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Synthesis of an Original Oxygenated Taxuspine X Analogue: a Versatile "Non-Natural" Natural Product with Remarkable P-gp Modulating Activity

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The diterpenoide paclitaxel (Taxol) (**1**, Figure 1) from the yew (*Taxus*) species is one of the best anticancer agents in clinical use today for the treatment of ovarian, breast, and lung cancer, as well as of Kaposi's sarcoma. Taxol belongs to the antimitotic drugs that bind to β-tubilin. However clinical use of this drug and related derivatives results in the development of cross resistance to a broad range of structurally and functionally unrelated compounds (multidrug resistance, MDR). The most supported mechanism of MDR is the overexpression of ABC transporters localized in the cell membrane, which determines this pharmacological effect by effluxing a variety of chemotherapeutic drugs from tumor cells. The mostly involved ABC transporter in MDR is ABCB1 (ABCB subfamily) well known as P-glycoprotein (Pgp).[2] Among the number of taxol-related compounds isolated from the Japanese yew *Taxus Cuspidata*,[1] taxuspine X (**2**)[3] has shown to be a potent MDR reversing agent increasing accumulation of vincristine in MDR cell lines. These natural products were however isolated in very poor yields from the natural source thus requiring the intervention of the synthetic chemistry to generate a sufficient amount of compound to fully explore the biological potential of taxuspine analogues. Accordingly, our molecular modeling group has recently proposed the simplified analogue **3** as a promising and versatile advanced key intermediate (Figure 1): whereas the functionalization of C13 with the taxol® side chain should permit generation of potential anticancer agents, the introduction of a cynnamoyl group in C5 may lead to Taxuspine X analogues potentially active as MDR reversing agents.[4]

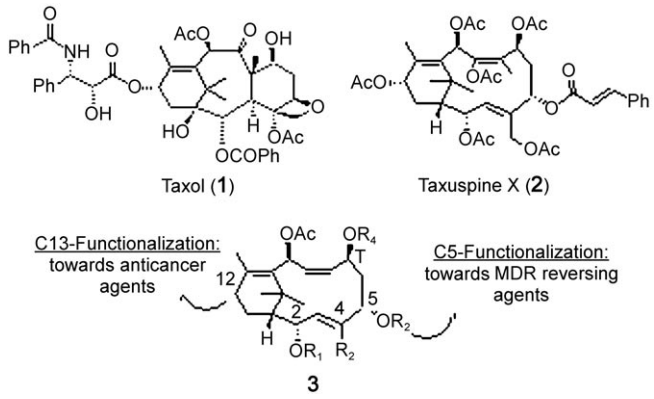
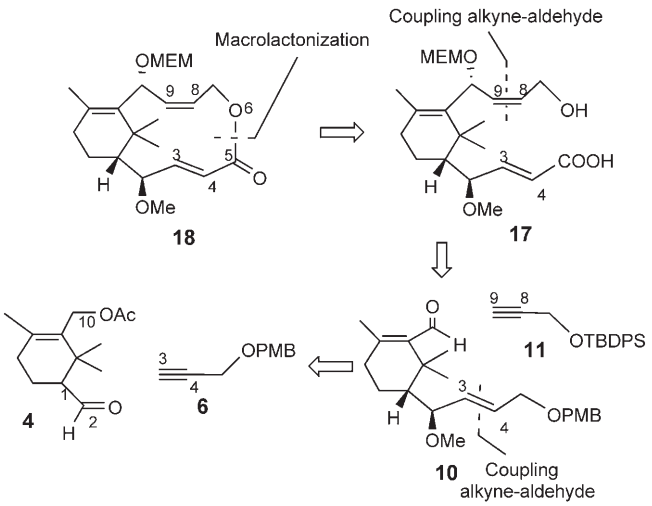


Figure 1. Taxol (**1**) and taxol-related compounds.

