Synthesis of Taxoids with Improved Cytotoxicity and Solubility for Use in Tumor-Specific Delivery

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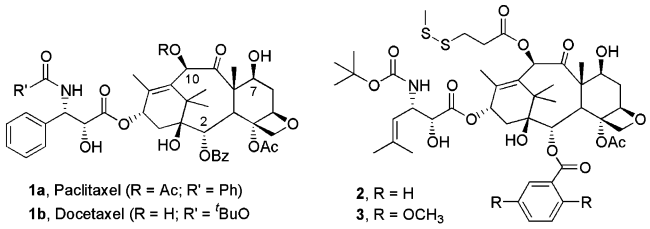
Abstract: To develop effective taxane−antibody immunoconjugates, we have prepared a series of modified taxanes that have both improved toxicity and solubility in aqueous systems as compared to paclitaxel (**1a**). These taxanes have been modified at either the C-10 or C-7 position and were found to be very cytotoxic against both normal and multi-drug-resistant (MDR) cells, as well as up to 30 times more soluble than paclitaxel in various buffer systems.

Recently, the potential for the use of monoclonal antibodies in cancer therapy has been recognized with the approvals of Rituxan,[1] for lymphoma, Herceptin,[2] for breast cancer and Erbitux,[3] for colorectal cancer. While these antibodies have proven their therapeutic value, they are only moderately cytotoxic and therefore are often more effective when used in combination with other chemotherapeutic drugs. Nevertheless, a vast majority of monoclonal antibodies are only weakly potent and thus not useful as anticancer agents. However, the exquisite selectivity of these antibodies for binding to tumor-associated antigens renders them excellent vehicles for the targeted delivery of highly cytotoxic small molecular weight drugs.[4] Linkage of cytotoxic drugs to monoclonal antibodies takes advantage of the long in vivo half-life of the antibody (typically several weeks for a humanized antibody). The drug conjugate remains nontoxic during circulation and is activated only upon binding to the tumor cell surface, followed by internalization and release of the fully active drug inside the target cell.

Paclitaxel (**1a**)[5] and docetaxel (**1b**)[6] are among the most active anticancer agents in clinical use today, being commonly used against ovarian cancer, breast cancer, and non small cell lung cancer. Despite their contribution to chemotherapy, these taxanes suffer a number of undesirable difficulties, such as the poor selectivity for killing normal vs cancer cells, the development of multidrug resistance (MDR), and a severe lack of solubility in aqueous systems. While the issue of selectivity can potentially be addressed through the development of an immunoconjugate selective for a tumor marker on the surface of a cancer cell,[7] it remains of interest to develop taxanes that overcome the MDR phenotype while possessing increased water solubility to facilitate conjugation to antibodies in aqueous solutions.

Previously, we have described the synthesis of disulfide-containing taxanes such as **2** that were linked to monoclonal antibodies generating an immunoconjugate.[8] In addition, our initial structure−activity relationship (SAR) studies revealed that certain C-2 modified taxanes (i.e. **3**) were found to be between 3 and 20-fold more potent than **1b** against various tumor cell lines.[9] In light of this increased potency we became interested in continuing to explore the SAR of these disulfide-containing taxanes with the goal of developing highly cytotoxic taxanes with improved aqueous solubility.



To pursue these efforts, we became interested in incorporating both heterocyclic and poly(ethylene glycol) (PEG) substituents within the disulfide linker to explore their effects on potency and solubility. Previously, it has been shown that the introduction of a piperazinyl substituent at the C-10 position had a dramatic effect on the solubility of various taxanes.[10] As a result of these findings, we were interested in preparing taxoids in which a piperazinyl ring was incorporated between the C-10 position and the disulfide linker of taxanes such as **2** and **3**. As shown in Scheme 1, taxane **7** was prepared through the coupling of the previously described taxane **4**[8] with *p*-nitrophenyl chloroformate followed by the addition of piperazinyl disulfide **6** to give the desired C-10 carbamate. Removal of the silyl protecting groups with HF/pyridine gave the desired taxane in good overall yield. In addition, to further explore the effects on cytotoxicity of taxoids with a 3,5-dimethoxybenzoyl substituent at C-2, we also prepared taxoid **8** using a slightly different procedure. Thus, treatment of taxane **5**[9] with carbonyl diimidazole (CDI) followed by the addition of **6** and subsequent removal of the silyl protecting groups gave **8** in good isolated yields.

