Chapter 3. Androgen-receptor-targeted paclitaxel and docetaxel analogs for treatment of hormone refractory prostate cancer (HRPC)

3.1 Overview of prostate cancer

Prostate cancer occurs in the prostate gland when cells within the prostate grow uncontrollably. It is one of the most common cancers for men in America, and one out of six American men develops prostate cancer. More than 200,000 American men are diagnosed with prostate cancer each year and about 30,000 men died from this disease every year in the US.[1] The mortality rate of prostate cancer among American men was reported to be 25 per 100,000 in 2004, and it was estimated that there were about two million prostate cancer survivors in the US in 2004. For reasons not yet fully understood yet, African-American men have the highest risk of developing prostate cancer and the highest death rate from this disease all over the world. On the other hand, prostate cancer is relatively rare in southern and eastern Asia, suggesting that either genetic or dietary factors are the reason for this difference. Prostate cancer is also dependent on age. It tends to develop in men over the age of 50, and the average age of diagnosis is 70.[2] Some studies indicate that eighty percent of men develop prostate cancer in their 70's.[3]

Prostate cancer is typically a slow-growing tumor. In its early stages, it is comprised of multiple small primary tumors in the prostate gland and is curable, with a survival rate of 90% or better. Detection of the disease in the early stages is difficult because prostate cancer does not have any symptoms most of the time. Fortunately, this issue has been solved by the development of several detecting methods such as physical examination,

measurement of prostate specific antigen (PSA), or biopsy, and now about 90% of American men with prostate cancer are diagnosed in its early stages. Once prostate cancer spreads to other organisms through the lymph nodes and progresses to advanced prostate cancer, the cure rate decreased significantly.

Current treatments of prostate cancer include surgery, radiotherapy, chemotherapy, and hormone therapy. The primary options for treatment of early-stage prostate cancer are surgery and radiotherapy, whereas hormone therapy and chemotherapy are usually used at the advanced prostate cancer stage. Treatment options are always on a case by case basis, depending on the patient's age, life expectancy, health situation, personal preference, and concerns about the side effects.

3.1.1 Hormone refractory prostate cancer (HRPC)

Androgens play an essential role in cell growth, and generally serve as the main fuel for prostate cancer cell growth and thus have been recognized as a major therapeutic target.[4] One of the successful examples is hormone therapy, also known as androgen-deprivation therapy (ADT), which is designed to stop the production of testosterone (the primary androgen in men's body) or to prevent testosterone from acting on prostate cancer cells.[5] There are four typical methods for androgen-deprivation therapy: castration, estrogen treatment, anti-androgen therapy, and combined androgen blockade. Hormone therapy is of great importance in managing advanced prostate cancer and is likely a necessary part of the therapeutic regimen at some point in fighting against advanced prostate cancer, but it is not a perfect therapy for prostate cancer and cannot cure it. It was found that the majority of prostate cancer cells stop growing after hormone therapy,

but some cells do not respond to the removal of testosterone and grow independent of testosterone. Thus, these hormone-independent cells continue uncontrolled growth and over time, hormone therapy becomes less and less effective to prevent the cancer cell growth until prostate cancer progresses to hormone-refractory prostate cancer (HRPC). At the HRPC stage, the prostate cancer cells become unaffected by hormone therapy at all, and thus prostate cancer can metastasize through lymph nodes and become incurable if no further therapy is taken. The most widely used therapy for HRPC is chemotherapy. Many different chemotherapeutic agents have been tested for the treatment of HRPC,[6] and show efficacy to some extent, but none of them are completely curative. Therefore, the development of a strongly effective treatment of HRPC is a high priority.

3.1.2 The androgen receptor (AR)

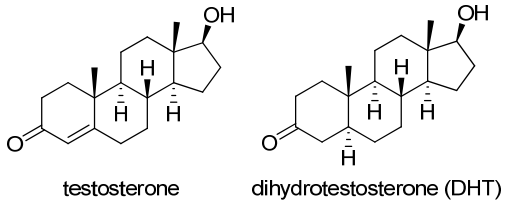


Figure 3.1 Testosterone and dihydrotestosterone (DHT)

Androgen action involves the testicular synthesis of testosterone, its transportation to target tissues such as the prostate, and the conversion of testosterone by 5α-reductase to the more active metabolite 5α-dihydrotestosterone (DHT) (Figure 3.1). Testosterone and DHT exert their biological effects through binding to the androgen receptor (AR) in the cytoplasm and inducing AR transcriptional activity in the nucleus.[7]

Androgen receptors are a class of proteins that are responsible for sensing the presence of steroid and thyroid hormones in the cell. In prostate cancer cells, the androgen receptor binds to heat shock protein in the cytosol and remains inactive until DHT enters the cell and binds to it,[8] leading to the dissociation of androgen receptor from the protein. The activated androgen receptor then moves through the nuclear membrane into the nucleus, activates the androgen-responsive genes and triggers transcriptional activity in the nucleus.[4,9] Androgen receptors are expressed throughout prostate cancer progression.[10] Even hormone refractory prostate cancer still needs AR expression for growth. The functional status of the androgen receptor is an important mediator in prostate cancer growth and survival.[7]

