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Synthesis of potent taxoids for tumor-specific delivery using monoclonal antibodies

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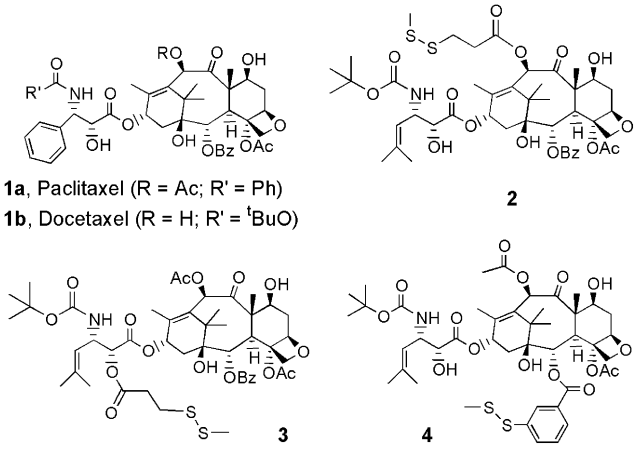
Abstract—The targeted delivery of taxoids, in the form of taxane–antibody immunoconjugates, requires the preparation of taxoids containing moieties suitable for their conjugation to monoclonal antibodies. A series of taxoids incorporating a disulfide-containing linker at various positions of the taxoid framework have been prepared to investigate the most suitable position for conjugation. A second series of taxoids modified at the C-2 position aimed at increasing the potency of these taxanes has also been prepared.

Cancer chemotherapy has long been based on the expectation that cytotoxic agents will preferentially kill rapidly proliferating tumor cells as opposed to healthy normal cells. However, in humans, these agents display only a modest selectivity for the tumor cells. Thus, administration of a large dose of the chemotherapeutic agent is required to ensure delivery of sufficient drug to the tumor site to be clinically effective. Such high doses often result in severe toxicity, and unacceptable damage to normal tissues. Thus, it remains of utmost interest to develop new cytotoxic agents with greater selectivity towards tumor cells and lower systemic toxicity.

The discovery that many tumors over-express specific determinants on their cell surface suggests the possibility of selectively targeting tumor cells.[1] Thus, a tumor-associated antigen could be targeted with a monoclonal antibody (mAb) that has shown a high specificity and binding affinity for the antigen. The value of monoclonal antibodies in cancer therapy has been recognized with the recent approvals of both Rituxan®,[2] for lymphoma and Herceptin®,[3] for breast cancer. Despite their high specificity for antigens most naked antibodies are only weakly cytotoxic and thus, are not very effective as anti-cancer agents. However, the antigen-selectivity of these antibodies render them excellent vehicles for the targeted delivery of cytotoxic drugs to the tumor. The linkage of a potent cytotoxic drug to an antibody, such as through a disulfide bond, generates an immunoconjugate that is stable and nontoxic in circulation in vivo. The conjugate is specifically activated upon binding to the tumor surface, followed by internalization and subsequent cleavage of the disulfide bond between the antibody and the drug to release fully active drug inside the tumor cell.[1] In fact, immunoconjugates have already been shown to exhibit high potency and selectivity with



Keywords: Taxoid; Antibody conjugate; Tumor specific; Targeted delivery.
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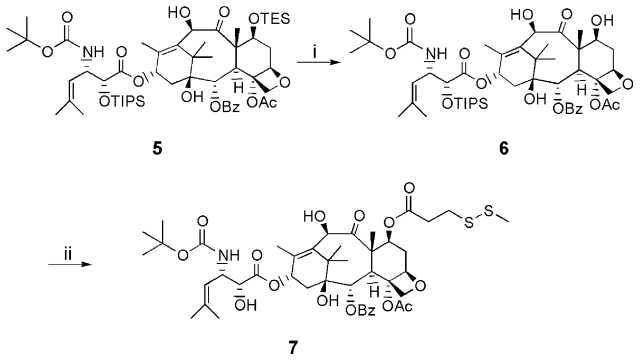
cytotoxic drugs such as calicheamicin,[4] maytansinoids,[5] and the CC-1065 analogues.[6]

Paclitaxel (**1a**)[7] and docetaxel (**1b**)[8] are two of the most commonly used anticancer agents found in the clinic today, with activity against ovarian, breast, and non-small cell lung cancers. Despite their contribution to chemotherapy, the therapeutic potential of these taxanes is limited by their nonspecific toxicity towards normal cells, as well as the development of multi-drug resistance. The tumor-selectivity, and hence, the overall therapeutic index of taxanes can potentially be greatly enhanced by targeted delivery using monoclonal antibodies. Recently, we and others have reported on the development of the first generation of taxane–antibody immunoconjugates.[9–11]

