Pergamon

Tetrahedron Letters, Vol. 37, No. 43, pp. 7837-7840, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01744-3

Synthesis of 7,9-Nitrogen-Substituted Paclitaxel Derivatives[1]

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Abstract Reaction of 10-deacetyl-10-dehydrobaccatin III-type taxanes with hydrazine afforded 7,9-pyrazoline adducts, which retained anticancer activity. Copyright © 1996 Elsevier Science Ltd

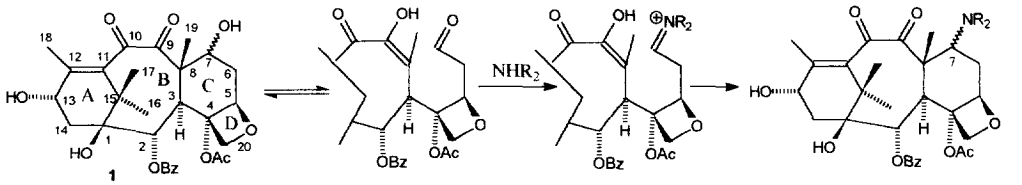
The synthesis of analogues of paclitaxel (= Taxol®) has been an active area of research, spurred by the possibility to improve the pharmaceutical profile of the natural product and to shed light on the structure-activity relationships within antitumor taxoids.[2] As a result, the literature is full of reports describing the systematic modification of the complex functionality pattern of the terpenoid core and the aminoacidic side-chain of paclitaxel. One attractive modification to improve the poor water solubility of the natural product is the introduction of a nitrogen function at C-7. Since the anticancer activity is relatively tolerant to chemical modification in the northern hemisphere,[2] retention of biological activity can be foreseen for these paclitaxel derivatives.

Initial attempts[3] to introduce a nitrogen substituent at C-7 by nucleophilic displacement of a leaving group were thwarted by the propensity of the taxane skeleton to undergo rearrangement to 7,19-cycloderivatives, via a corner-protonated cyclopropane intermediate.[4] Under more forced conditions, elimination to compounds of the $\Delta^{6,7}$-type[5] was observed.[6] Recently, using phase-transfer conditions, a successful protocol for the displacement of a 7β-triflate by an azide group was eventually reported, and applied to the synthesis of 7-deoxy-7-α-aminopaclitaxel.[6] We report here a different approach to the synthesis of nitrogen-substituted paclitaxel derivatives, capitalising on the nucleophilic trapping of C-*seco* aldehydes in equilibrium with taxanes of the 7-hydroxy-9-oxo type.

In basic medium, baccatin III derivatives epimerize at C-7 through a retro-aldol mechanism.[7] Attempts to trap the intermediate C-*seco* aldehyde failed, but the presence of a keto group at C-10 was shown to stabilise by conjugation this intermediate, allowing its trapping with hydrides.[8] We became intrigued by the possibility to effect this trapping with nitrogen nucleophiles. This would generate an electrophilic iminium ion, leading, after realdolisation, to baccatin III derivatives bearing a nitrogen function at C-7 (Scheme 1).

Attempts to implement this strategy with ammonia or simple amines (aniline, dimethylamine, pyrrolidine) failed. No reaction was observed when **1**[8] was treated with these nitrogen nucleophiles in buffered or mildly acidic medium, whereas rearrangement to the 7,8-*seco*-8,12-cycloderivatives **2**[9] took place in basic