In addition to looking into the incorporation of a piperazinyl substituent at C-10, we were also interested in preparing taxanes that possessed a small poly(ethylene glycol) (PEG) linker to enhance the solubility.[11] In light of this, we prepared two small PEG linkers containing either 4- or 10-ethyleneoxy (4-PEG or 10-PEG) units that still had the desired disulfide moiety present. The synthesis of the 4-PEG disulfide containing carboxylic acid **12** is shown in Scheme 2. The synthesis began with the treatment of tetraethylene glycol (**9**) with *tert*-butyl acrylate in the presence of 1 mol % Na followed by tosylation of the remaining free hydroxyl group to give **10**.[12] Reaction of **10** with the potassium salt of *O*-ethyl xanthic acid in ethanol gave xanthate **11** in 92% yield.[13] Conversion to the free thiol was accomplished with the use of hydroxylamine, followed by the generation of the methyl disulfide using methyl methanethiosulfonate in a phosphate buffer. Finally, removal of the *tert*-butyl ester in the presence of TFA and triethylsilane gave the desired free acid **12** in good overall yield.

The 10-PEG linker **16** was prepared through a similar path. Thus, treatment of hexaethylene glycol (**13**) with

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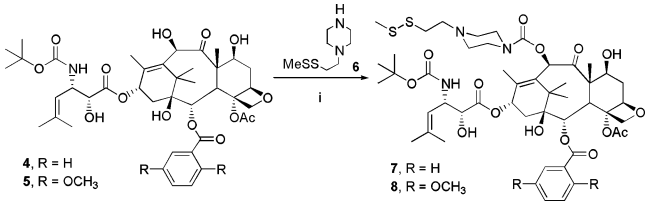
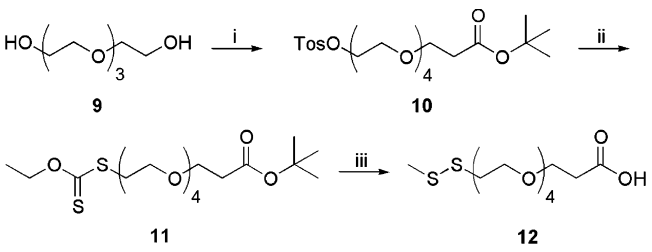
Scheme 1[a]



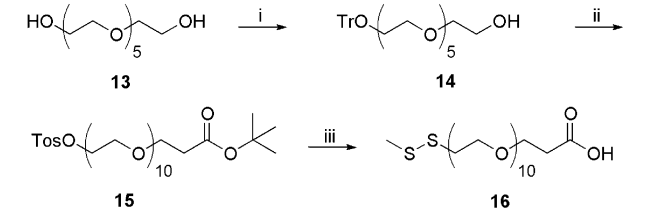
[a] Reagents and conditions: (i) for **7**, (a) Et_3N, *p*-nitrophenyl chloroformate, then **6**, 71%. (b) HF/pyridine, CH_3CN, 73%. For **8**, (a) CDI, imidazole, pyridine, 60 °C, then **6**, 60 °C 14 h, 63%. (b) HF/pyridine, CH_3CN, 92%.

Scheme 2[a]



[a] Reagents and conditions: (i) (a) 1 mol % Na, *tert*-butyl acrylate, 20 h, 78%. (b) tosyl chloride, Et_3N, DIC, CH_3CN, 80%. (ii) $EtOCS_2K$, EtOH, rt, 4 h, 92%. (iii) (a) 0.5 M $H_2NOH{\cdot}HCl$ (pH = 7.5), $EtOH/H_2O$. (b) $CH_3SO_2SCH_3$, 1.0 M NaH_2PO_4 (pH = 7.0), EtOH, 50% two steps. (c) TFA, Et_3SiH, CH_2Cl_2, 67%.

