Use of Fluorine in the Medicinal Chemistry and Chemical Biology of Bioactive Compounds—A Case Study on Fluorinated Taxane Anticancer Agents

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Introduction

It has been shown that the introduction of fluorine to a bioactive molecule causes minimal steric alterations and, hence, can facilitate interactions of that biomolecule with enzyme active sites, receptor recognition sites, transport mechanisms, and other biological systems.[1,2] At the same time, however, the introduction of fluorine significantly alters the physico-chemical properties of the bioactive molecule because of its large electronegativity. Thus, this type of modification can also induce modified biological responses.[1,2]

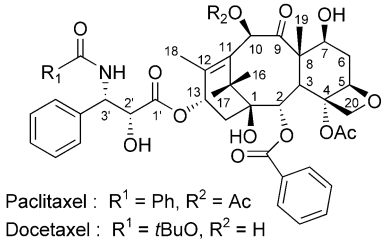
Rational designs exploiting these special properties of fluorine have been successful in the development of new and effective biochemical tools as well as medicinal and therapeutic agents.[2] Fluorinated congeners can also serve as excellent probes for the investigation of biochemical mechanisms. ^{19}F NMR spectroscopy can provide unique and powerful tools for mechanistic investigations in biology.[2]

This minireview describes the exploitation of the unique nature of fluorine in the medicinal chemistry and chemical biology of taxane anticancer agents as a showcase in this field of research.

Taxane Anticancer Drugs

Paclitaxel (Taxol®) and docetaxel (Taxotère®) are two of the most important anticancer drugs, approved for clinical use in chemotherapy against various human tumors, for example, metastatic breast cancer, advanced ovarian cancer, non-small-cell lung cancer, and Kaposi's sarcoma.[3,4]

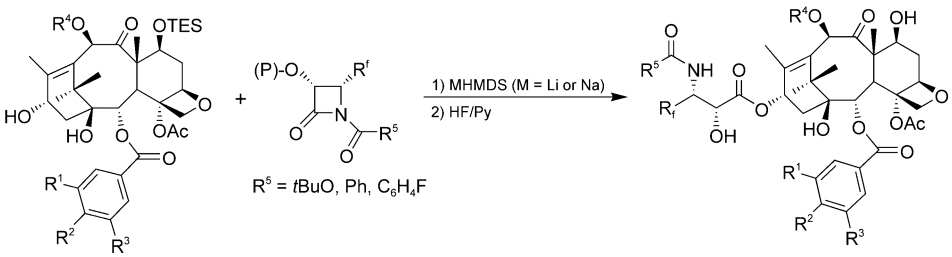
These drugs are currently undergoing clinical trials worldwide for the treatment of other cancers, such as head and neck, prostate, and cervical cancers. Effective chemotherapy in combination with other anticancer agents like cisplatin, carboplatin, or doxorubicin has also been developed. These "taxane" anticancer drugs bind to the β-tubulin subunit, accelerate the polymerization of tubulin, and stabilize the resultant microtubules, thereby inhibiting their depolymerization. This results in the arrest of the cell-division cycle, mainly at the G2/M stage, and leads to apoptosis through the cell-signaling cascade.[5,6] Although both paclitaxel and docetaxel possess potent antitumor activity, recent reports have shown that treatment with these drugs often encounters undesirable side effects as well as drug resistance.[3,4,7] Therefore, it is important to develop new taxane anticancer agents with fewer side effects, superior pharmacological properties, and improved activity against various classes of tumors, especially against drug-resistant human cancer.



Paclitaxel : R^1 = Ph, R^2 = Ac
Docetaxel : R^1 = *t*BuO, R^2 = H

Fluorotaxane Anticancer Agents

In the course of our extensive studies on the design, synthesis, and structure–activity relationships (SAR) of taxane anticancer agents, we synthesized fluorine-containing taxanes by means of the β-lactam synthon method[8–10] (Scheme 1) to investigate the effects of fluorine incorporation on cytotoxicity and the blockage of known metabolic pathways.[11–13] The primary sites



Scheme 1. *Synthesis of fluorine-containing taxanes by means of the β-lactam synthon method. HMDS = hexamethyldisilazide.*

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of metabolism on the paclitaxel molecule for the P450 (CYP) family of enzymes are the *para*-position of the C-3′ phenyl, the *meta*-position of the C-2 benzoate, the C-6 methylene, and the C-19 methyl groups.[12,13] A substantial decrease in the rates of enzymatic oxidation is anticipated for the replacement of a C–H bond with a C–F bond at these sites; this laid the basis for our design of fluorine-containing taxanes possessing high cytotoxicity coupled with enhanced metabolic stability.

In addition, we envisaged the use of these fluorine-containing taxanes as probes for conformational analysis to aid our efforts to discover the biologically active conformation of paclitaxel. We have studied these taxane probes by NMR and molecular modeling methods and postulated likely recognition and binding conformations for paclitaxel, both in solution and at its binding site on the microtubules.