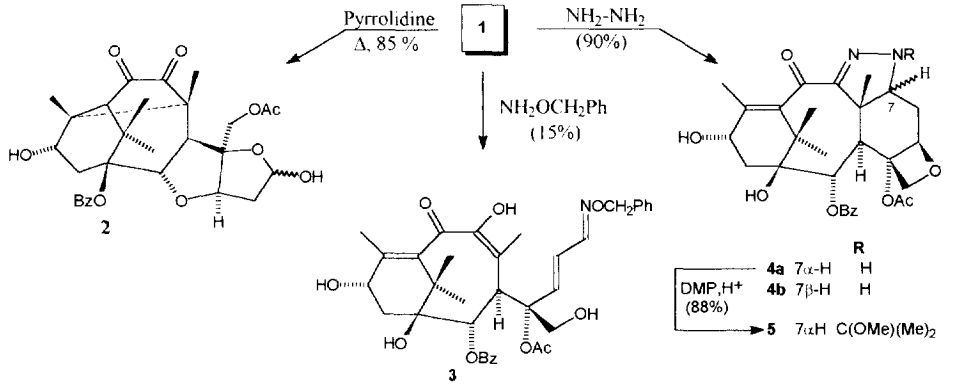
conditions, showing that intramolecular Michael reaction was faster than imine formation and realdolisation (Scheme 2).



*Scheme 1. Synthesis of nitrogen-bearing baccatins by nucleophilic trapping of C-*seco *aldehydes.*

Since the carbonyl adducts of α-nucleophiles are more stable than those from simple amines, we reasoned that compounds like hydroxylamine and hydrazine might act as more efficient traps of the 7-aldehyde carbonyl. In the event, the reaction of **1** with *O*-benzyl hydroxylamine gave, in low yield, the α,β-unsaturated oxime **3** (Scheme 2),[10] the result of the nucleophilic trapping of the C-*seco* aldehyde, followed by opening of the oxetane ring via a β-elimination reaction. Also 7-dehydropaclitaxel[11a] and 7-dehydrobaccatin III derivatives[11b] are easily turned into their corresponding D-seco-Δ^5-derivatives via an E1cb -type reaction. Thus, a β-carbonyl (imino) group has a detrimental effect on the stability of the oxetane ring, an observation which raised a major concern on the feasibility of the nucleophilic trapping of C-7 aldehydes of the oxetane-type.

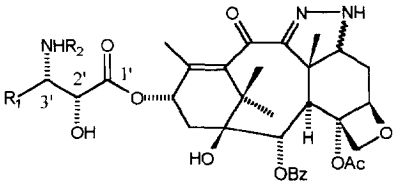
With hydrazine, however, an easily separable mixture of the pyrazolines **4a** and **4b** was obtained in high yield (90%).[12] In this case, realdolisation of the C-*seco* hydrazone was faster than the opening of the oxetane ring or the intramolecular Michael reaction, presumably because the closure of the pyrazoline ring provides an extra drive for the aldolisation.[13] Interestingly, the major pyrazoline was the 7β-isomer, in contrast to what was observed in baccatin III derivatives, whose retro-aldol equilibration affords instead mainly the 7α-aldol instead.[7] Reaction of **4a** with 2,2-dimethoxypropane afforded the aminal **5**, a suitable starting material for the esterification with protected aminoacidic side-chains.[14]



Scheme 2. Reaction of 10-dehydro-10-deacetylbaccatin III (V) **(1)** *with nitrogen nucleophiles*

Alternatively, the reaction with hydrazine was directly applied to 10-dehydro-10-deacetyl baccatin III derivatives bearing an aminoacidic side chain at C-7. Starting from docetaxel, and the 10-deacetyl derivatives[15] of paclitaxel and *N*-debenzoyl-*N*-Boc-3'-dephenyl-3'-isobutyldocetaxel,[16] the pyrazolines **6-8**[17] were synthesised by C-10 oxidation [$Cu(OAc)_2$, MeOH, 90-95%][11b] and reaction with hydrazine (50-70%).[18] **6-8** displayed *in vitro* anticancer activity comparable to that of the corresponding starting compounds, showing that isosteric substitution at C-7 and C-9 as well as closure of an extra ring in the north-eastern hemisphere are compatible with high cytotoxicity.[19]

The obtaining of the **4a,b** from the reaction of **1** with hydrazine is a remarkable example of how heterocyclic ring closure can steer reactivity along an otherwise unfavourable path, and paves the way to the synthesis of new classes of isosterically substituted paclitaxel derivatives.



