Pergamon

Tetrahedron Letters, Vol. 37, No. 36, pp. 6495-6498, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01420-7

Diastereoselective Addition of Grignard Reagents To Azetidine-2,3-dione: Synthesis of Novel Taxol® Analogues

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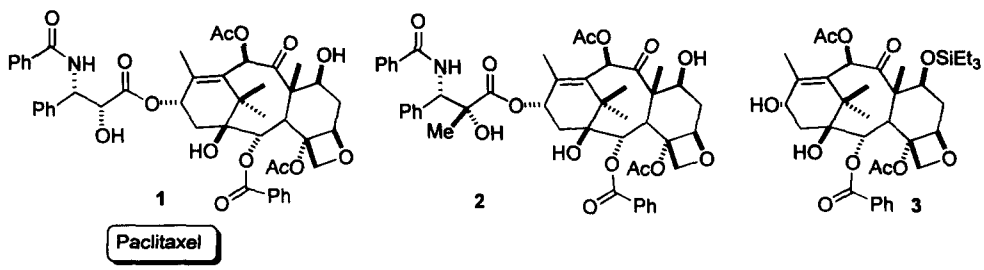
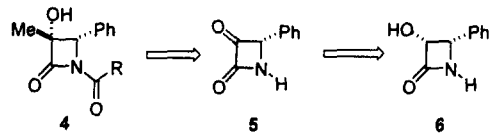
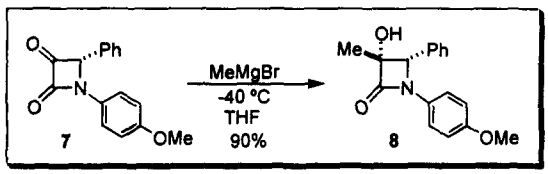
Abstract: Synthesis and cytotoxicity properties of novel C-2' analogues of paclitaxel are described. The analogues were synthesized using Holton's β-lactam approach to append the side chain on baccatin III. The key intermediate to the synthesis of novel analogues was prepared employing an unprecedented stereocontrolled addition of Grignard reagent to a chiral azetidine-2,3-dione.

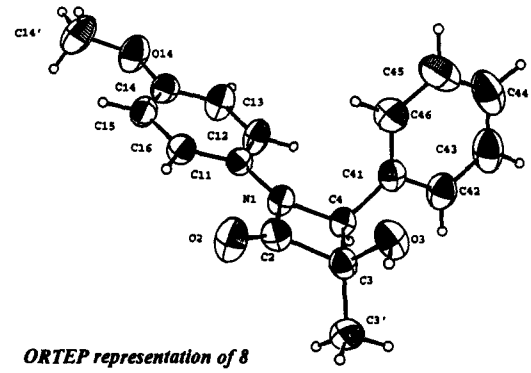
Recent approval of Taxol® (paclitaxel) (**1**) for the treatment of metastatic carcinoma of the ovary has generated considerable interest to synthesize novel analogues with the goal of designing more effective antitumor drugs.[1] Paclitaxel, a naturally occurring diterpene,[2] inhibits cell replication in the mitotic phase of the cell cycle by promoting polymerization of microtubules which are stable and abnormally resistant to depolymerization.[3] To understand the features of the paclitaxel binding site on microtubules and to develop compounds having more desirable properties than the prototype, we were interested in designing analogues with modified C-13 amino acid side chains. Conformational studies on paclitaxel suggest that the C-13 side chain has a high degree of freedom, and therefore, adopts a variety of conformations.[4] To probe the conformation necessary for binding to microtubules, we decided to introduce a methyl group at the C-2' position. This group should create some additional torsional strain (vs. H) for rotation around C-2'/C-3', thus reducing the number of viable conformations, and therefore providing some indirect information on the nature of the active conformation. This letter describes the synthesis, biological evaluation, and NMR studies of our target compound **2**.