Fluorine-Containing Analogues of Paclitaxel and Docetaxel for Metabolism Studies

Novel fluorine-containing paclitaxel and docetaxel analogues, 3′-(4-fluorophenyl)paclitaxel (**1**), 2-(3-fluorobenzoyl)paclitaxel (**2**), and 2-(3-fluorobenzoyl)docetaxel (**3**) have been used as substrates to probe the metabolic pathways of taxane anticancer drugs, with a focus on the action of the key CYP family of enzymes, for example, subfamilies CYP 2C (responsible for 6α-hydroxylation of paclitaxel) and CYP 3A (responsible for hydroxylation of the *t*Boc group in docetaxel).[12,13] As predicted, introduction of a fluorine atom at the primary site of enzymatic hydroxylation in the paclitaxel molecule, that is, the *para*-position of C-3′ phenyl group to give 3′-(4-fluorophenyl)paclitaxel (**1**), was found to block the enzymatic hydroxylation by CYP completely.[12,13] A striking result was observed for 2-(3-fluorobenzoyl)paclitaxel (**2**), that is, the hydroxylation occurred only at C-6, while no hydroxylation was observed at the 3′-phenyl ring; this suggests a strong allosteric effect of the fluorine-containing 2-benzoate moiety on the action of the enzyme (Scheme 2).[12,13]

However, the corresponding docetaxel analogue, 2-(3-fluorobenzoyl)docetaxel (**3**), was shown to undergo the usual side chain hydroxylation, which may well be due to the greater reactivity of the *tert*-butyl group as compared to the 3′-phenyl of paclitaxel toward enzymatic hydroxylation by CYP (Scheme 3).[12,13] Further studies with novel taxoids containing strategically placed fluorine atoms are underway to elucidate these metabolic events in greater detail.

Fluorotaxane Anticancer Agents Bearing CF_3 and CF_2H Groups at the C3′-Position

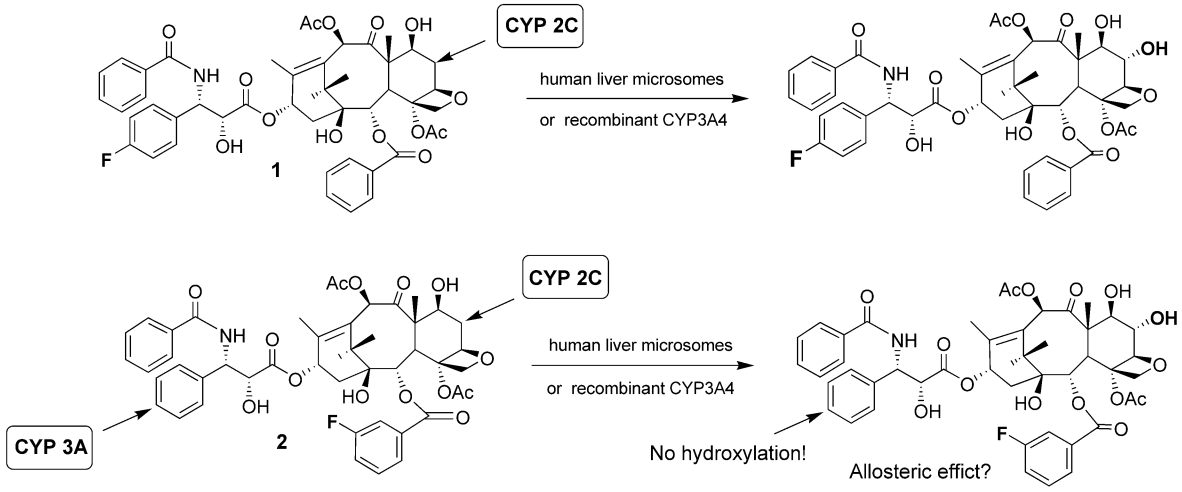
Synthesis

A series of 3′-CF_3-docetaxel congeners was synthesized through ring-opening coupling reactions of an excess of racemic *N*-*t*Boc-4-CF_3-β-lactam (*rac*-**4**, 2.5 equiv) with 7-TES-baccatins (**5**) (Scheme 4).[14] The reaction gave the desired (2′*R*,3′*R*)-3′-CF_3-docetaxel congeners (**6**) with diastereomeric ratios of 9:1 to >30:1 (by ^{19}F NMR analysis) through high-level kinetic resolution of the β-lactam. In particular, the desired diastereomers of three 3′-CF_3-taxanes (**6**: R = H, Ac, and Me_2NCO) were obtained exclusively (Table 1). The rationale for this highly impressive level of kinetic resolution is illustrated in Figure 1. Other second-generation 3′-CF_3-taxanes were synthesized by means of the β-lactam synthon method with the enantiopure *N*-*t*-Boc-4-CF_3-β-lactam, following the general process shown in Scheme 1.[15]