	R_1	R_2
6	Ph	Boc
7	Ph	Bz
8	isobutyl	Boc

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10. CI-MS (NH_3): 665 $(M + NH_4)^+$ $(C_{36}H_{41}NO_{10} + NH_4)^+$ (80). ^1H NMR (400 MHz, $CDCl_3$, TMS as reference, 60° C): δ 7.93 (br d, J=7.7 Hz, Bz), 7.82 (d, J=9.5 Hz, H-7), 7.51 (t, J=7.7 Hz, Bz), *ca* 7.40 (m, Bnz), 7.33 (br t, J=7.7 Hz, Bz), 6.32 (s, 9-OH), 6.20 (d, J=17.0 Hz, H-5), 6.05 (dd, J=17.0, 9.5 Hz, H-6), 5.55 (d, J=8.0 Hz, H-2), 5.12 (d, J=12.0 Hz, H-20a), 5.10 (d, J=12.0Hz, H-20b), 4.92 (m, H-13), 4.33 (d, J=8.0 Hz, H-3), 4.06 (d, J=11.5 Hz, Bnz), 3.96 (d, J=11.5 Hz, Bnz), 2.60 (dd, J=16.0, 6.0 Hz, H-14a), 2.48 (dd, J=16.0, 6.5 Hz, H-14b), 2.16 (s, Ac), 1.92 (s, H-19), 1.91 (d, J=1.5 Hz, H-18), 1.14, 1.06 (s, H-16 and H-17).
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12. To a suspension of **1** (1.028 g, 1.84 mmol) in MeOH (15 ml), an ethanol solution of hydrazine (11.7 ml, 10%, 36.9 mmol, 20 mol. equiv.) was added. After refluxing for 2 h, the mixture was diluted with water (100 ml) and dil. HCl (50 ml), and extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4) and evaporated. The residue was purified by CC (hexane-EtOAc 6:4) to give 709 mg **4a** and 194 mg **4b** (overall yield: 90%). **4a**: mp. 195°C; $[\alpha]_D^{25}$ -8 (MeOH, c 0.6); IRν_{max} (KBr): 3474, 1719, 1273, 1248, 1111, 1026, 758 cm^{-1}; CI-MS (NH_3): 556 $(M + NH_4)^+$ $(C_{29}H_{34}N_2O_8 + NH_4)^+$ (100). ^1H NMR (400 MHz, $CDCl_3$, TMS as reference): δ 8.14 (br d, J=7.6 Hz, Bz), 7.62 (br t, J=7.6 Hz, Bz), 7.49 (br t, J=7.6 Hz, Bz), 6.44 br s, (NH), 5.80 (br d, J=8.6 Hz, H-2), 5.04 (dd, J=9.5, 4.5 Hz, H-5), 4.69 (br t, J=6.5Hz, H-13), 4.41 (d, J=8.6 Hz, H-20a), 4.33 (d, J=8.6 Hz, H-20b), 4.20 (ddd, J=13.5, 4.5, 3.0 Hz, H-7), 3,16 (d, J=8.6 Hz, H-3), 2.43 (ddd, J=13.5, 9.5, 4.5 Hz, H-6a), 2.34 (m, H-14a,b), 2.33 (br s, OH), 2.26 (s, Ac), 2.20 (dd, J=13.5, 13.5, 4.5 Hz, H-6b), 1.87 (br s, OH), 1.66 (br s, H-18), 1.50 (s, H-19), 1.23 (s, H-16), 1.15 (s, H-17). ^{13}C NMR (100 MHz, $CDCl_3$, TMS as reference): δ 191.4 (s, C-10), 170.6 (s, Ac), 167.5 (s, Bz), 157.3 (s, C-9), 137.9 (s, C-12), 137.9 (s, C-11), 133.7 (d, *p*-Bz), 130.1 (d, *o*-Bz), 129.4 (s, *i*-Bz), 128.6 (d, *m*-Bz), 85.3 (d, C-5), 84.0 (s, C-4), 79.6 (s, C-1), 77.6 (t, C-20), 74.6 (d, C-2), 70.2 (d, C-7), 66.4 (d, C-13), 49.5 (s, C-8), 48.7 (d, C-3), 40.1 (s, C-15), 38.5 (t, C-14), 28.4 (t, C-6), 25.9 (q, C-17),