In our initial studies to prepare these conjugates we synthesized a series of taxanes (i.e. **2**–**4**) incorporating a disulfide-containing linker at various positions of the taxoid framework.[9] The immunoconjugates were then prepared by cleavage of the disulfide to the corresponding thiol, followed by disulfide exchange with an appropriately modified antibody.[9] From these studies it was apparent that the site of incorporation of the disulfide-containing linker could greatly affect the potency of the drug. Thus, incorporation of the linker at C-2 (taxoid **4**) or C-2′ (taxoid **3**) resulted in an unacceptable loss in potency.

As a result of these studies, we wanted to investigate the incorporation of the desired disulfide at other positions of the taxoid framework. When targeting tumor cells using immunoconjugates, one must be aware that there are limitations based on (a) the number of available antigens present on the cell surface (typically 10^5 antigen molecules per cell) and (b) the extent of internalization of the bound conjugate. Based on these limitations, and with data from previous studies with immunoconjugates, it was determined that the drug component had to be highly potent with an IC_{50} value between 10^{-10} and 10^{-11} M in order to be effective.[1] Thus, another objective of the current study was to develop disulfide-containing taxanes, which possessed considerably higher potency than that of paclitaxel or docetaxel.

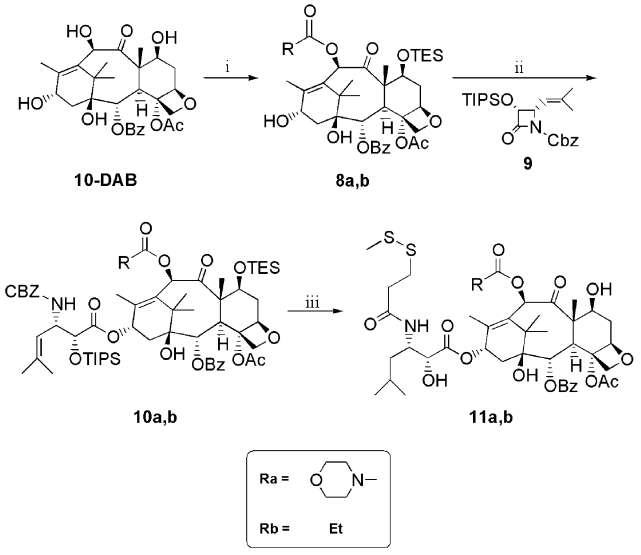


Scheme 1. Reagents and conditions: (i) 0.1 M aq HCl, rt, 16 h, 85%; (ii) (a) 3-methyldithio-propionic acid, DMAP, EDC, rt, 74%, (b) HF/pryidine, CH_3CN, 88%.

In order to further look into the positional scanning of the disulfide linker, taxoid **7**, bearing a disulfide containing linker at C-7, was prepared as shown in Scheme 1. Thus, taxane **5**[9] was treated with 0.1 N HCl to remove only the triethylsilyl (TES) protecting group followed by the selective coupling at C-7 with 3-methyldithio-propionic acid using EDC as the coupling agent. Removal of the remaining triisopropylsilyl (TIPS) protecting group with HF/pyridine gave taxoid **7** in high yield.

We next looked at the incorporation of the disulfide linker at the C-3′*N*-position. As shown in Scheme 2, we prepared two C-10 modified baccatins (**8a**,**b**), which had previously been shown to possess excellent cytotoxicity against five different human cancer cell lines.[12] Using the β-lactam synthon method[12–14] we coupled β-lactam **9**, possessing a benzyloxycarbonyl (Cbz) protecting group, with the baccatins to give taxanes **10a**,**b**. Treatment of these taxanes with hydrogen in the presence of Pd/C resulted in removal of the Cbz protecting group, as well as reduction of the alkene at the C-3′ position, to generate the free amine. Acylation of the amine with 3-methyldithio-propionic acid in the presence of EDC, and subsequent removal of the silyl protecting groups using HF/pyridine completed the synthesis to give taxanes **11a**,**b** in high yield.

