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Synthesis of a Conformationally Restricted Analogue of Paclitaxel

Luciano Barboni,[a]* Catia Lambertucci,[a] Roberto Ballini,[a] Giovanni Appendino,[b]* and Ezio Bombardelli[c]

a: Dipartimento di Scienze Chimiche, via S. Agostino 1, 62032 Camerino (MC), Italy

b: Dipartimento di Scienza e Tecnologia del Farmaco via Giuria 9, 10125 Torino, Italy

c: Indena S.p.A., Viale Ortles 12, 20141 Milano, Italy

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Abstract: A conformationally constrained analogue of the paclitaxel side-chain was synthesised in an enantioselective way from ethyl 1*H*-indene-2-carboxylate. Coupling with 7-triethylsilylbaccatin III and deprotection afforded 2',2"-methylenepaclitaxel, a novel analogue of the natural product retaining anticancer activity.

Despite extensive structure/activity work[1] and spectacular progress in tubulin crystallography,[2] the biologically active conformation of the anticancer drug paclitaxel (= Taxol®, **1a**) is still a matter of hot debate.[3] The paclitaxel diterpenoid core is rigid, but hydrophobic interactions between its pendant ester groups lead to an oligo-conformational system, which assumes different geometries according to the polarity of the medium.[3] In this context, flexibility around the C-2'-C-3' bond is pre-eminent in terms of conformational motion, and underlies the clustering, in polar media, of the C-3' phenyl, the C-2 benzoate, and the 4-acetate ("hydrophobic collapse").[4] Based on crystal data[5] and extensive NMR investigations, three conformational domains were proposed for the aminoacidic side chain of paclitaxel: A, typical of apolar media, and B/C, relevant in polar solvents (Figure 1).[3]

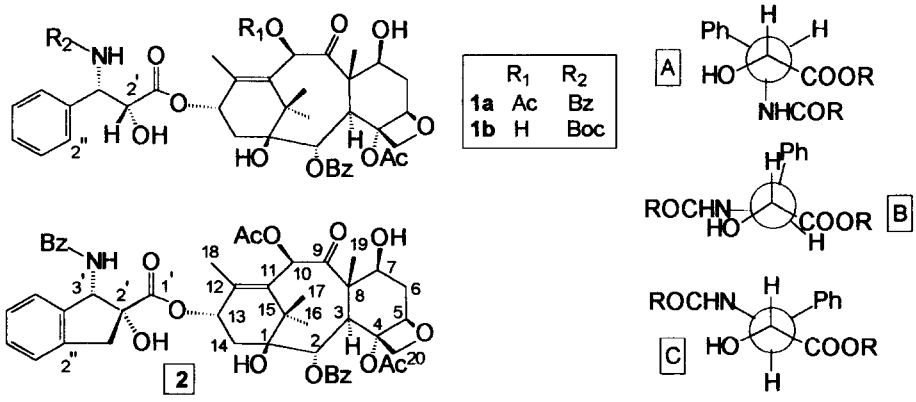

Figure 1. Structural formulae of paclitaxel (**1a**), *docetaxel* (**1b**), *and 2',2"-methylenepaclitaxel* (**2**). *A,B,C: Major conformations of the paclitaxel side chain in apolar (A) and polar (B,C) solvents.*

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Cyclic compounds are frequently used to mimic bioactive conformations of linear sequences,[6] and we report here the synthesis of a conformationally constrained analogue of paclitaxel where two carbons of the amino-acid side-chain are tethered by an extra 2',2"-methylene bridge. Introduction of a methyl group on the 2' position of the side-chain of paclitaxel and docetaxel (= Taxotere®, **1b**) is well-tolerated in terms of biological activity.[7] Differences in activity between paclitaxel and a tethered analogue where the extra C-2'-carbon is also bound to the *ortho* carbon of the 3'-phenyl (C-2") would thus be directly related to the conformational bias introduced by the methylene bridge. Because of the modest puckering of cyclopentene derivatives, the presence of this tether is expected to shift the conformational equilibrium towards a conformational domain with a reduced CH_2-(corresponding to H-2' in **1a,b**)-C-2'-C-3'-Ph torsion angle, approaching the conformation B of paclitaxel (see Figure 1). Furthermore, the 3'-phenyl would now be locked in an orthogonal orientation towards the plane passing through C-1', C-2' and the 2'-hydroxyl, a situation quite different from the one generally found in antitumour taxoids, where these elements lie in almost parallel planes,[5] and found so far only in one of the two conformations assumed by paclitaxel in the solid state.[5a]