22.2 (q, Ac), 20.1 (q, C-16), 14.2 (q, C-18), 11.0 (q, C-19). **4b**: mp.222°C; $[\alpha]_D^{25}$ -23 (MeOH, c 0.6); IR ν_{max} (KBr): 3428, 1748, 1717, 1649, 1516, 1373, 1273, 1111, 1071, 712 cm^{-1}; CI-MS (NH_3): 556 $(M + NH_4)^+$ $(C_{29}H_{34}N_2O_8 + NH_4)^+$ (100); ^1H NMR (400 MHz, $CDCl_3$, TMS as reference): δ 8.12 (br d, J=7.6 Hz, Bz), 7.64 (br t, J=7.6 Hz, Bz), 7.50 (br t, J=7.6 Hz, Bz), 6.34 br s, NH), 6.04 (br d, J=6.0 Hz, H-2), 4.93 (br d, J=2.5 Hz, H-5), 4.79 (br dd, J=10.0, 6.5Hz, H-13), 4.39 (dd, J=14.0, 4.2 Hz, H-7), 4.38 (br s, H-20a + H-20b), 3.71 (d, J=6.0 Hz, H-3), 2.63 (br s, OH), 2.46 (dd, J=14.0, 10.0 Hz, H-14a), 2.36 (s, Ac), 2.06 (td, J=14.0, 14.0, 2.5 Hz, H-6a), 2.00 (br s, OH), 1.88 (dd, J=14.0, 6.5 Hz, H-14b), 1.85 (m, H-6b),1.75 (br s, H-18), 1.70 (s, H-19), 1.33 (s, H-16), 1.23 (s, H-17); ^{13}C NMR (100 MHz, $CDCl_3$, TMS as reference): δ 192.4 (s, C-10), 173.0 (s, Ac), 166.6 (s, Bz), 150.4 (s, C-9), 139.6 (s, C-12), 139.0 (s, C-11), 133.8 (d, *p*-Bz), 130.0 (d, *o*-Bz), 129.1 (s, *i*-Bz), 128.7 (d, *m*-Bz), 84.8 (d, C-5), 81.9 (s, C-4), 78.5 (s, C-1), 76.3 (t, C-20), 75.3 (d, C-2), 69.5 (d, C-7), 67.4 (d, C-13), 51.1 (s, C-8), 41.6 (s, C-15), 40.8 (d, C-3), 40.6 (t, C-14), 28.6 (t, C-6), 25.3 (q, C-17), 22.9 (q, Ac), 22.9 (q, C-19), 21.1 (q, C-16), 14.3 (q, C-18). The ^1H and ^{13}C NMR spectra were assigned with the aid of NOE-inspection and the HMBC spectra.