Scheme 3[a]

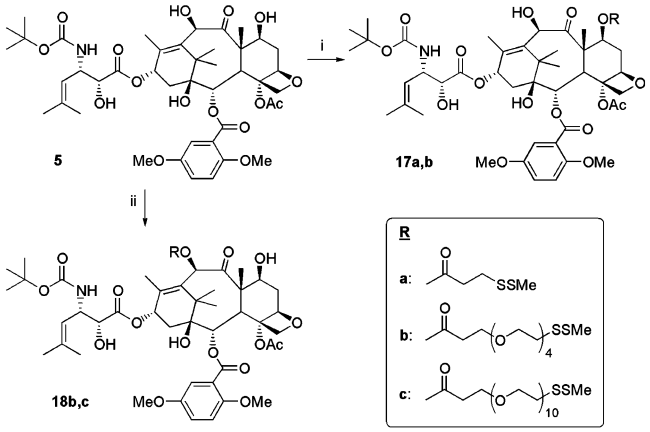


[a] Reagents and conditions: (i) Trityl chloride, pyridine, 74%. (ii) (a) $tBuO^-K^+$, toluene, then **10**, 67%. (b) H_2, 10% Pd/C, MeOH, 77%. (c) Tosyl chloride, Et_3N, DIC, CH_3CN, 86%. (iii) (a) $EtOCS_2K$, EtOH, rt, 86%. (b) 0.5 M $H_2NOH{\cdot}HCl$ (pH = 7.5), $EtOH/H_2O$. (c) $CH_3SO_2SCH_3$, 1.0 M NaH_2PO_4 (pH = 7.0), EtOH, 60% two steps. (d) TFA, Et_3SiH, CH_2Cl_2, 82%.

trityl chloride gave the monoprotected glycol **14**.[14] Treatment of **14** with potassium *tert*-butoxide followed by the addition of the tosylated PEG **10** gave the desired 10-PEG. Removal of the trityl protecing group with hydrogen over Pd/C, followed by reaction with tosyl chloride, gave **15** which was then easily converted to the required 10-PEG disulfide linker **16** following the same procedure described earlier for the conversion of **10** to **12**.

With the desired PEG coupling units in hand, we next turned our attention to the synthesis of taxoids **18b**,**c** as well as their C-7 modified counterparts **17a**,**b**. As shown in Scheme 4, the approach to preparing taxane **17a** began by treating **5** with 5% HCl in ethanol to give the free hydroxyls at both the C-7 and C-10 positions. Treatment of this intermediate with 1 equiv of methyldithioproprionic acid in the presence of EDC (2 equiv) and DMAP (1 equiv) resulted in selective acylation at the C-7 position giving the desired ester in 75% isolated yield. Removal of the silyl protecting groups with HF/pyridine gave the desired taxoid **17a** in good yield. Taxoid **17b** was prepared following an identical procedure to that described for **17a** using acid **12**, giving the

Scheme 4[a]



[a] Reagents and conditions: (i) (a) 5% HCl in ethanol, 79%. (b) EDC (2 equiv), DMAP (1 equiv), acid **a** or **b** (1 equiv), **a**: 75%, **b**: 51%. (c) HF/pyridine, CH_3CN, **a**: 83%, **b**: 88%. (ii) (a) DIC (3−10 equiv), DMAP (1−3 equiv), CH_2Cl_2 or toluene, acid **b**, or **c** (3−10 equiv), rt or 60 °C, **b**: 72%, **c**: 68%. (b) HF/pyridine, CH_3CN, **b**: 74%, **c**: 67%.