The synthesis of **2** requires the protected chiral non-racemic carboxylic acid **3**. The known[8] 1*H*-indene-2-carboxylate **4** seemed a logical precursor, since several methods to convert cinnamic acid derivatives to the side chain of antitumour taxoids have been developed.[9] Sharpless asymmetric aminohydroxylation (AA)[10] of **4** occurred with excellent regioselectivity, but gave a racemic product under a variety of experimental protocols.[10] Attempts to resolve (±)-**3** or various intermediates *en route* to it failed. Since the antitumour activity of taxoids is critically dependent on the stereochemistry of the side-chain,[9,11] an alternative procedure was pursued. We thus considered a recent modification[12] of the synthesis of the paclitaxel side-chain mediated by the asymmetric dihydroxylation (AD) of cinnamate esters, a process first developed by Sharpless.[13] The asymmetric dihydroxylation of the trisubstituted double bond of **4** took place with satisfactory enantioselectivity [92% e.e., based on the HPLC analysis of the diastereomeric 3'(paclitaxel numbering) menthylcarbonate] (Figure 2).[14] After conventional functional group manipulation, the protected oxazolidine **3** was obtained in overall 10% yield from **4**. In spite of the increased encumbrance, **3** could be smoothly coupled to 7-triethylsilylbaccatin III.[15] After deprotection, **2** was obtained in a rewarding and unoptimised 35% yield from 7-triethylsilylbaccatin III. The ^{1}H-NMR spectra of crude **2** did not show the presence of the small amounts of the 2',3'-diastereomer expected from the 92% e.e. of the acid **3**, suggesting that kinetic resolution had taken place in the esterification of 7-triethylsilylbaccatin III.[16]

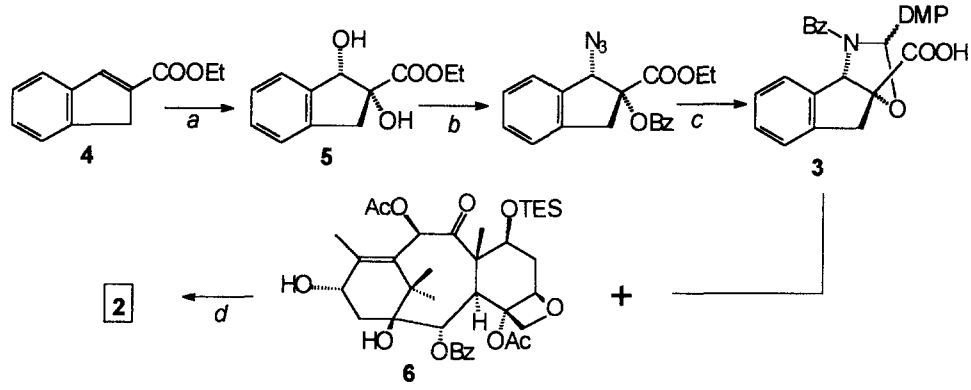

Figure 2. Synthesis of 2. a) AD-mix-α, t-BuOH-H_2O 1:1, 65%; b) 1. Trimethylorthobenzoate, PTSA,CH_2Cl_2; then AcBr. 2: NaN_3., DMF, 45°C. c): 1. H_2, Pd/C, EtOH; 2. 2,4-dimethoxybenzaldehyde dimethylacetal, PPTS, toluene, Δ 3:K_2CO_3, MeOH-H_2O (16% from 5). d): 1. DCC, DMAP, toluene,room temp., 52%. 2. 0.1 M HCl, MeOH, 68%.

Comparison of the ^{1}H-NMR data of paclitaxel (**1a**) and its constrained analogue **2**[17] revealed a dramatic upfield shift of the 4-acetate methyl in **2**, both in apolar ($CDCl_3$, $\Delta\delta$ -0.51 ppm) and in polar solvents (DMSO-d_6-D_2O 3:1, $\Delta\delta$ -0.38 ppm). No NOEs between the 3'-phenyl and the 2-benzoate were observed, but the detection of NOEs between the 4-acetate and H-3', and between the 4-acetate and the proton of the methylene bridge *cis* to H-3' (δ 3.30) showed that the side chain and the 4-acetate are spatially close, suggesting that the upfield shift of the latter is due to anisotropic shielding from the 3'-phenyl.[18] Anisotropic shifts of this type have never been reported for paclitaxel and its derivatives in a variety of solvents. For conformationally unrestrained antitumour taxoids, the geometry of **2** (reduced C-2'-C-3' torsion angle[19] and orthogonal arrangement of the 3'-phenyl and the plane through C-1', C-2' and the 2'-hydroxyl) is thus seemingly not significantly populated in solution. Notwithstanding these observations, **2** showed a high degree of cytotoxicity, comparable to that of paclitaxel in several cell lines.[20] The rather broad tolerance in the spatial arrangement of what are apparently key elements of the taxoid pharmacophore is totally surprising.

In conclusion, we have shown that the synthesis of conformationally constrained probes can be a powerful approach to study the dynamic behaviour of anticancer taxoids and focus on their bioactive conformation(s).[21] Furthermore, the anticancer activity of the 2',2"-methylene analogue **2** paves the way to the synthesis of taxoids where the key elements of side chain pharmacophore are enshrined in polycyclic structures.