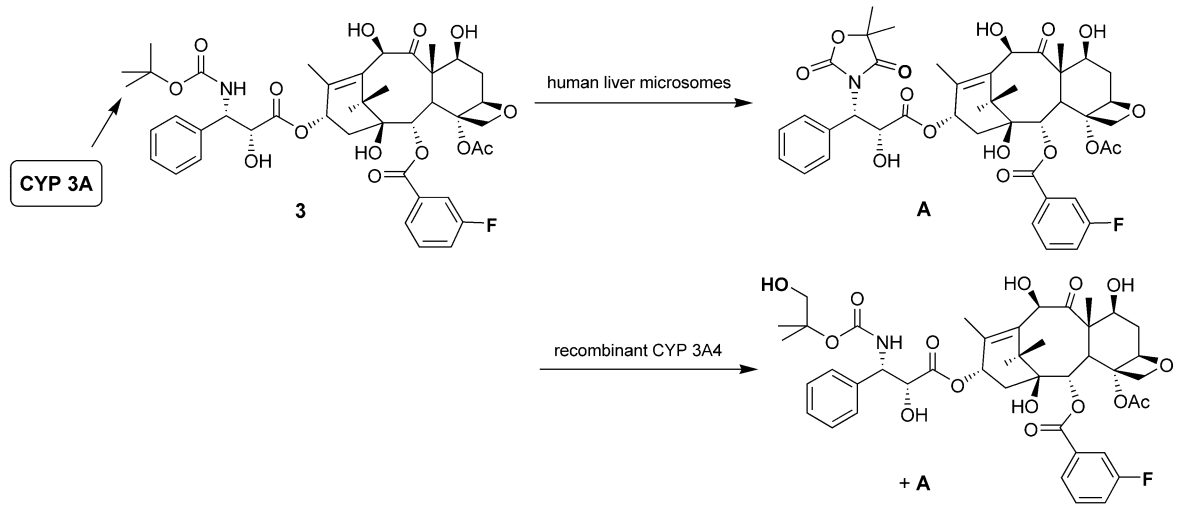
A series of 3′-CF_2H-taxanes (**12**) was synthesized from *N*-*t*Boc-4-CF_2H-β-lactam (**11**), obtained from its enantiopure precursor (3*R*,4*R*)-3-TIPSO-4-(2-methyl-1-propenyl)-β-lactam (**7**) by ozonolysis, followed by treatment with (diethylamino)sulfur trifluoride (Scheme 5). Coupling of 7-TES-baccatins (**5**) with the 4-CF_2H-β-lactam (**11**) under standard conditions gave the desired 3′-CF_2H-taxanes (**12**) in moderate to good yields.[16]

Anticancer activity

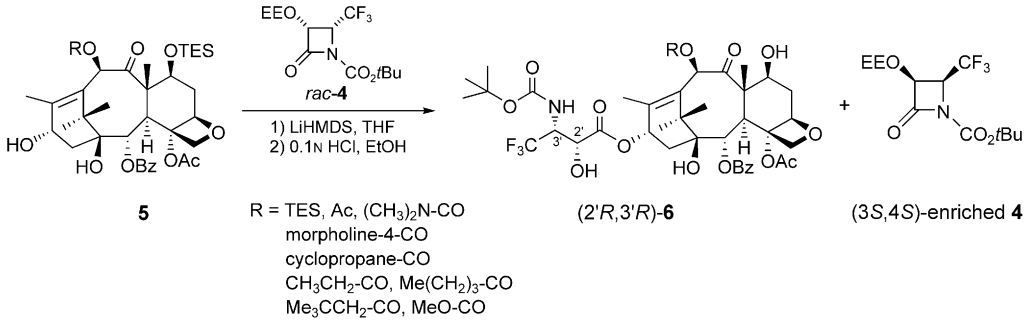
Cytotoxicities of these 3′-CF_3-taxanes (**6**) were evaluated against five human cancer cell lines (Table 2). All the 3′-CF_3-taxanes (**6**) are significantly more potent than either paclitaxel or



Scheme 2. *Metabolism of 3′-(4-fluorophenyl)paclitaxel (**1**) and 2-(3-fluorobenzoyl)paclitaxel (**2**) by CYP.*



Scheme 3. *Metabolism of 2-(3-fluorobenzoyl)docetaxel (**3**) by CYP.*



Scheme 4. *Synthesis of 3′-CF_3-taxanes through ring-opening coupling with kinetic resolution.*

docetaxel in every case. The most remarkable results are, however, *the two orders of magnitude* better activities of the 10-acyl-3′-CF_3-taxanes (**6**: R = EtCO, Me_2NCO, MeOCO) relative to paclitaxel and docetaxel against the drug-resistant breast cancer cell lines MCF7-MDR and LCC6-MDR.[15,17]