The biological activity of taxanes **7**, **11a**, and **11b** along with those of the taxoids reported earlier,[9] are shown in Table 1. These compounds represent a series of taxoids modified with the desired disulfide linker at five different positions around the taxoid framework. Comparison of the corresponding cytotoxicities should provide insight as to which of these five positions proves to be most acceptable for the incorporation of this new handle for the development of immunoconjugates. Interestingly, modification of the C-3′*N*-position (**11a**,**b**) was found to be completely detrimental to cytotoxicity against both



Scheme 2. Reagents and conditions: (i) see Ref. 12; (ii) **9**, LiHMDS, THF, −40 °C, 30 min, 83%; (iii) (a) 10% Pd/C, 1 atm H_2, rt, 95%, (b) 3-methyldithio-propionic acid, DMAP, EDC, rt, 82%, (c) HF/pyridine, CH_3CN, 86%.

Table 1. In vitro cytotoxicity of taxoids

Taxoid	IC_{50} nM[a]	
	A431[b]	A-549[c]
Paclitaxel, **1a**		3.0
Docetaxel, **1b**		1.0
2	0.5	0.8
3	>3.0	0.9
4	>3.0	>3.0
7	0.3	0.2
11a	2.8	>3.0
11b	>3.0	>3.0

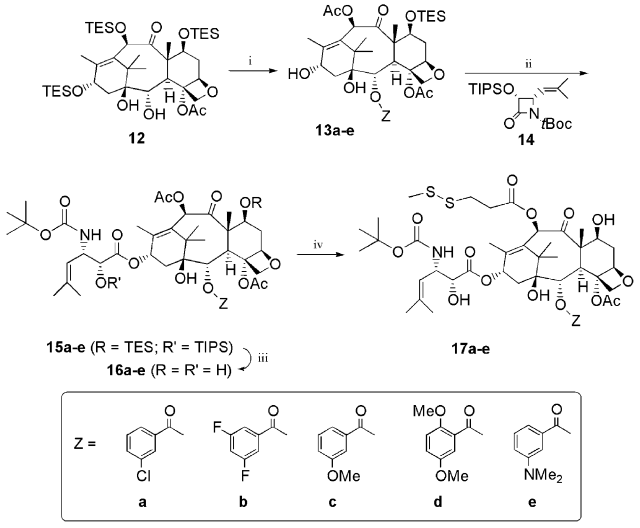
[a] The concentration of compound that inhibits 50% of the growth of cancer cell line after 72 h of drug exposure.
[b] Human epidermoid carcinoma.
[c] Nonsmall cell lung carcinoma.

cell lines, as was modification at C-2 (**4**). However, incorporation of the disulfide linker at either the C-10 (**2**) or C-7 (**7**) positions was found to be well tolerated, giving taxanes with cytotoxicity greater than that of either paclitaxel or docetaxel against the nonsmall cell lung carcinoma cell line A-549. This data is in agreement with previous SAR studies that found that modifications in the northern part (C-7–C-10) of the molecule were generally found to be better tolerated toward cytotoxicity.[15]

While these taxoids were indeed more potent than paclitaxel, they did not possess the desired toxicity to be effective as immunoconjugates. It has previously been shown that substitution of the C-2 benzoyl group of paclitaxel or docetaxel with other substituted aromatic groups gave taxoids with exceptional activities.[16,17] In our own studies we have prepared a series of C-2 modified second-generation taxoids, which possessed subnanomolar activities against both drug sensitive and drug resistant cell lines.[18] In light of these findings we set out to prepare a series of C-2 modified disulfide containing taxoids with increased potency for the development of taxane–mAb immunoconjugates.

As shown in Scheme 3, the synthesis of the C-2 modified baccatin precursors began by coupling of baccatin precursor **12**[18] with various carboxylic acids (**a**–**e**) in the presence of either DCC/4-pp[17] or DIC/DMAP[18] to give the C-2 modified baccatins **13a**–**e** after a few functional group manipulations as previously described.[18] Coupling of baccatins **13a**–**e** with β-lactam **14**[12] using Li-HMDS gave the fully protected taxoids **15a**–**e**. Removal of the silyl protecting groups using HF/pyridine gave the final C-10 acetyl taxoids **16a**–**e** in high yield.[19] In order to prepare the disulfide containing taxoids, the C-10 acetyl group of **15a**–**e** was removed using hydrazine monohydrate[20] in ethanol followed by esterification of the free hydroxyl with 3-methyldithio-propionic acid. Finally, the silyl protecting groups were removed to give taxoids **17a**–**e**, which now have the desired disulfide linker attached at the C-10 position.

Cytotoxicity assays were performed on taxoids **16a**–**e** and **17a**–**e** against the nonsmall cell lung carcinoma cell line A-549 and the human breast cancer cell line MCF-7.



