Communications to the Editor

12,13-Isotaxanes: Synthesis of New Potent Analogs and X-ray Crystallographic Confirmation of Structure

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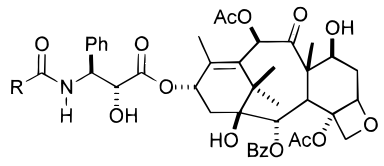
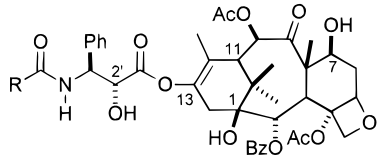
In a previous communication,[1] we described a new structural modification of taxanes in which, in effect, the normal 11,12-double bond of taxol[2] (**1**, R = Ph) has been transposed into the 12,13-position creating enol ester structures as shown for **2**. We have named these isomeric taxanes as the 12,13-isotaxanes. We have also described, in a separate communication,[3] the synthesis



1, normal taxanes



2, 12,13-*iso*-taxanes

of a series of taxol analogs in which modifications of the C-ring such as a 7α-fluoro,[4] a 7*β*,8*β*-methano (cyclopropyl),[5] or a 6,7-olefin[6] were combined with *tert*-butyloxycarbonyl (BOC)[7] or *tert*-butylurea (TBU)[8] variations of the side chain. *In vitro* assay results indicated enhanced antitumor potency in the 6,7-olefinic analogs when compared to the otherwise identical 7-hydroxyl analogs. In this communication, we outline the preparation of a series of 12,13-isotaxol analogs having the 7*β*,8*β*-methano, 6,7-olefin, BOC side chain, and TBU side chain modifications and describe an improved synthesis of these compounds. We give *in vivo* assay results for these new analogs and report an X-ray crystallographic study showing both the enol ester and cyclopropane structural features. As illustrated by the results described below, the 12,13-isotaxanes are chemically stable under essentially all of the reaction conditions to which they have been exposed and they also retain antitumor potency which in some cases is enhanced over that of the normal 11,12-taxanes.

Our first preparation of C-ring-modified 12,13-isotaxanes (see Scheme 1) began with compound **3**, whose synthesis was reported previously.[1] As before,[3] the 2′-hydroxyl group was protected with a troc group, and this protected compound (**4**) was subjected to reaction with methyl-DAST.[9] This reaction produced 7α-fluoro (**5**, 8%), 7*β*,8*β*-methano (**6**, cyclopropyl, 54%), and 6,7-olefin (**7**, 17%) analogs; the ratio of 1:7:2 is significantly different from the ratios of approximately 9:4:1 (fluoro:cyclopropyl:olefin) observed previously.[3] Removal of the the 2′-troc protecting groups from **5**−**7** gave new 12,-13-isotaxol analogs **8**−**10**, respectively. The method of synthesis used for preparation of **8**−**10** has the advantage of generating three new analogs in one reaction, but this becomes a disadvantage when larger quantities of a specific analog are needed for further pharmacological evaluation. Therefore, we have developed the alternate synthesis shown in Scheme 2. This synthetic scheme has additional versatility in that it can be used for the introduction of various side chain amino substituents; of particular interest to us is the TBU modification of the side chain. The synthesis begins with 12,13-isobaccatin III 7-*O*-TES (**11**), obtained from the zinc−acetic acid reduction of 7-*O*-TES-13-ketobaccatin III and used without purification.[1] The enol **11** is acylated with carbobenzyloxy (CBZ) side chain precursor **12**, giving the coupled product **13** (74%) as a mixture of diastereoisomers. The 7-*O*-TES and side chain oxazolidine protecting groups of **13** are removed in acidic methanol to give **14** (87%). A methanolic sodium bicarbonate solution containing **14** was generated during our workup of this reaction, and as the scale of the reaction increased, the resulting increased time involved in the workup led to generation of small quantities (5−15%) of the byproduct **15** in which hydrolysis of the 10-acetate has also occurred.[10] The 2′-hydroxyl group of **14** is protected with a triethylsilyl group (**16**, 86%), and the 7-*O*-triflate **17** (97% crude yield) is prepared[3] and serves as the key intermediate for the subsequent synthetic steps.

The triflate **17** is used without purification for conversion to the 7*β*,8*β*-methano intermediate **18** by stirring with silica gel ($ClCH_2CH_2Cl$, 60 °C). The cyclopropane formation giving **18** (67% from **16**) is accompanied by some elimination to give the 6,7-olefin **19** (18% from **16**), which is combined with additional **19** obtained below. The next steps in the synthesis are carried out without chromatographic purification until the final analog is obtained. First, the Cbz group of **18** is removed by hydrogenolysis to give the amine **20**. Reaction of the amine with BOC anhydride gives the BOC side chain compound **21** from which the 2′-TES protecting group is removed with $Et_3N{\cdot}(HF)_3$ to give the analog **9** (70% from **18**). Alternately, reaction of amine **20** with *tert*-butyl isocyanate produces the TBU side chain compound **22** from which removal of the 2′-*O*-TES protecting group gives the analog **23** (81% from **18**).

