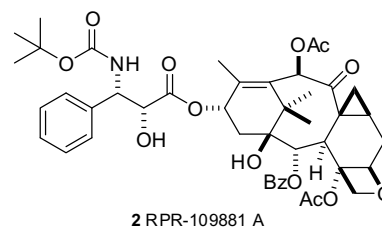
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The structurally and biologically unique diterpenoid paclitaxel (**1a**, Fig. 1),¹ and its semisynthetic derivative docetaxel (**1b**, Fig. 1), are among the most effective anticancer agents known for the treatment of metastatic breast cancer, refractory ovarian cancer and Kaposi's sarcoma.^{2,3} Paclitaxel suffers from a number of disadvantages, including low aqueous solubility, low oral bioavailability, development of drug resistance, and inability to cross the blood–brain barrier (BBB).⁴ Therefore, the continued synthesis and evaluation of new analogues has been important and has led to promising new results.⁵ For example, butitaxel analogue BMS-275183 (**1e**, Fig. 1),⁶ is orally bioavailable, RPR-109881 A (**2**, Fig. 1)⁷ is effective against MDR-positive and taxane resistant human tumor xenografts, and TX-67 (**1d**, Fig. 1)⁸ was shown to permeate the BBB *in situ*.

The efficient semisynthesis of paclitaxel analogues can be achieved by reacting baccatin III derivatives with optically pure β -lactams, the precursors for the C13 *N*-acyl-3'-phenylisoserine side chain. Enantiopure (3*R*,4*S*)- β -lactams can be obtained through an asymmetric ester enolate-imine cyclocondensation or via enzyme-catalyzed resolutions.^{9–13} An alternative semisynthesis, involving the kinetic resolution of racemic β -lactams with 7-*O*-triethylsilylbaccatin III derivatives has been studied for more than 10 years after being first described by Holton et al.¹⁴ This method has



- 1a** Paclitaxel, $R^1 = R^2 = \text{Ph}$, $R^3 = R^4 = \text{Ac}$
1b Docetaxel, $R^1 = t\text{-BuO}$, $R^2 = \text{Ph}$, $R^3 = \text{H}$, $R^4 = \text{Ac}$
1c Butitaxel, $R^1 = t\text{-BuO}$, $R^2 = t\text{-Bu}$, $R^3 = \text{H}$, $R^4 = \text{Ac}$
1d TX-67, $R^1 = R^2 = \text{Ph}$, $R^3 = \text{COCH}_2\text{CH}_2\text{CO}_2\text{H}$, $R^4 = \text{Ac}$
1e BMS-275183, $R^1 = t\text{-BuO}$, $R^2 = t\text{-Bu}$, $R^3 = \text{Ac}$, $R^4 = \text{CO}_2\text{Me}$



2 RPR-109881 A

Figure 1. Structures of paclitaxel (**1a**), docetaxel (**1b**), butitaxel (**1c**), TX-67 (**1d**), BMS-275183 (**1e**), and RPR-109881 A (**2**).

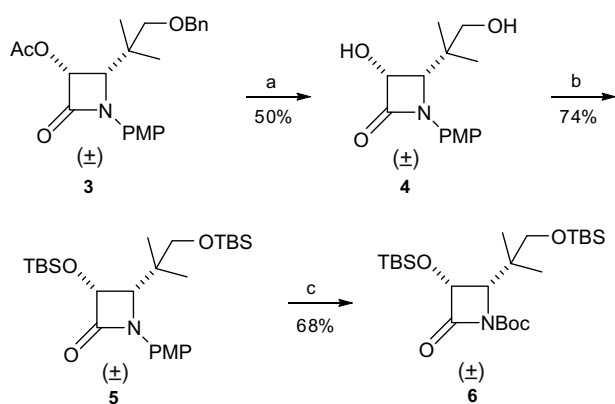
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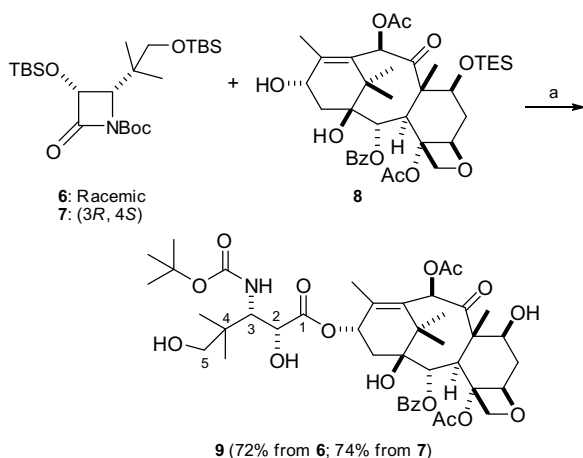
not been used widely for the semisynthesis of paclitaxel analogues because of unpredictable diastereoselectivities obtained during the kinetic resolution.^{6,15,16}