The 3′-CF_2H-taxanes (**12**) follow the pattern of the 3′-CF_3-taxanes by exhibiting activities greater than those of paclitaxel and docetaxel against both drug-sensitive and drug-resistant cell lines. Most notably, these 3′-CF_2H-taxanes exhibit single-digit nanomolar-level IC_{50} values against the drug-resistant human breast cancer cell line LCC6-MDR, thereby showing *two orders of magnitude* greater potency than paclitaxel and *three orders of magnitude* greater potency than doxorubicin.[15,16]

Table 1. *Kinetic resolution coupling of β-lactams with baccatins.*

Taxoid	R	*T* [°C]	Conversion [%][a]	Yield [%][a]	Isomer ratio[c] (2′*R*,3′*R*):(2′*S*,3′*S*)
a	H (TES)	−40 to −20	80	54	single isomer
b	Ac	−40 to −10	72	41	single isomer
c	$(CH_3)_2NCO$	−40 to 0	100	63	single isomer
d	morpholine-4-CO	−40 to −20	100	60	23:1
e	cyclopropane-CO	−40 to 0	100	64	10:1
f	CH_3CH_2CO	−40 to −15	100	74	>30:1
g	$CH_3(CH_2)_3CO$	−40 to −10	100	56	9:1
h	$(CH_3)_3CCH_2CO$	−40 to −20	91	59	22:1
i	MeOCO	−40 to −20	93	54	24:1

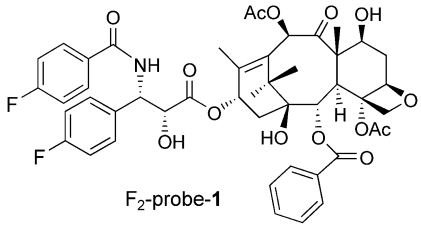
[a] Based on consumed Baccatin. [b] Two-step yield. [c] Determined by ^{19}F NMR analysis.

Fluorine-Probe Approach to the Study of Bioactive Conformations of Taxanes

Solution-phase structure and dynamics of paclitaxel

The wide dispersion of ^{19}F NMR chemical shifts makes this type of spectroscopy particularly amenable to the observation of dynamic conformational equilibria through the freezing of conformers at low temperature. Thus, we envisaged using fluorine-containing taxanes as probes for NMR analysis, in conjunction with molecular modeling, of the conformational dynamics of paclitaxel, a poorly investigated area.

Analysis of low-temperature, variable-temperature (^{19}F and ^{1}H) NMR and $^{19}F,^{1}H$ heteronuclear NOE spectra of 3′-(4-fluorophenyl)-3′-*N*-(4-fluorobenzoyl)paclitaxel (F_2-probe-**1**) in conjunction with molecular modeling has revealed the presence of an equilibrium between two conformers in protic solvent systems.[18] Interpretation of the temperature dependence of the coupling constants between H2′ and H3′ for the F_2-probe-**1** shows that one of these conformers is *anti*. The other conformer has an unusual near-eclipsed arrangement around the H2′-C2′-C3′-H3′ dihedral angle, which is found to be more prevalent at ambient temperature. Strong support for this unique conformer can be found in its close resemblance to a proposed solution structure of a water-soluble paclitaxel analogue, paclitaxel-7-MPA (MPA = *N*-methylpyridinium acetate).[19] This previously unrecognized conformer might play an important role at the binding site(s) on the microtubules.