We decided to use Holton's chemistry[5] to append the C-13 side chain on 7-(triethylsilyl)baccatin III (**3**)[6]. Therefore, the chiral azetidinone **4** was viewed as an ideal synthon for **2**. The synthesis of **4** was envisioned via an unprecedented addition of methylmagnesium bromide (MeMgBr) to azetidine-2,3-dione (**5**), readily available from **6**.[7] To put our proposal to practice and to determine the stereochemical outcome of the reaction between the Grignard reagent and azetidine-2,3-dione, racemic **7** [8] was treated with MeMgBr in THF at -40 °C for 60 min. Interestingly, the reaction afforded only one diastereomer in 90% yield along with recovered starting material. Preliminary nOe studies indicated the azetidinone to be **8** which was unequivocally confirmed by X-ray analysis.[9] The high degree of diastereofacial selectivity can be attributed to the phenyl group which is directing the transfer of the methyl group to the less hindered face of the carbon-oxygen double bond.

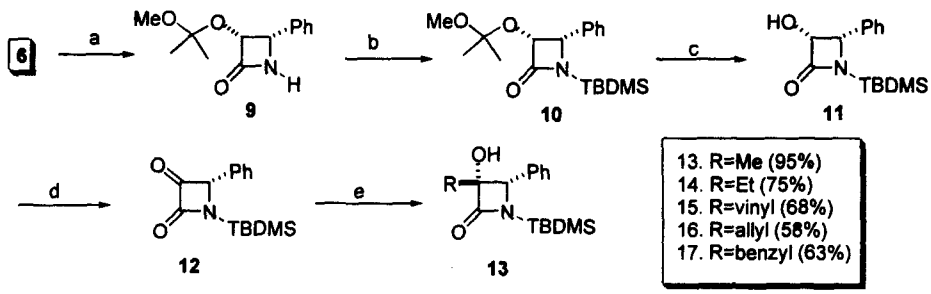




ORTEP representation of 8

Next, we focused on the synthesis of our chiral key intermediate **13**; after protection/deprotection sequence followed by oxidation and the addition of MeMgBr, we were able to synthesize azetidinone **13** from **6** in good yield and high diastereoselectivity (>99%) (Scheme I). Since the 1,2-addition of MeMgBr proceeded with a high diastereoselectivity, we decided to explore the scope of this reaction.

Scheme I

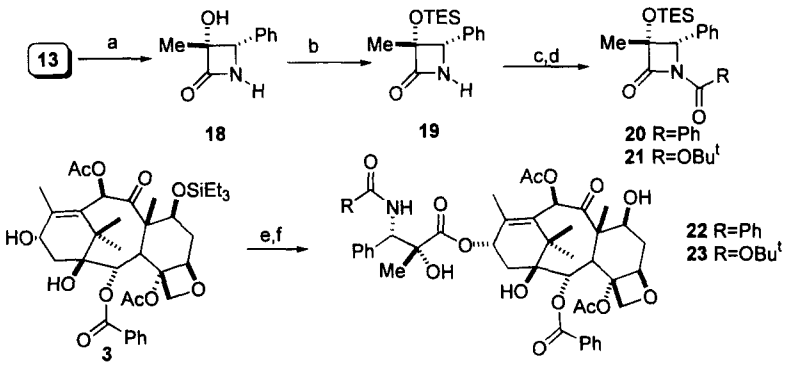


13. R=Me (95%)
14. R=Et (75%)
15. R=vinyl (68%)
16. R=allyl (58%)
17. R=benzyl (63%)

Conditions: a. 2-methoxypropene, ppts, acetone, 20h (90%); b. LiHMDS, TBDMSiCl, -40 °C, THF, 2h (92%); c. 0.1N HCl, THF, rt, 60 min (100%); d. $Me_2S{\cdot}Br_2$, CH_2Cl_2, 0 °C, 2h (80%); e. RMgBr, THF, -40 °C, 2-3h