Our recent study of the kinetic resolution of racemic β -lactams with 7-*O*-triethylsilylbaccatin III¹⁷ showed that the structure of the β -lactam played an important role in controlling the diastereoselectivity of the kinetic resolution. High diastereoselectivities were observed when sterically demanding C3-hydroxy protecting groups or sterically demanding C4 substituents at the β -lactam rings were present. In this communication we are reporting the semisynthesis of 10-*O*-acetyl-5'-hydroxybutitaxel (**9**) via kinetic resolution with racemic β -lactam **6** that carries two sterically demanding groups: the *tert*-butylsilyloxy group at C3 and the 1-hydroxy-2-methylpropan-2-yl group at C4 of the β -lactam ring (Scheme 2). The new hydroxylated butitaxel analogue **9** was expected to possess better aqueous solubility ($\text{cLogP} = 3.7$) than paclitaxel ($\text{cLogP} = 4.2$). In addition, analogue **9** is a potential template for structure-activity studies as it allows the facile attachment of a variety of groups at the 5'-hydroxyl group of the C13 side chain.¹⁸

The synthesis of the racemic *cis*- β -lactam **6** is shown in Scheme 1, starting with the hydrolysis (KOH) of the acetate in β -lactam **3**,¹⁹ followed by deprotection of the benzyl group to provide diol **4**. Protection of the two hydroxyl groups of **4** with *tert*-butyldimethylsilyl chloride provided intermediate **5**. The 4-methoxyphenyl moiety



Scheme 1. Synthesis of racemic β -lactam **6**. Reagents and conditions: (a) KOH, THF, 0 °C; then 10% Pd/C, HCO_2NH_4 , MeOH, reflux; (b) TBSCl, imidazole, DMF, 35 °C; (c) CAN, CH_3CN , –10 °C; then Boc_2O , DMAP, Et_3N , CH_2Cl_2 , rt.



Scheme 2. Synthesis of 10-*O*-acetyl-5'-hydroxybutitaxel. Reagents and conditions: (a) LiHMDS, THF, –40 °C; then HF-pyridine, pyridine, 0 °C–rt.

Table 1

ED₅₀ values for tubulin assembly and cytotoxicity assays

Compound	Assembly ^a (μM)	MCF7 ^b (nM)	NCI/ADR-RES (μM)
Paclitaxel (1a)	1.2	2.1	2.0
9	2.2	560	12.5

^a The assembly assay was conducted as described in Ref. 23.

^b The cytotoxicity assay was conducted as described in Ref. 24.

(PMP) of **5** was removed oxidatively with ammonium cerium(IV) nitrate (CAN),²⁰ and the resulting *N*-unsubstituted β -lactam was then treated with *tert*-butyl dicarbonate to afford racemic *N*-Boc- β -lactam **6**.