F_2-probe-**1**

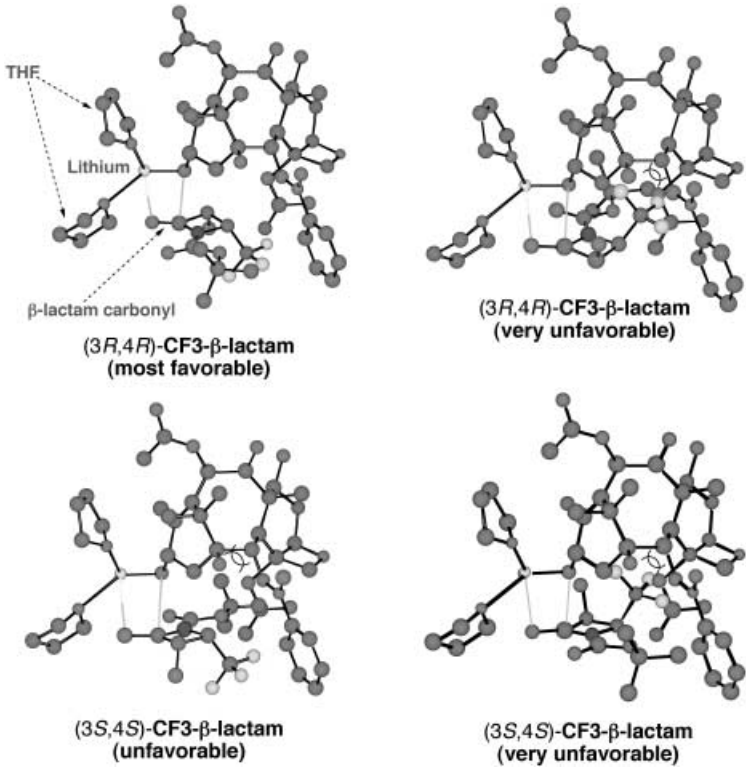
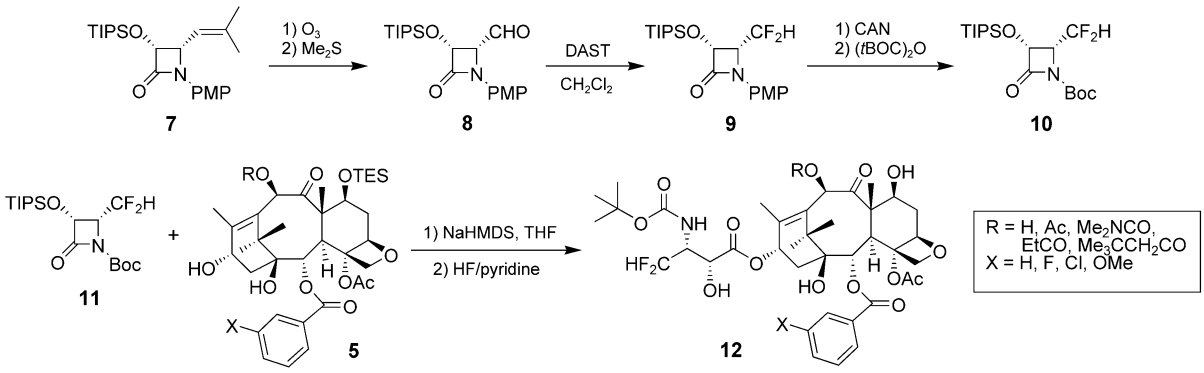


Figure 1. *Rationale for highly efficient kinetic resolution in the ring-opening coupling of* N-t*Boc-4-CF_3-β-lactams (**4**) with baccatins (**5**). Note the steric hindrance in the three unfavorable approaches. (The ethoxyethyl protecting group on the 3-hydroxyl moiety of the β-lactams is omitted for clarity.)*

Protein-bound conformation of paclitaxel using fluorine probes in solid-state ^{19}F NMR.

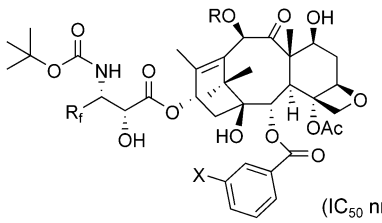
We have successfully extended the fluorine-probe approach to estimating the F–F distance in the microtubule-bound 2-(4-fluorobenzoyl)-3′-(4-fluorophenyl)-10-Ac-docetaxel (F_2-probe-**2**) using solid-state magic-angle spinning (SS MAS) ^{19}F NMR analysis with radio-frequency driven dipolar recoupling (RFDR). Our preliminary studies revealed that, in the microtubule-bound complex, the distance between the two fluorine atoms in the F_2-probe was 6.5 ± 0.5 Å.[13] Restrained high-temperature molecular dynamics were conducted for F_2-probe-**2** while maintaining a distance restraint of 6.5 Å between the two fluorine atoms. This analysis indicated that this distance (6.5 Å) could be maintained by a couple of energetically similar conformers.

In the mean time, another SS NMR method, rotational echo double resonance (REDOR), was applied to the same problem by Schaefer and co-workers using 2-(4-fluorobenzoyl)-C3′(^{13}C)-C3′N(^{15}N)-(^{13}CO-benzoyl)paclitaxel.[20] This led them to propose a microtubule-bound paclitaxel conformation, which supported our proposed conformation based on photoaffinity labeling studies,[21] that is, a structure close to one of the two X-ray crystal structures of paclitaxel.[22] Thus, two SS NMR methods that can determine the F–F or F–^{13}C distance have become



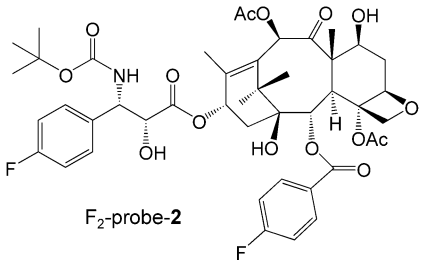
Scheme 5. *Synthesis of 3′-CF_2H-taxanes (**12**).*

Table 2. Cytotoxicity of 3′-CF_3-taxanes and 3′-CF_2H-taxanes against human cancer cell lines.