Crystals of **9** suitable for X-ray crystallography were prepared, and the single-crystal X-ray structure provided confirmation of the unusual enol−ester bond in

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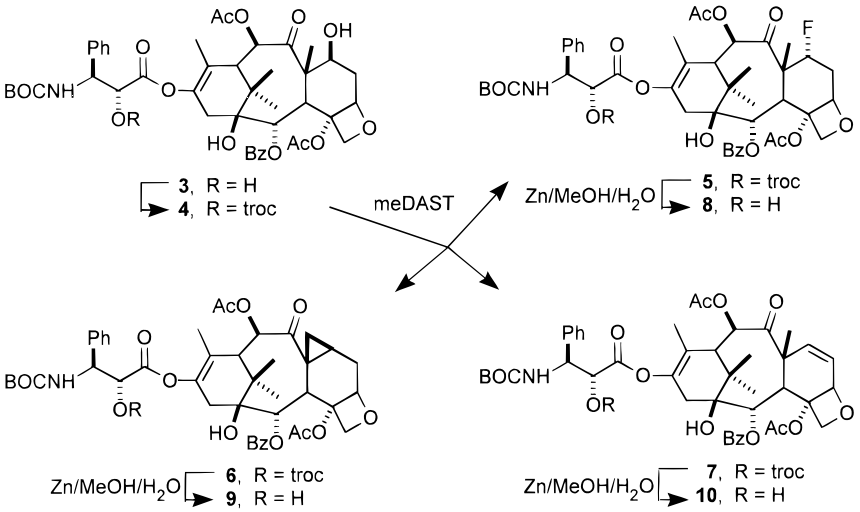
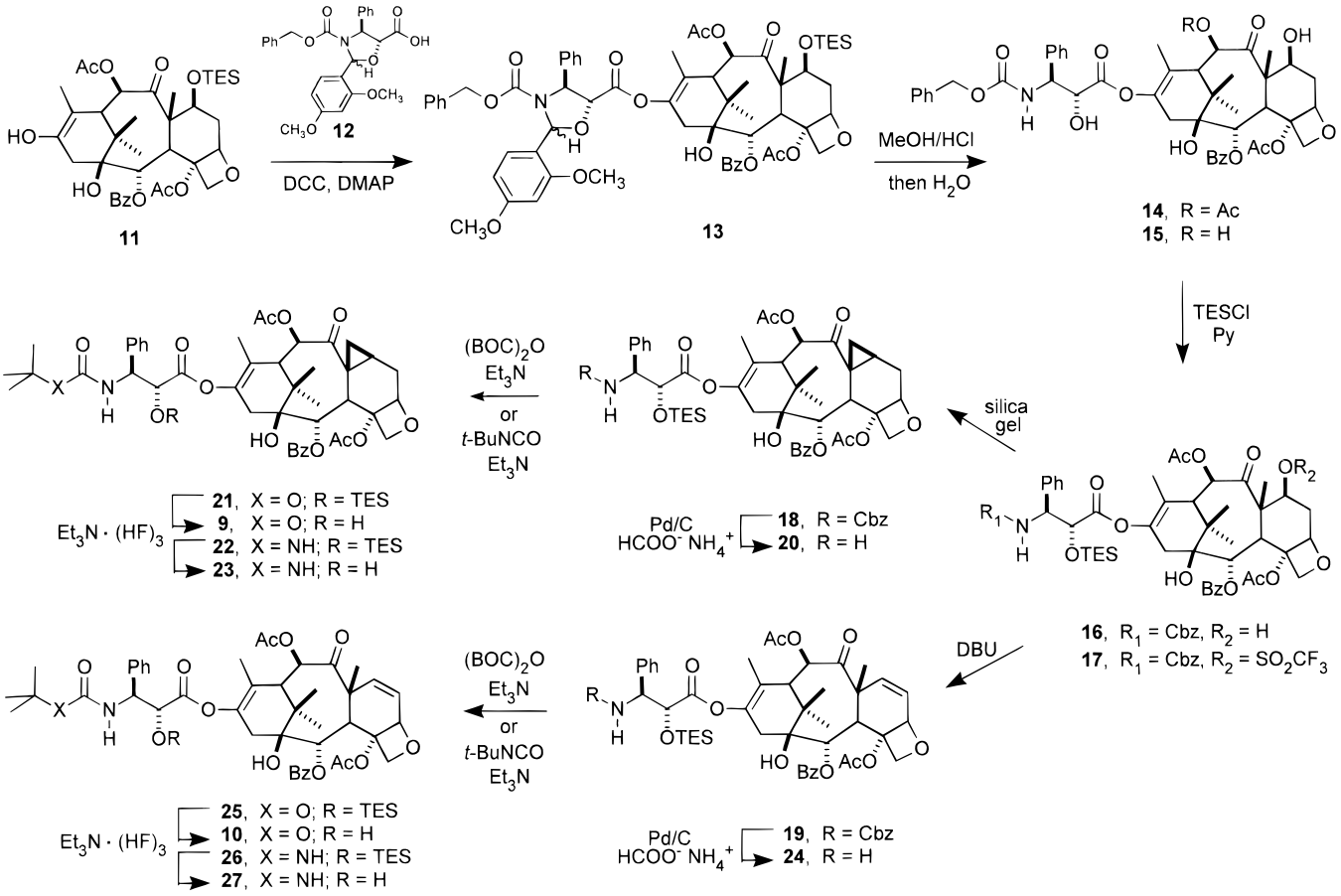
Scheme 1