Several RCM (ring closes metathesis)-based synthetic approaches for the synthesis of **3** have been thoroughly investigated in the past few years. Unfortunately, the RCM approach proved to be relatively inefficient and unpractical, giving the desired compounds in low to moderate yields. These shortcomings led us to propose the macrolactone **18** as a synthetically more accessible oxygenate analogue of **3**, retaining much of the pharmacophoric features of the original target compound (Scheme 1). This derivative may best be described as a "non-natural" natural product,[5] as it still retains most of the (two-dimensional) structural features of the natural product lead; at the same time it is structurally unique, as it is outside of the general scope of nature's biosynthetic machinery.[6] The structure of **18** consists of a twelve-membered macrolactone condensed with ring A of taxol in which the macrocyclic C6 of Taxuspine X has been replaced by its bioisosteric oxygen atom. Once more, the modifications on this 6-oxa-bicyclo-[9.3.1]pentadecane skeleton, by introduction of the paclitaxel



Scheme 1. Retrosynthetic approach.

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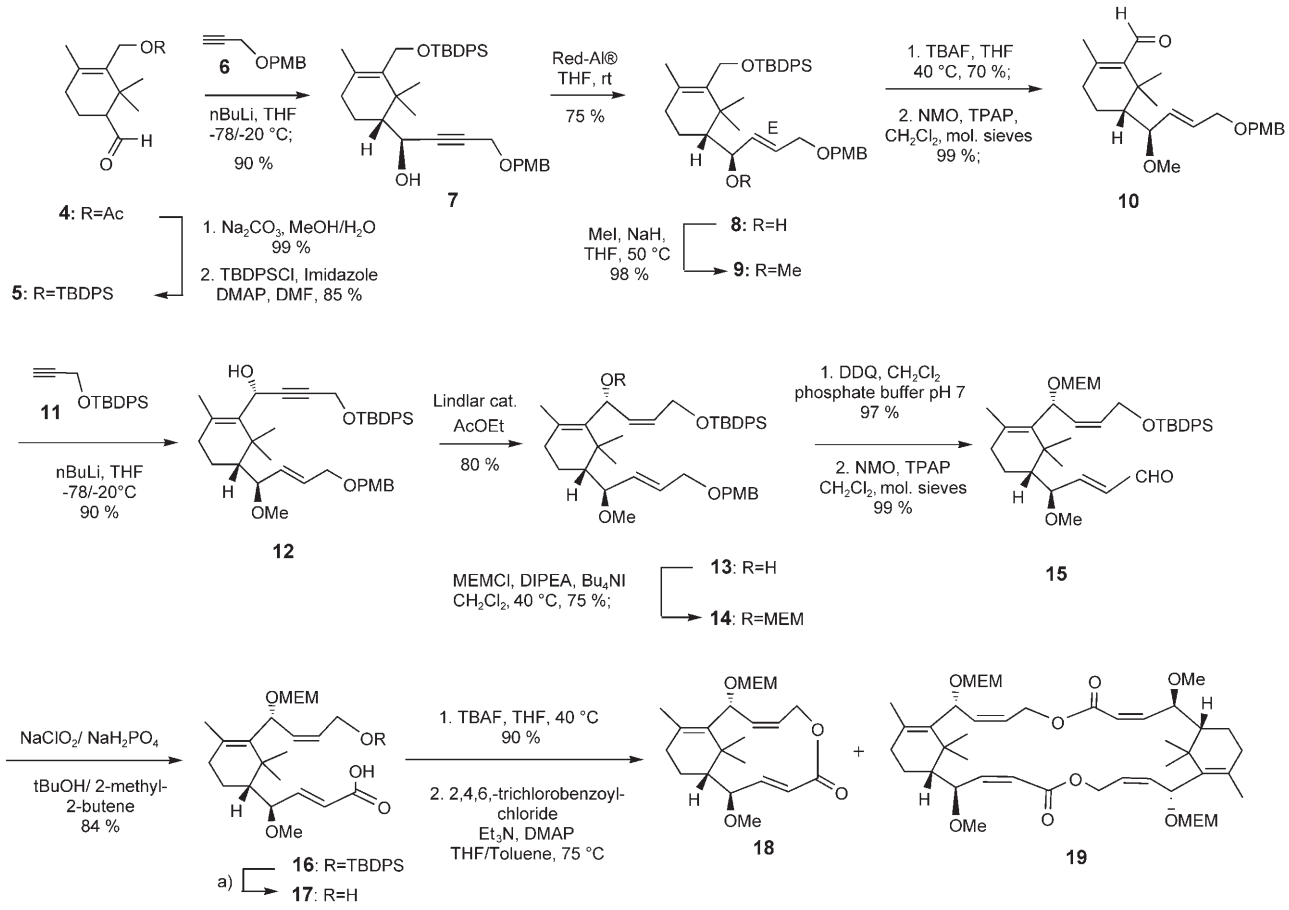
side chain at position C13 or *trans*-cinnamoyl moiety at position C5 could lead to potential anticancer and/or MDR reversing agents. In this communication we report a straightforward synthetic strategy to prepare the original key intermediate **18** and its remarkable P-gp modulating activity.