F Taxoid	R^f	R	X	MCF7 (breast)	MCF7-R (breast)	*R/S*[b]	LCC6WT (breast)	LCC6-MDR (breast)	*R/S*[b]	H460 (ovarian)	HT-29 (colon)	A549 (NSCLC)
				(IC_{50} nM)[a]								
Paclitaxel	Ph	Ac	H	1.8	484	269	3.4	216	64	5.5	3.6	3.6
Docetaxel	Ph	H	H	1.1	359	343	–	–	–	–	1.0	1.2
Doxorubicin				–	–	–	180	2900	16	–	–	–
SB-T-12841-2	CF_3	CH_3CH_2CO	H	0.5	16	32	–	–	–	–	0.3	0.4
SB-T-12842-4	CF_3	$(CH_3)_2NCO$	N_3	0.5	4.2	8.4	1.4	2.2	1.6	0.3	0.5	–
SB-T-12844-1	CF_3	CH_3CH_2CO	N_3	0.4	2.6	6.5	1.2	1.6	1.3	0.2	0.4	–
SB-T-12844-2	CF_3	CH_3OCO	N_3	0.5	4.7	9.4	1.2	2.5	2.1	0.2	0.4	–
SB-T-12842	CF_2H	CH_3CH_2CO	F	–	–	–	1–0	5–7	8.9	–	–	–
SB-T-12843	CF_2H	$(CH_3)_3CCH_2CO$	Cl	–	–	–	1.3	5.0	5.7	–	–	–
SB-T-128221	CF_2H	$(CH_3)_2NCO$	MeO	0.7	10	14	0.8	7.0	3.9	0.2	0.4	–
SB-T-12821-3	CF_2H	CH_3CH_2CO	F	0.6	6.4	11	0.6	3.1	5.2	0.3	0.5	–
SB-T-128221-3	CF_2H	CH_3OCO	H	1.1	8.1	7.4	1.2	6.7	5.6	0.3	0.5	–
SB-T-12823-3	CF_2H	CH_3OCO	H	0.8	14	18	1.0	8.3	8.3	0.3	0.5	–

[a] The concentration of compound which inhibits 50% (IC_{50}) of the growth of the human tumor cell line after 72 h drug exposure. [b] *R/S* = drug resistance factor = IC_{50}(drug-resistant cell line)/IC_{50}(drug-sensitive cell line).



F_2-probe-**2**

available to investigate interesting problems in chemical biology.

Taxane–Antibody Immunoconjugates for Tumor-Specific Delivery of Anticancer Agents

Current cancer chemotherapy is based on the premise that rapidly proliferating tumor cells are more likely to be killed by cytotoxic drugs. Unfortunately, the difference in activity of current drugs against tumor tissues relative to healthy tissues is relatively small. The amount of a drug required to achieve a clinically effective level of activity against the targeted tumor cells often causes severe damage to actively propagating non-malignant cells such as those of the gastrointestinal tract and bone marrow; this results in a variety of undesirable side effects. Therefore, it is very important to develop new chemotherapeutic agents with improved tumor specificity.

The discovery of antigens that are particularly over-expressed on the surface of cancer cells suggests that, by using certain antibodies to selectively "mark" tumor cells, malignant tissues could be distinguished from normal tissues. Monoclonal antibodies (mAbs), which have shown high binding specificity for tumor-specific antigens, could fulfill this task. In fact, these mAbs could be used as vehicles to deliver cytotoxic drugs selectively to tumor cells. A drug–mAb conjugate would target the tumor cells by binding to the antigens on their surfaces. The conjugate would then be internalized and release the original cytotoxic agent in its active form. This type of immunoconjugate can be categorized as "tumor-activated prodrugs" (TAPs; Figure 2).[23] Ideally, a TAP should be stable during circulation (no premature release of the drug) and should not bind to normal tissue cells.

The practical efficacy of such immunoconjugates heavily depends on the nature of the cytotoxic agents, the tumor specificity of mAbs, and the property of the linker unit. An mAb–calcheamicin conjugate "Mylotarg" has been approved for clinical use.[24] Maytansinoids and CC-1065 analogues have been conjugated to mAbs for treatment of tumors and showed encouraging potency and selectivity in preclinical models.[25,26]

Paclitaxel and docetaxel have made a significant impact on current cancer chemotherapy, mainly because of their unique mechanism of action[8] but seriously suffer from a lack of tumor specificity and multidrug resistance (MDR). Thus, it is beneficial to develop immunoconjugates of these drugs. Indeed, two research groups have recently reported paclitaxel–mAb conjugates as potential tumor-specific anticancer agents. However, one of the conjugates was only tested in vitro and the other showed only limited efficacy in vivo. It is clear from the current understanding of the requirements for effective immunoconjugates that the cytotoxicity level of paclitaxel or docetaxel is not sufficient as the cytotoxic component of the conjugate for human clinical use.[23] In addition, it is an-