Interestingly, treatment of **12** with a variety of Grignard reagents afforded 1,2-addition products (**14-17**) as single diastereomers in moderate yields along with **11** (10-20%). The formation of **11** was not surprising as Grignard reagents containing β-proton are known to reduce ketones via a six centered transition state.[10] The reaction was found to be general enough to be utilized in organic synthesis, especially in β-lactam and peptide chemistry.[11] To proceed with the synthesis of our target analogues, the chiral lactam **13** was subjected to a deprotection-reprotection sequence before the *N*-acylation step. Treatment of **3** with *n*-BuLi followed by the addition of β-lactams **20** and **21**, for the elaboration of paclitaxel and docetaxel (Taxotere™) side chains, afforded novel analogues **22** and **23** in good yields. (Scheme II).

Scheme II



Conditions: a. TBAF, THF, 0 °C, 2h (90%); b. TESCl, Imidazole, THF, 16h (91%); c. BzCl, *i*-Pr_2 NEt DMAP(cat), CH_2Cl_2, 0 °C, 2h (65%); d. BOC_2O, *i*-Pr_2NEt, DMAP(cat), CH_2Cl_2, 0 °C, 2h (85%); e LiHMDS, THF, **20** or **21**, -40 °C to 0 °C, 60 min (R=Ph, 55%, R=OBu^t, 68%); f. 6N HCl, CH_3CN, -5 °C, 3h (60-65%).

Compound	IC_{50} (nM) HCT 116	Tubulin Ratio
Paclitaxel	3.2	1.0
22	2.0	0.7
23	1.7	0.7

The 2' methyl paclitaxel **22** and docetaxel **23** analogues were found to be more cytotoxic than the parent paclitaxel when evaluated in an *in-vitro* assay using HCT116 human colon carcinoma cell lines.[12] The analogues also displayed increased binding affinity to microtubules compared to paclitaxel.[13] The ^{1}H NMR of **22** and **23** were found to be similar to paclitaxel in the d_6-DMSO solvent system: strong to moderate nOe interactions were observed between H-3'/OAc-4, H-3'/*o*-Ph-3', and *o*-Ph-3'/OAc-4 in **22** and **23**; similar nOe's have been observed in paclitaxel.[14] Interestingly, these analogues exhibited better microtubule assembly properties (ca. 1.5 fold increase) than paclitaxel; the enhanced potency due to the presence of the C-2' methyl group may be a result of the postulated reduction in the degree of freedom of rotation at the C-2'/C-3' bond, or simply to some additional hydrophobic binding interaction of the methyl group with the microtubule binding site. These interesting C-2' methyl analogues open avenues for further studies which will be reported in the future.[15]

Acknowledgement: We would like to thank Dr. Terry Doyle for valuable suggestions and Mr. Scott Peterson, BMS summer research participant, for his technical assistance.

References and Notes

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9. Crystal data for **8**: the crystal (0.08x 0.27x 0.36 mm), grown from CH_2Cl_2, monoclinic, space group $P2_{1/a}$, a=11.7212(8), b=9.6233(5), c=13.672(1) Å,β=102.86(1)°, V=1503.4(2) $Å^3$, Z=4, Dx=1.252g/cm^3. 3008 refelections (θ = 70°) were measured (ω/2θ) at room temperature on an Enraf-Nonius CAD4 diffractometer with graphic monochromated Cu Kα radiation (λ=1.5418 Å). The structure was solved by direct methods and was refined by full matrix least squares techniques. The final R(F)=0.042 and *w*R(F)=0.037 for 191 variables and 2240 reflections with unit weights.

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13. Tubulin polymerization potency is expressed as an $EC_{0.01}$ value, which is defined as the extrapolated concentration yielding an initial slope of 0.01 absorbancy unit increase per minute for turbidity of a 1 mg/mL solution of calf brain tubulin incubated at 37°C. Relative potencies are expressed as the ratio of the $EC_{0.01}$ of analog divided by $EC_{0.01}$ of paclitaxel.

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(*Received in USA* 27 *February* 1996; *revised* 15 *July* 1996; *accepted* 18 *July* 1996)