According to the retrosynthetic strategy outlined in Scheme 1, compound **18** could be prepared through Yamaguchi twelve-membered macrolactonization[7] of the corresponding *seco*-acid **17** which could in turn be obtained after two consecutive alkyne-aldehyde couplings. Aldehyde **4**, available from ethyl acetoacetate in six steps[8] was readily transformed into the aldehyde **10** by a sequence of protecting group manipulations (Scheme 2). Coupling of **5** with the lithium derivate of the alkyne **6**[9] in THF at −78 °C gave preferentially the propargyl alcohols **7**. The nucleophilic attack from the less hindered side, opposite to the gem-dimethyl group, favored the formation of the *syn*-compound **7** (the high diastereoselectivity of the nucleophilic addition to aldehyde **4** has been previously noted by Fallis et al.).[10] Red-Al® mediated reduction of **7**[11] gave the *E*-olefine **8** in good yield and the hydroxyl group was protected as a methyl ether (Scheme 2). Removal of the TBDPS group, followed by oxidation of the primary alcohol with NMO-TPAP, led to the aldehyde **10** in excellent yield. For the C8–C9 bond formation, the aldehyde **10** was reacted with a lithiated derivative of alkyne **11**[12] affording a diastereoisomeric mixture of propargyl alcohols (**12** was the major isomer together with traces of the other isomer). Partial hydrogenation of the alkyne **12** with Lindlar catalyst and quinoline furnished the desired *Z*-olefin **13**, which was then protected as methoxyethoxymethyl ether, to give **14**. The acid moiety was then introduced in three steps: oxidative cleavage of PMB under buffered conditions, oxidation of the resulting primary alcohol to aldehyde, and further oxidation of the aldehyde to carboxylic acid. Among the numerous methods developed for the oxidation of α,β-unsaturated aldehydes to the corresponding acids,[13] the mild Pinnick procedure[13b] was chosen because of the high sensitivity of our substrate. Treatment of **15** with $NaClO_2/NaH_2PO_4$ in *t*BuOH/2-methyl-2-butene at room temperature afforded the unsaturated acid **16** in high yield (Scheme 2). After deprotection of the primary alcohol from the silyl ether, the Yamaguchi macrolactonization of *seco*-acid **17** was carried out in 3.5 mM toluene solution, furnishing the desired macrolactone **18** in 52% yield, together with 11% of dimer **19**.[14]

The macrocycle **18** and the dimer **19** were initially tested for their antitumor activity on H460 Cells (Human lung cancer): both compounds showed moderate anticancer activity (IC_{50} = 22 and 30 μg mL^{-1}, respectively). The effect of **18** and **19** on P-glycoprotein ATPase was then investigated with the use of rat small intestine brush border membrane vesicles by assaying



Scheme 2. Synthesis of the macrolactone **5**.