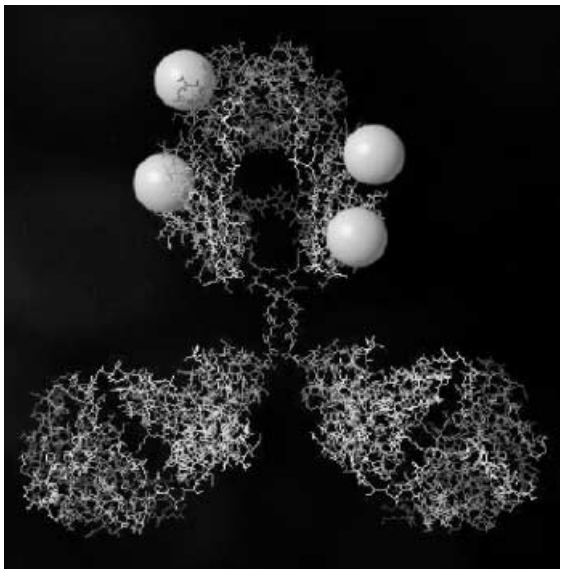


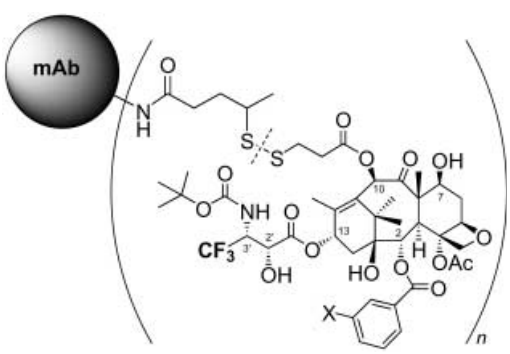
Figure 2. *A model for tumor-activated prodrug (TAP) with mAb.*

ticipated that those conjugates will be inactive against tumors expressing MDR phenotypes.

On the basis of our structure–activity relationship study of taxanes, we have developed a series of highly potent second-generation taxanes.[27–32] Most of these taxanes exhibited 2–3 orders of magnitude higher potency than those of paclitaxel and docetaxel against drug-resistant cell lines expressing MDR phenotypes. One of these second-generation taxanes, "ortataxel" (SB-T-110131; IDN5109; BAY59-8862), exhibited an excellent pharmacological profile in preclinical studies[33–35] and is currently undergoing phase II human clinical trials, sponsored by Bayer, against several cancers. Accordingly, in principle we should be able to develop novel chemotherapeutic agents with high potency and exceptional tumor specificity by linking these new-generation taxanes with mAbs. Use of an appropriate linker between a taxane and a mAb is crucial for the efficacy of the resulting immunoconjugate. The linker must be stable for an extended period of time during storage and during circulation in vivo, but readily cleavable once inside a cancer cell. Among possible linker units reported, we chose to employ a disulfide linker because of its favorable characteristics. It is expected that the mAb component of the conjugate binds to the specific antigens on a tumor surface, and the whole conjugate is internalized by endocytosis. The disulfide bond is then cleaved by an intracellular thiol, such as glutathione, to release the taxoid in its active form.

Recently, we launched the development of taxane–mAb conjugates as TAPs and obtained extremely promising results in human cancer xenografts in SCID (severe combined immune deficiency) mice. We clearly demonstrated the tumor-specific delivery of a taxane anticancer agent without any noticeable toxicity to the animals, and all the animals tested were cured.[36] Thus, we are currently investigating the efficacy of the fluorine-containing taxane anticancer agents mentioned above as the taxane portion of taxane–mAb conjugates.

However, the first-generation taxane–mAb conjugates have a modification at the C-10 position with a sulfhydrylpropanoyl group (see Scheme 6). The modification at the C-10 position with this group was found to be the most effective on the basis of in vitro assay results, although the C-7 and the C-2′ positions were other possible modification sites.[36] The introduction of a sulfhydryl group reduces the taxane's cytotoxicity by 8–10 times relative to a propanoyl group at the same position.[36] Accordingly, we have been developing new short linkers in combination with the disulfide linkage, which can release the parent taxane anticancer agent highly efficiently. One of these approaches is the glutathione-triggered cascade drug release, which forms a thiolactone as a side product (Scheme 7). This mechanism-based drug-release concept has been nicely proven in a model system by monitoring the reaction by ^{19}F NMR with fluorine-labeled compounds (Figure 3). The strategic incorporation of a fluorine substituent at the