Scheme 2



this molecule. The X-ray structure also confirmed the presence and configuration assigned to the cyclopropane moiety in this compound.[11]

When the triflate **17** is subjected to reaction with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), the 6,7-olefin **19** is generated in 68% yield for the two steps from **16**. Removal of the Cbz group from **19** by hydrogenolysis gives the amine **24** which is acylated with BOC anhydride to produce the BOC side chain compound **25**. Removal of the 2′-*O*-TES protecting group leads to analog **10** (87% from **19**). Likewise, acylation of amine **24** with *tert*-butyl isocyanate gives the TBU side chain compound **26** from which removal of the 2′-*O*-TES group gives analog **27** (89% from **19**).

Several biological assays were used to characterize the anticancer potential of the new analogs **9**, **10**, **23**, and **27** in comparison to taxol and taxotere. Preparations of semipurified bovine brain microtubule protein were incubated with analogs at 20 °C, and the rate of polymerization of tubulin dimers into microtubules was determined by light scattering.[12] Results (not presented) show that the potency of the four new isotaxol analogs is comparable to that of taxol and taxotere in promoting tubulin polymerization. This finding is

Table 1. Assay Results for 12,13-Isotaxol Analogs Compared to Taxol and Taxotere

	in vitro assays		*in vivo* assay	
			ip-implanted B16 melanoma[c]	
compd	human A2780 ovarian carcinoma cell growth,[a] IC_{50}, nM	murine B16 melanoma cell mitotic block,[b] conctn inducing 20% mitotic index, nM	optimal dose (total), ng/kg, days 1 & 3, iv	%T/C[d]
taxol	3.4 ± 0.6[e]	28	250[f]	161[f]
taxotere	1.8 ± 0.4[e]	15	100	177 ± 9[g]
9	1.9	7	25	184, 210[h]
23	0.6	10	50	213, 225[h]
10	1.2	10	50	181, 181[h]
27	0.5	10	25, 50[h]	232, 241[h]

[a] Human A2780 ovarian carcinoma cells (ref 13) were cultured in 96-well plates with 72 h drug exposure. Cell growth was estimated by an MTT dye reduction method. [b] Mitotic index was determined by a method adapted from ref 14. [c] Method adapted from ref 15. [d] %T/C = ratio of median survival time in the group receiving the optimal dose to median survival time in the vehicle control group × 100. [e] Mean ± SD, $n = 14$. [f] Dosing schedule was days 1−5. [g] Mean ± SD, $n = 17$. [h] Individual values from two experiments.

consistent with microtubule overstabilization, a biochemical effect which is implicated in cytotoxicity of the taxanes.[12] An *in vitro* cytotoxicity assay using human A2780 ovarian cells in culture[13] shows that the potency of the four analogs is about 2−7 times greater than that of taxol (see Table 1).

Measurements of mitotic index[14] in drug-treated B16 melanoma cells in culture show dose-dependent accumulation of cells in mitosis for taxol, taxotere, and the four analogs (see Table 1). These results establish that the mechanism of cytotoxicity of the analogs at the cellular level is consistent with their biochemical effects and with previously characterized taxanes. Introduction of an enol−ester bond between the baccatin nucleus and the side chain of these analogs has not modified the mechanism by which they express cytotoxicity.

In B6D2F1 mice bearing ip-implanted B16 melanoma,[15] the four isotaxol analogs lengthened survival time by about 80−140% over vehicle-treated control mice (see Table 1). Optimal antitumor doses of the isotaxol analogs are 5−10-fold lower than optimal doses of taxol and 2−4-fold lower than that for taxotere. Analogs **23** and **27** appear to be significantly more efficacious at their optimal doses than both taxol and taxotere.

Supporting Information Available: Experimental details for preparation of and analytical data for compounds **4**−**11**, **13**, **14**, and **16**−**27** (12 pages). Ordering information is given on any current masthead page.

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