We then carried out the kinetic resolution between racemic β -lactam **6** and 7-*O*-triethylsilylbaccatin III (**8**)²¹ using the previously reported reaction conditions (Scheme 2).¹⁷ Thus, four equivalents of **6** were used to react with the baccatin derivative **8** in the presence of 1.5 equivalents of LiHMDS in THF at –40 °C. The crude reaction product was subsequently dissolved in pyridine and treated with hydrogen fluoride to furnish derivative **9**. The isomeric ratio of **9** was determined by HPLC analysis as described before.²² Excellent diastereoselectivity (216:1) was observed. In our previous study we had observed that diastereoselectivities increased with the size of the C4-substituents of *N*-Boc-3-OTBS- β -lactams. The 4-phenyl- β -lactam showed a 41:1 diastereomeric ratio and the 4-*tert*-butyl- β -lactam an 82:1 diastereomeric ratio.¹⁷ In the present study, the even more sterically demanding 4-(2-*tert*-butyldimethylsilyloxy-1,1-dimethyl)ethyl group in β -lactam **6** provided an enhanced diastereomeric ratio (216:1), further supporting the hypothesis that C4-substituents play an important role in controlling the diastereoselectivity of this kinetic resolution.

To verify the efficiency of the kinetic resolution, we coupled **8** with enantiopure β -lactam **7** prepared according to the reported procedure¹⁹ under the standard reaction conditions to generate analogue **9** in 74% yield after deprotection of the silyl groups (Scheme 2). Spectral data and HPLC retention time of this analogue are identical to the product obtained in the kinetic resolution. This result demonstrated the highly efficient feature of the kinetic resolution method.

Analogue **9** was tested in a tubulin assembly assay and as an inhibitor of proliferation of the MCF7 and NCI/ADR-RES breast cancer lines (Table 1).^{23,24} In the tubulin assembly assay analogue **9** was just slightly less active than paclitaxel. However, it showed significantly reduced activity against MCF7 proliferation (267-fold).

In conclusion, a new paclitaxel analogue was synthesized through a highly efficient kinetic resolution of racemic β -lactam **6** with 7-*O*-triethylsilylbaccatin III. The observed high diastereoselectivity confirms earlier observations that sterically demanding C4- β -lactams substituents are a key factor in the efficiency of this kinetic resolution. Although the new paclitaxel analogue showed excellent activity in the tubulin assembly assay, it had significantly decreased activity against MCF7 cell proliferation. The results suggest that the compound may not enter the cells as well as paclitaxel because it is more hydrophilic or possibly because it is a much better substrate for the multidrug transporter than paclitaxel. A similar result was observed for the phenolic paclitaxel metabolite that carries a 4-hydroxy group at the C3 phenyl group.²⁵