Scheme 3. Reagents and conditions: (i) (a) acid **a**–**e** (5–20 equiv) and either DIC/DMAP or DCC/4-pp, (b) HF/pyridine, rt, 40 h, (c) TESCl, imi., rt, (d) LiHMDS, AcCl; (ii) **14**, LiHMDS, THF, −40 °C; (iii) HF/pyridine, CH_3CN; (iv) (a) H_2NNH_2–H_2O, EtOH, (b) 3-methyldithio-propionic acid, DMAP, EDC, rt, (c) HF/pyridine, CH_3CN.

As Table 2 shows, the C-2 modified 10-acetyl taxoids **16a**–**d** all showed a significant improvement in potency (up to 26 fold for **16a**,**d**) as compared to that of the parent taxoid **2** against both cell lines tested. Interestingly, the disulfide containing taxoids **17a**–**e**, with the exception of **17e**, all showed potency superior to that of **2** being from 2 to 10 fold more potent, while being only slightly less toxic than their 10-acetyl counterpart. In particular, taxoids **17a**,**c**, and **17d** were all found to be highly potent, possessing an IC_{50} in the 30–80 pM range, in addition to possessing the desired disulfide linkage making them suitable candidates for the development of immunoconjugates.

The use of taxanes as immunoconjugates requires that a suitable linker be attached in order to link the drug with

Table 2. In vitro cytotoxicity of taxoids **16** and **17a**–**e**

Taxoid	IC_{50} nM[a]	
	A-549[b]	MCF-7[c]
Paclitaxel, **1a**	3.00	1.70
Docetaxel, **1b**	1.00	1.00
2	0.80	—
16a	0.03	0.02
16b	0.10	0.04
16c	0.04	0.02
16d	0.03	0.03
16e	1.00	1.00
17a	0.29	0.08
17b	0.36	0.15
17c	0.10	0.038
17d	0.08	0.045
17e	>3.00	3.00

[a] The concentration of compound that inhibits 50% of the growth of cancer cell line after 72 h of drug exposure.
[b] Nonsmall cell lung carcinoma.
[c] Human breast carcinoma.

the antibody. We have prepared a series of taxanes in which a simple disulfide bond has been incorporated at various positions. This positional scanning approach has identified that both the C-7 and C-10 positions are most suitable for this endeavor. Using the C-10 position to maintain the disulfide, we also prepared a series of C-2 modified taxanes. This led to the development of 'linkable' taxanes with exceptional potency (**17a**,**c**,**d**). These taxanes have the desired potency needed and can be coupled to an antibody for evaluation as immunoconjugates. The preparation and pre-clinical evaluation of these immunoconjugates will be reported in due course.

Acknowledgements

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19. Experimental data example for taxoid **17a**: ^{1}H NMR ($CDCl_3$) δ 1.15 (s, 3H), 1.26 (s, 3H), 1.35 (s, 9H), 1.68 (s, 3H), 1.75 (s, 3H), 1.76 (s, 3H), 1.85 (m, 1H), 1.88 (m, 1H), 1.91 (s, 3H), 2.32 (m, 1H), 2.37 (s, 3H), 2.43 (s, 3H), 2.57 (m, 1H), 3.00 (m, 4H), 3.82 (d, $J = 7.2$ Hz, 1H), 4.16 (d, $J = 8.4$ Hz, 1H), 4.20 (d, $J = 2.4$ Hz, 1H), 4.30 (d, $J = 8.4$ Hz, 1H), 4.42 (dd, $J = 6.8$, 10.8 Hz, 1H), 4.74 (m, 2H), 4.98 (d, $J = 8.0$ Hz, 1H), 5.32 (br s, 1H), 5.62 (d, $J = 7.2$ Hz, 1H), 6.15 (t, $J = 8.8$ Hz, 1H), 6.34 (s, 1H), 7.43 (t, $J = 7.6$ Hz, 1H), 7.59 (d, $J = 7.6$ Hz, 1H), 7.99 (d, $J = 7.6$ Hz, 1H), 8.12 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 9.5, 15.0, 16.6, 22.3, 23.2, 25.7, 26.6, 28.0, 32.1, 34.2, 35.5, 35.6, 43.1, 45.6, 51.6, 58.5, 72.2, 72.3, 73.7, 75.4, 75.8, 76.3, 79.3, 81.0, 84.4, 116.5, 120.5, 128.4, 130.0, 130.2, 131.0, 132.5, 133.7, 134.8, 143.0, 152.1, 155.4, 165.6, 170.0, 172.1, 203.5. HRMS (FAB): m/z HRMS for $C_{45}H_{60}ClNO_{15}S_2Na^+$: calcd: 976.2991; found: 976.2984 ($\Delta = 0.7$ ppm).
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