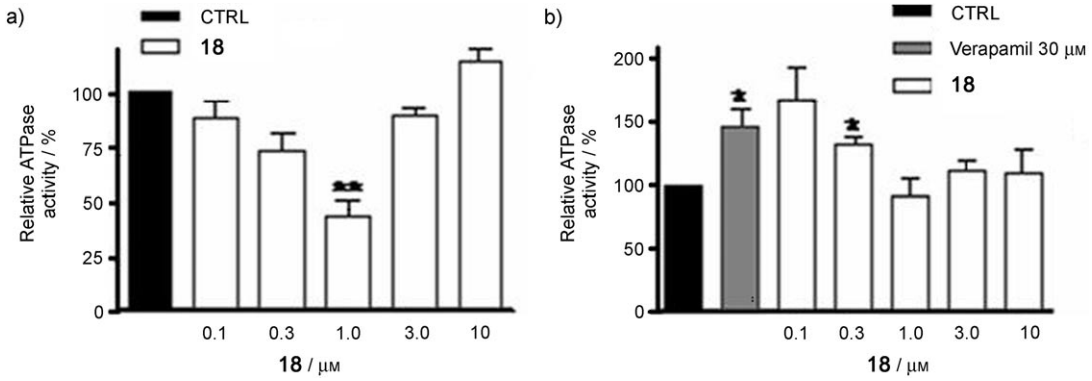


Figure 2. Effects of **18** on P-gp-dependent, rat jejunal ATPase activity. a) Modulation by **18** of basal P-gp ATPase activity. One sample t-test: $^{**}P < 0.01$ versus control, $n = 3$. b) Modulation by **18** of the P-gp ATPase activity stimulated by verapamil. One sample t-test: $^{*}P < 0.05$ versus control; one sample t-test: NS versus Verapamil; ANOVA followed by Dunnet: $P < 0.055$ versus Verapramil, $n = 3$.

both basal and verapamil-stimulated ATPase activity. Permeability glycoprotein (P-gp) is a multidrug transporter responsible for resistance to anticancer chemotherapy (MDR) and physiologically involved in absorption, distribution, and excretion of a large number of lipophilic uncharged and cationic drugs.[15] On the assumption of the existence of multiple binding sites on P-gp, both basal ATPase activity of P-gp and ATPase activity stimulated by the substrate verapamil must be taken into account when investigating P-gp modulators. To test the effects of **18** and **19** on P-gp ATPase activity, plasma membrane vesicles were prepared from homogenates of rat small intestine mucosa.[16] On these P-gp-containing membrane vesicles ATPase activity measurements were performed at 37 °C by a spectrophotometric method based on continuous monitoring of ADP formation, regenerated in ATP by a coupled enzyme system consisting of pyruvate kinase/phosphoenolpyruvate and lactate dehydrogenase/NADH, according to the method of Scharschmidt and co-workers by monitoring NADH absorbance decay with time at 340 nm. Membrane ATPases not related to P-gp , that is, mitochondrial ATPases, Na+, K+ATPases, and Ca-ATPases, were inhibited by addition of 10 mM sodium azide, 0.5 mM ouabain, and 1 mM EGTA, respectively. Residual ATPase activity was fully ascribable to P-gp owing to its complete inhibition by low μM concentrations of Na-orthovanadate. Whereas compound **19** did not show any significant effect (maximum concentration used = 10 μM), compound **18** inhibited P-gp ATPase activity in a concentration-dependent fashion up to 1 μM at which inhibition averaged 60% (Figure 2a). However at higher concentrations inhibition vanished, P-gp ATPase activity recovering at 10 μM **18** even higher values than those observed under control conditions. As shown in Figure 2b, 30 μM verapamil stimulated by about 50% P-gp ATPase activity. **18** at ≤ 1 μM concentrations fully antagonised the verapamil effect.[17] These findings indicate a clear antagonism of **18** towards verapamil activation of P-gp ATPase activity which needs further clarification as to its mechanism. The bimodal effect of **18** on basal P-gp ATPase activity suggests a complex interaction, at ≤ 1 μM concentrations **18** behaving like an inhibitor, at higher concentrations like a substrate of P-gp ATPase; both effects are noteworthy in terms of biological activity.

In summary, a concise strategy for the construction of a new macrolactone **18** with peculiar structural features and high functionalization has been achieved. The effects of macrolactone **18** and dimer **19** on P-glycoprotein ATPase have been investigated by assaying both its basal and verapamil-stimulated ATPase activity. These preliminary data suggest a competitive relationship between **18**/**19** and verapamil. The unique chemical structure and the interesting biological properties of **18**, make it an promising P-gp modulator. The further functionalization of this advanced key intermediate can lead to novel oxygenated Taxuspine X analogues potentially active as MDR reversal agents.

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Keywords: "non-natural" natural product · p-gp modulating agents · taxuspine analogues · yamaguchi macrolactonization

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