Table 1. In Vitro Cytotoxicity of Taxoids

	IC_{50}, nM[a]	
taxoid	A549[b]	MCF7[c]
paclitaxel, **1a**[d]	3.0	1.7
docetaxel, **1b**[d]	1.0	1.0
2	0.80 ± 0.02	1.0 ± 0.04
3	0.09 ± 0.01	0.05 ± 0.016
7	1.10 ± 0.06	1.0 ± 0.1
8	0.30 ± 0.02	0.039 ± 0.015
17a	0.13 ± 0.03	0.073 ± 0.006
17b	1.0 ± 0.05[e]	0.32 ± 0.004[e]
18b	0.29 ± 0.02	0.063 ± 0.015
18c	1.1 ± 0.13[e]	0.58 ± 0.07[e]

[a] The concentration of compound that inhibits 50% of the growth of cancer cell line after 72 h of drug exposure and are expressed as the mean ± SD based on three separate determinations. [b] Non small cell lung carcinoma. [c] Human breast carcinoma. [d] See ref 8. [e] IC_{50} based on two separate determinations.

desired C-7 4-PEG-modified taxoid. The C-10-modified taxoids **18b**,**c** were prepared by treating **5** with the desired carboxylic acid (**12** or **16**) in the presence of DIC and DMAP. Reaction with the 10-PEG acid **16** required that the reaction be heated to 60 °C for an extended period of time (2−5 days) in order for the reaction to go to completion, but no decomposition of the reaction was observed. Finally, removal of the protecting groups with HF/pyridine gave taxoids **18b**,**c** in good overall yield.

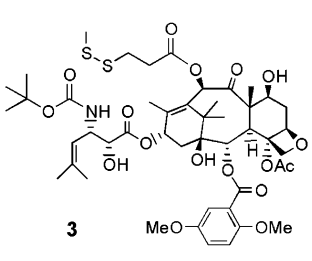
The biological activities of taxanes **7**, **8**, **17a**,**b**, and **18b**,**c** were measured against the non small cell lung carcinoma, A549, and the human breast carcinoma cell line, MCF-7. As was seen with taxoids **2** and **3**,[9] the effects of modification at C-2 were found to be critical for achieving high potency. As shown in Table 1, taxoid **7**, with a C-2 benzoyl group, was found to be very similar in potency to parent taxoid **2**. However, the C-2 dimethoxybenzoyl-modified taxoid **8** was found to be significantly more potent, being some 25-fold more potent against MCF7 cells.

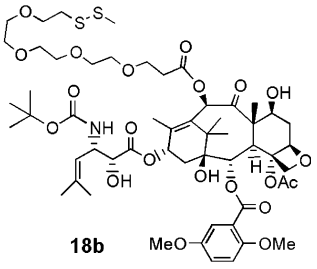
Incorporation of the disulfide linker at the C-7 position was also shown to be well tolerated toward potency, as **17a** was found to be between 6- and 13-fold more potent than **2** against both cell lines as a result of the C-2 modification. The results for the PEG-ylated taxoids

were found to be quite interesting. Modification of C-10 with either the 4-PEG (**18b**) or the 10-PEG (**18c**) disulfide gave taxoids that showed high potency. It was anticipated that as the size of the PEG was increased, there would be a decrease in potency, and indeed the 10-PEG taxane **18c** is about 10-fold less potent than the corresponding non-PEGylated taxoid **3** against both cell lines tested. Interestingly, the 4-PEG-containing taxane **18b** shows only a slight loss in activity as compared to **3** but had the potential to be substantially more soluble as a result of the presence of the short PEG linker.

Since the conjugation of cytotoxic drugs with antibodies must be carried out in aqueous systems, only small amounts of organic solvents may be used without an effect on the antibody. Typically, suitable amounts of organic cosolvents used vary from about 5 to 10%. To test the enhancement in solubility for these modified taxoids, we chose a system that contained 10% of an organic solvent (DMA, DMSO, or ethanol) in the typical conjugation media (0.1 M potassium phosphate buffer with 1 mM EDTA (pH = 6.5)). Thus, 200 μg of taxane was dissolved in 50 μL of organic solvent and then diluted to a volume of 500 μL with buffer. The mixture was then vortexed and centrifuged, and the supernatant was analyzed spectrophotometrically to determine the concentration of taxane in solution.