13. An alternative mechanism for the formation of the pyrazolines, triggered by the formation of a 9-hydrazone seems unlikely, since the 9-carbonyl of baccatin III-type taxanes is unreactive towards nucleophilic attack.[2] The importance of pyrazoline ring closure is highlighted by the observation that no reaction occurred when **1** was treated with tosylhydrazine, phenylhydrazine or *N,N*-dimethylhydrazine.
14. Deprotection of the pyrazoline nitrogen could be effected with wet silica gel (EtOAc, RT, 12 h, 92%).
15. Prepared by reaction of the 10-acetates with hydrazine in ethanol (see reference 5).
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17. Data for **8** as representative: 7α-H-isomer: CI-MS (NH_3): 801 $(M + NH_4)^+$ $(C_{41}H_{57}N_3O_{12} + NH_4)^+$ (100). ^1H NMR (400 MHz, $CDCl_3$, TMS as reference): δ 8.14 (br d, J=8.1 Hz, Bz), 7.60 (br t, J=8.1 Hz, Bz), 7.48 (br t, J=8.1 Hz, Bz), 6.29 (br d, J=2.5 Hz, 7-NH), 5.98 (br dd, J=9.0, 7.5 Hz, H-13), 5.86 (d, J=8.5 Hz, H-2), 4.99 (dd, J=9.5, 4.5 Hz, H-5), 4.61 (br d, J=10 Hz, Boc-NH), 4.45 (d, J=9.1 Hz, H-20a), 4.31 (d, J=9.1 Hz, H-20b), 4.19 (dd, J=4.5, 2.5 Hz, H-2'), 4.15 (m, H-3'), 4.13 (m, H-7), 3.29 (br s, 2'-OH), 3.19 (d, J=8.5 Hz, H-3), 2.40 (m, H-6a), 2.40-2.30 (m, H-14a + H-14b), 2.35 (s, Ac), 2.22 (m, H-6b), 1.80-1.70 (m, H-5'), 1.65-1.55 (m H-4'a), 1.60 (d, J=1.5 Hz, H-18), 1.53 (s, H-19), 1.45 (s, Boc), 1.45-1.35 (m, H-4'b), 1.28 (s, H-16), 1.25 (s, H-17), 0.99 (d, J=7.0 Hz, 5'-Me), 0.98 (d, J=7.0 Hz, 5'-Me). 7β-H-isomer: CI-MS (NH_3): 801 $(M + NH_4)^+$ $(C_{41}H_{57}N_3O_{12} + NH_4)^+$ (100). ^1H NMR (400 MHz, $CDCl_3$, TMS as reference): δ 8.13 (br d, J=8.1 Hz, Bz), 7.62 (br t, J=8.1 Hz, Bz), 7.49 (br t, J=8.1 Hz, Bz), 6.18 (br d, J=2.5 Hz, 7-NH), 6.11 (d, J=6.0 Hz, H-2), 6.01 (br dd, J=9.0, 7.5 Hz, H-13), 5.01 (br d, J=4.0 Hz, H-5), 4.68 (br d, J=10 Hz, Boc-NH), 4.43 (dd, J=13.0, 4.0 Hz, H-7), 4.37 (br s, H-20a + H-20b), 4.24 (dd, J=4.5, 2.0 Hz, H-2'), 4.15 (m, H-3'), 3.76 (br s, 2'-OH), 3.59 (d, J=6.0 Hz, H-3), 2.51 (dd, J=15.0, 9.0 Hz, H-14a), 2.34 (s, Ac), 2.16 (dd, J=15.0, 7.0 Hz, H-14b), 2.10 (ddd, J=14.0, 13.0, 1.5 Hz, H-6a), 1.84 (ddd, J=14.0, 4.0, 4.0 Hz, H-6b), 1.80-1.70 (m H-5'), 1.66 (d, J=1.5 Hz, H-18), 1.65-1.55 (m, H-4'a), 1.45-1.35 (m, H-4'b), 1.39 (s, Boc), 1.37, 1.35, 1.32 (s, H-16, H-17, H-19), 0.97 (d, J=7.0 Hz, 5'-Me), 0.96 (d, J=7.0 Hz, 5'-Me).
18. The lower yield compared to **1** was due to the hydrolytic loss of the C-13 side chain, with formation of variable amounts of **4a,b**.
19. Only the major 7α-H epimers were tested. The IC_{50} values, measured on MDA-MB231 cells, were: 1.4, 7.6 and 2.6 nM, respectively. Under these conditions, the corresponding values for docetaxel, paclitaxel and *N*-debenzoyl-*N*-Boc-3'-dephenyl-3'-isobutyltaxol were 0.8, 2.4 and 4.7 nM, respectively.

(*Received in UK* 9 *July* 1996; *revised* 3 *September* 1996; *accepted* 6 *September* 1996)