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References and notes

- Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325.
- For review: Spencer, C. M.; Faulds, D. *Drugs* **1994**, *48*, 794.
- For review: Zhou, J.; Giannakakou, P. *Curr. Med. Chem. Anticancer Agents* **2005**, *5*, 65.
- Ge, H.; Vasandani, V.; Huff, J. K.; Audus, K. L.; Himes, R. H.; Seelig, A.; Georg, G. I. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 433.
- For review: Kingston, D. G. I.; Jagtap, P. G.; Yuan, H.; Samala, L. *Prog. Chem. Org. Nat. Prod.* **2002**, *84*, 53.
- Mastalerz, H.; Cook, D.; Fairchild, C. R.; Hansel, S.; Johnson, W.; Kadow, J. F.; Long, B. H.; Rose, W. C.; Tarrant, J.; Wu, M.-J.; Xue, M.-Q.; Zhang, G.-F.; Zoeckler, M.; Vyas, D. M. *Bioorg. Med. Chem.* **2003**, *11*, 4315.
- Gelmon, K. A.; Latreille, J.; Tolcher, A.; Genier, L.; Fisher, B.; Forand, D.; D'Aloisio, S.; Vernillet, L.; Daigneault, L.; Lebecq, A.; Besenval, M.; Eisenhauser, E. *J. Clin. Oncol.* **2001**, *18*, 4098.
- Rice, A.; Liu, Y.; Michaelis, M. L.; Himes, R. H.; Georg, G. I.; Audus, K. J. *Med. Chem.* **2005**, *48*, 832.
- For review: Boge, T. C.; Georg, G. I. The medicinal chemistry of β -amino acids: paclitaxel as an illustrative example. In *Enantioselective Synthesis of β -Amino Acids*; Juaristi, E., Ed.; Wiley-VCH: New York, 1997; p 1.
- Kayser, M. M.; Mihovilovic, M. D.; Kearns, J.; Feicht, A.; Stewart, J. D. *J. Org. Chem.* **1999**, *64*, 6603.
- a Kayser, M. M.; Yang, Y.; Mihovilovic, M. D.; Feicht, A.; Rochon, F. D. *Can. J. Chem.* **2002**, *80*, 796; b Yang, Y.; Wang, F.-Y.; Rochon, F. D.; Kayser, M. M. *Can. J. Chem.* **2005**, *83*, 28.
- Patel, R. N.; Howell, J.; Chidambaram, R.; Benoit, S.; Kant, J. *Tetrahedron: Asymmetry* **2003**, *14*, 3673.
- Carr, J. C.; Al-Azemi, T. F.; Long, T. E.; Shim, J.-Y.; Coates, C. M.; Turos, E.; Bisht, K. S. *Tetrahedron* **2003**, *59*, 9147.
- Holton, R. A.; Biedinger, R. J.; Boatman, P. D. Semisynthesis of taxol and taxotere. In *Taxol® Science and Applications*; Suffness, M., Ed.; CRC: Boca Raton, FL, 1995; p 97.
- Ojima, I.; Slater, J. C. *Chirality* **1997**, *9*, 487.
- Takeda, Y.; Uoto, K.; Iwahana, M.; Jimbo, T.; Nagata, M.; Atsumi, R.; Ono, C.; Tanaka, N.; Terasawa, H.; Soga, T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3209.
- Ge, H.; Spletstoser, J. T.; Yang, Y.; Kayser, M.; Georg, G. I. *J. Org. Chem.* **2007**, *72*, 756.
- For review: Zefirova, O. N.; Nurieva, E. V.; Ryzhov, A. N.; Zyk, N. V.; Zefirov, N. S. *Russ. J. Org. Chem.* **2005**, *41*, 315.
- Yang, Y.; Wang, J.; Kayser, M. M. *Tetrahedron: Asymmetry* **2007**, *18*, 2021.
- Georg, G. I.; Kant, J.; Gill, H. J. *J. Am. Chem. Soc.* **1987**, *109*, 1129.
- Denis, J.-N.; Greene, A. E.; Guénard, D.; Guéritte-Voegelein, F.; Mangatal, L.; Potier, P. *J. Am. Chem. Soc.* **1988**, *110*, 5917.
- HPLC conditions. Jupiter 5 μ C4-reverse phase column (10 \times 250 mm) from Phenomenex USA, employing a gradient of H₂O–CH₃CN (0–70%, v/v) with 0.1% TFA as the solvent system with a flow rate of 2.0 mL/min for 100 min. Average retention time for the major isomer (2'R,3'S)-**9** = 65.75 min; minor isomer (2'S,3'R)-**9** = 64.98 min. All reaction intermediates and the final products showed spectroscopic properties in agreement with their structures.
- Barron, D. M.; Chatterjee, S. K.; Ravindra, R.; Roof, R.; Baloglu, E.; Kingston, D. G.; Bane, S. *Anal. Biochem.* **2003**, *315*, 49.
- Liu, Y.; Ali, S. M.; Boge, T. C.; Georg, G. I.; Victory, S.; Zygmunt, J.; Marquez, R. T.; Himes, R. H. *Comb. Chem. High Through. Screen.* **2002**, *5*, 39.
- Park, H.; Hepperle, M.; Boge, T. C.; Himes, R. H.; Georg, G. I. *J. Med. Chem.* **1996**, *39*, 2705.