As shown in Table 2, the solubility of paclitaxel in these three solvent systems is very low, ranging between 4 and 8 μg/mL in all. The modified parent taxanes **3** and **17a** were both found to have solubility that was roughly the same as that which was observed for paclitaxel. Taxoid **8** was found be from 4 to 8-fold more soluble as a result of the piperazinyl group at C-10. Interestingly, the taxanes in which the PEGs had been incorporated showed very different results. Taxane **17b**, with the 4-PEG at C-7, was shown to gain only very minimal advantages in solubility over **17a**, being at best only 6-fold more soluble. However, the taxanes modified at C-10 were found to show a very impressive increase in solubility as a result of these short PEG linkers. As seen with **18b**, the addition of the short 4-PEG linker was found to increase the solubility approximately 10 to 14-fold over that of **1a**. Taxoid **18c** displayed the highest degree of solubility with a 32-fold increase in solubility over **1a** in 10% EtOH; however, it's loss in potency makes it an unlikely candidate for further development.





On the basis of these results, we decided to further examine the cytotoxicity profile of taxoids **3** and **18b**. It has previously been reported that a series of C-2-modified taxoids were shown to be highly cytotoxic against both drug-sensitive and drug-resistant cell lines.[15] Therefore, we tested paclitaxel, **3** and **18b** against three different cancer cell lines (MCF7, LCC6/P9, and Namalwa) and their corresponding drug-resistant cancer cell lines (NCI ADR RES, LGM MDR, Namalwa MDR). As shown in Table 3, both taxanes were found to be significantly more potent than paclitaxel against all three pairs of cell lines tested. In fact, taxane **3** showed a greater than 50-fold increase in potency against the drug-resistant breast cancer cell line NCI ADR RES as compared to paclitaxel. It is interesting to note that taxane **18b**, despite the incorporated 4-PEG linker, maintains potency that is basically equivalent to that of **3** against both drug-sensitive and drug-resistant cell lines.

Table 2. Solubility of Selected Taxoids

taxoid	solubility (μg/mL)[a] 10% DMA	10% DMSO	10% EtOH
paclitaxel, **1a**	6	8	4
3	8	14	10
8	26	26	32
17a	10	4	2
17b	11	17	13
18b	79	82	55
18c	97	110	127

[a] Solubility measurements are expressed as the mean of three determinations. Standard deviations were generally found to be less than ± 5% for all cases.

Table 3. Cytotoxicity against Drug-Resistant Cell Lines

cell line	IC_{50} nM[a] paclitaxel (**1a**)	**3**	**18b**
MCF7[b]	1	0.4	0.5
NCI ADR RES[c]	830	15	19
R/S[d]	830	37.5	38
LCC6/P9[e]	2.2	1	1.6
LGM MDR[f]	110	4	18
R/S[d]	50	4	11.25
Namalwa[g]	1.6	0.53	0.45
Namalwa MDR[h]	15	0.65	0.9
R/S[d]	9.4	1.2	2

[a] The concentration of compound that inhibits 50% of the growth of cancer cell line after 72 h of drug exposure. [b] MCF7: human breast carcinoma. [c] NCI ADR RES:MDR human breast carcinoma. [d] Ratio of activities, drug-resistant (*R*) vs drug-sensitive (*S*) cell lines. [e] LCC6/P9: human breast carcinoma. [f] LGM MDR: MDR human breast carcinoma. [g] Namalwa:Burkitts lymphoma. [h] Namalwa MDR: MDR Burkitts lymphoma.

We have prepared a series of highly potent taxanes modified at C-2 with a 2,5-dimethoxybenzoyl group. In addition, these taxanes were found to be up to 50-fold more potent than paclitaxel against various drug-resistant cancer cell lines. With the additional incorporation of either heterocyclic or PEG linkers, these taxoids were also found to be significantly more soluble in an aqueous buffer system without compromising potency. Taxane **18b** was found to be highly potent and up to 14 times more soluble than paclitaxel. Efforts to further expand on the SAR of these taxanes, as well as the development of taxane−antibody immunoconjugates, are currently underway and will be reported in due course.

Acknowledgment. The authors thank Dr. Wei Zhang and Dr. Lintao Wang of ImmunoGen, Inc. for obtaining HRMS data for all compounds.

Supporting Information Available: Detailed synthetic procedures and characterization data for compounds **7**, **8**, **12**,

16, **17**, **18**, and their intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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