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16. Kinetic resolution in the esterification at C-13 of protected baccatin III derivatives with both acids (Denis, J.-N.; Kanezawa, A.M.; Greene, A.E. *Tetrahedron Lett.* **1994**, 35, 105-108) and β-lactams (Park, H.; Hepperle, M.; Boge, T.C.; Himes, R.H.; Georg, G.I. *J. Med. Chem.* **1996**, *39*, 2705-2709) has been previously reported.
17. Physical and spectroscopic data for **2**: colourless powder, mp: 254 °C (dec.); $[\alpha]_D^{25}$: -48 ($CHCl_3$, *c* 0.70); IR (KBr disc): 1745, 1457, 1383, 1203, 720 cm^{-1};^{1}H NMR (300 MHz, $CDCl_3$ at δ 7.27 as reference): δ 1.16 (s, H-16), 1.30 (s, H-19), 1.85 (ddd, J = 14.9, 11.0, 2.2 Hz, H-6β), 1.87 (s, 4-OAc), 1.93 (br s, H-18), 2.24 (s, 10-OAc), 2.35 (dd, J = 15.4, 9.2 Hz, H-14α), 2.44 (dd, J = 15.4, 9.2 Hz, H-14β), 2.50 (ddd, J = 14.9, 9.9, 6.8 Hz, H-6α), 3.30 (d, J = 16.5 Hz, 2',2"-β-CH_2), 3.79 (d, J = 7.3 Hz, H-3), 3.83 (d, J = 16.5 Hz, 2',2"-α-CH_2), 4.16 (d, J = 8.4 Hz, H-20β), 4.25 (d, J = 8.4 Hz, H-20α), 4.37 (dd, J = 10.8, 6.8 Hz, H-7), 4.85 (br d, J = 9.9 Hz, H-5), 5.69 (d, J = 7.3 Hz, H-2), 6.10 (d, J = 9.= Hz, H-3'), 6.29 (s, H-10), 6.42 (br t, J = 9.2 Hz, H-13), 6.78 (d, J = 9.0 Hz, NH), 7.33 (m, Ph), 7.4-7.6 (*m* OBz, *p*-OBz, *m*-NHBz, *o*-NHBz), 7.80 (AA',-NHBz), 8.08 (AA'-OBz). ^{13}C-NMR (75 MHz, $CDCl_3$ at δ 77.6 as reference): δ 203.6 (s, C-9), 175.1 (s, C-1'), 171.3 (s, 10-OAc), 170.4 (s, 4-OAc), 168.0 (s, NHBz), 166.9 (s, OBz), 142.0 (s, C-12), 139.1 (s, C-2"), 138.8 (s, *i*-NHBz), 133.6 (d, *p*-OBz), 133.4 (s, C-1"), 133.1 (s, C-11), 132.1 (d, C-4"), 130.2 (d, *o*-OBz), 129.1 (d, C-5"), 129.1 (s, *i*-OBz), 128.7 (d, *m*-NHBz), 128.7 (d, *m*-OBz), 127.9 (d, *p*-NHBz), 127.2 (d, *o*-NHBz), 125.2 (d, C-3"), 123.8 (d, C-6"), 84.4 (d, C-5), 82.1 (d, C-2'), 81.2 (s, C-4), 78.9 (s, C-1), 76.6 (t, C-20), 75.6 (d, C-10), 74.9 (d, C-2), 72.6 (d, C-13), 72.3 (d, C-7), 61.7 (d, C-3'), 58.7 (s, C-8), 45.6 (d, C-3), 43.9 (t, 2'(2")-CH_2), 43.3 (s, C-15), 35.7 (t, C-6 + C-14), 26.9 (q, C-17), 21.9 (q, C-16), 21.7 (4-OAc), 20.9 (10-OAc), 15.0 (q, C-18), 9.5 (q, C-19). CIMS (negative ions): m/z 865 $(M)^-(C_{48}H_{51}NO_{14})^-$.
18. Anisotropic shift of this type are well documented in taxoids, the most striking example being the negative (- 0.21 ppm) (!) chemical shift value of H-6α observed for taxine A in $CDCl_3$ (Barboni, L.; Gariboldi, P.; Appendino, G.; Enriù, R.; Gabetta, B.; Bombardelli, E. *Liebigs Ann.* **1995**, 345-349).
19. *Ab initio* full geometry optimisation of the C-13 side chain of 2 at Hartree-Fock level with a 6-31G (d,p) basis set gave a value of –30.3 ° for the torsion angle CH_2-C-2'-C-3'-Ph. We are grateful to Dr. Marco Milanesio (Dipartimento di Chimica IFM, Università di Torino) for these calculations.
20. **2** showed the following values of ED_{50}/ED_{50}(paclitaxel): 0.67 (T28-1); 0.88 (T19-7); 1.00 (N202-1B); 1.00 (RAS+); 7.5 (MDA-MB 231).
21. To address this problem, Nicolaou has suggested an alternative type of tethering, involving the 3'-phenyl and the 2-benzoate (Gomez Paloma, L.; Guy, R.K.; Wrasidlo, W.; Nicolaou, K.C. *Chem. Biol.* **1994**, *2*, 107-112). This interesting approach has not yet been implemented.