Scheme 6. *Fluorine-containing first-generation taxane–mAb conjugate.*

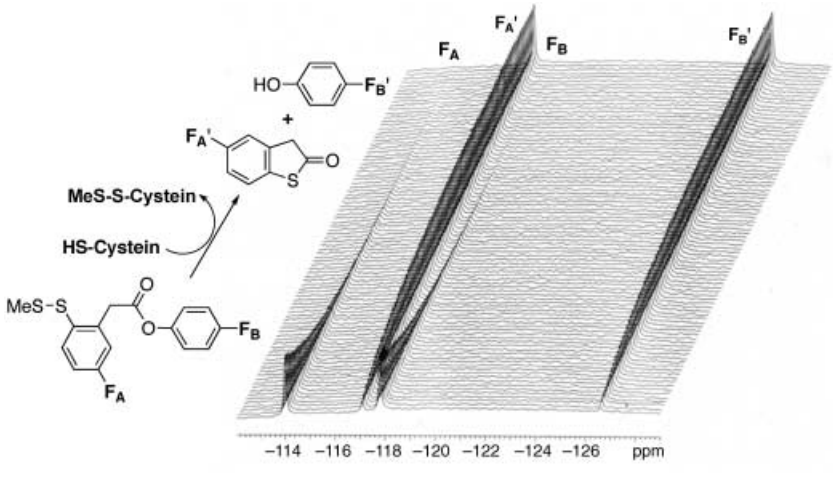
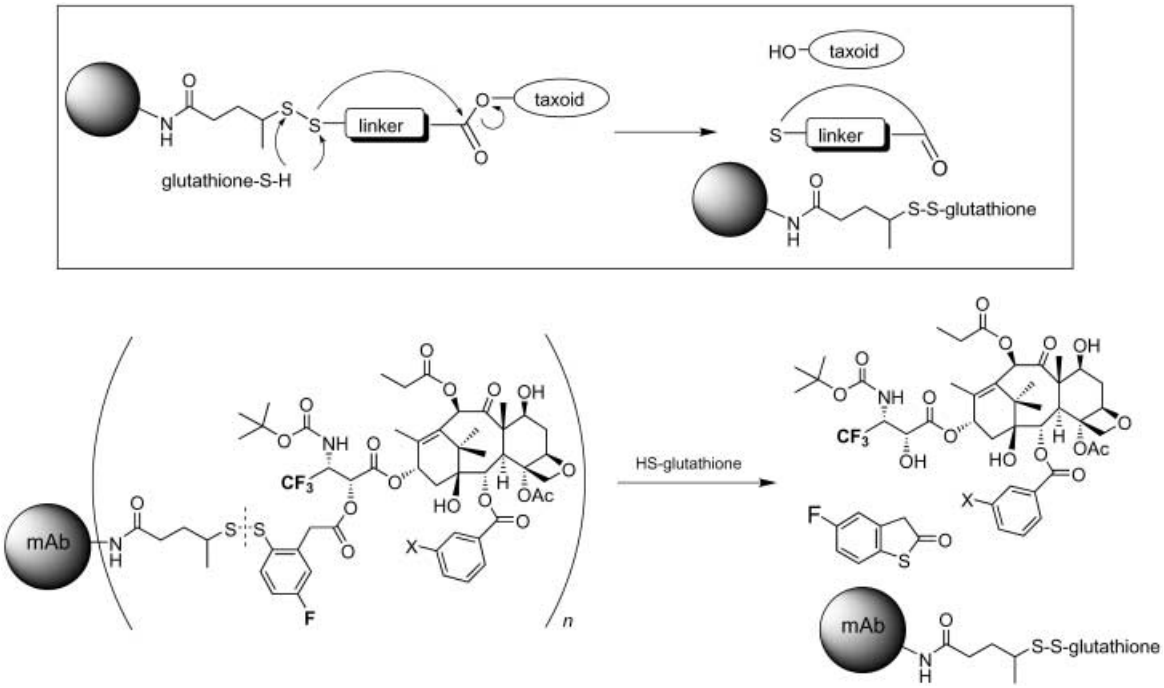


Figure 3. *A model system for the mechanism-based drug release.*



Scheme 7. *Proposed glutathione-triggered cascade drug release.*

para position to the disulfide linkage would direct the cleavage of this linkage by a thiol to generate the desired thiophenolate or sulfhydrylphenyl species for thiolactonization. Also, as described above, the incorporation of a fluorine substituent might increase the metabolic stability of the conjugate. This system has been successfully applied to an advanced model system by using a 3′-CF_3-taxane. We are now applying the fluorine-probe approach to monitoring drug release in real cancer cells.

Further applications of the strategic incorporation of fluorine(s) for the medicinal chemistry and chemical biology of biologically active compounds of medicinal interests are underway in these laboratories.

Keywords: antitumor agents · cancer · fluorine · immunochemistry · metabolism · taxane

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