It is widely accepted that the alteration of AR functions is involved in the mechanism for the progression of prostate cancer to hormone refractory prostate cancer. First, increased expression of the androgen receptor generally takes place in patients after hormone therapy, which allows prostate cancer cells to become sensitive to decreased androgen levels resulting from hormone therapy.[11] Similarly, changes in the androgen receptor may enable antiandrogens or other endogenous ligands such as adrenal androgens to activate its transcriptional activity, leading to the relaxation of androgen receptor specificity. In this case, antiandrogens or other endogenous ligands, which previously inhibited or had no effect on the androgen receptor, can now function as androgen receptor agonists.[12] Meanwhile, alteration in the expression of specific androgen receptor coactivators or mutation of coactivators may also contribute to the progression of prostate cancer to hormone refractory prostate cancer.[13] In general, the interaction between the AR and AR coactivators defines the range of ligands that can

activate the transcriptional activity of androgen receptor. Therefore, the mutation of AR coactivators may enhance the transcriptional sensitivity in response to other ligands and result in the relaxation of AR specificity.[13a] Besides, prostate cancer progression is often associated with the alteration of the growth factor as well.[7,14] In prostate cancer cells, growth factors can bind to receptors on the membrane and initiate a phosphorylation cascade that eventually leads to the phosphorylation of transcription factors such as the androgen receptor. Phosphorylation of the androgen receptor influences the interaction between it and its coactivators, and thus may contribute to the progression of prostate cancer to hormone refractory prostate cancer.

As discussed above, the androgen receptor plays an essential role throughout prostate cancer progression, as well as in hormone refractory prostate cancer. Therefore, it is a good chemotherapeutic target for treatment of hormone refractory prostate cancer. In this chapter, we describe the design and synthesis of androgen-receptor-targeted paclitaxel and docetaxel analogs for treatment of hormone refractory prostate cancer.

3.2 Design, synthesis and biological evaluation of androgen-receptor-targeted paclitaxel and docetaxel analogs

3.2.1 Design of the analogs

As mentioned in Chapter 2, taxane-based chemotherapy is the only form of HRPC treatment to prolong survival. Docetaxel (DTX) (Figure 3.2) is currently the best available chemotherapeutic agent which gives patients with hormone refractory prostate cancer survival and palliative benefits,[15] although survival benefits from docetaxel are

only 2 to 3 months.[16] Therefore, enhancement in the effectiveness of taxane-based chemotherapy for treatment of HRPC is highly needed. The objective of this research is to improve the chemotherapy for treatment of hormone refractory prostate cancer by targeting paclitaxel (PTX) or docetaxel (DTX) to the androgen receptor through an excellent androgen receptor ligand, cyanonilutamide (Figure 3.2).[17] We hypothesized that the conjugation of paclitaxel or docetaxel with an androgen receptor ligand will overcome the resistance mechanism of hormone refractory prostate cancer. On one hand, if well designed, the paclitaxel or docetaxel moiety would retain its tubulin assembly activity and antimitotic activity. On the other hand, if the androgen receptor ligand moiety can simultaneously bind to the androgen receptor with high affinity, a stable link between the taxane moiety and the AR ligand would allow the "cross-linking" of microtubules and the androgen receptor in the cytoplasm. This would sequester the androgen receptor in the cytoplasm, and thus keep it from entering the nucleus. This would then block its transcriptional activity, leading to suppression of cell growth.

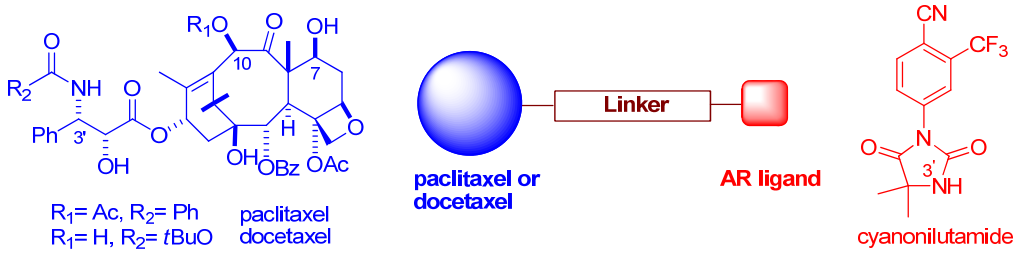


Figure 3.2 The structure of the designed conjugate

To achieve the two goals described above, the conjugation on the taxane moiety needs to be designed to retain its tubulin assembly activity, and an AR ligand with high affinity is needed as well. The SAR studies on paclitaxel and docetaxel, which were

discussed in Chapter 2, have revealed that paclitaxel or docetaxel can tolerate acetylation at the C7 or C10 positions with only minor effects on activity.[18] Another option is the C3' position, since the benzoyl group at this position in paclitaxel can be replaced with certain groups with minor effects on activity.[19] SAR studies showed that an acyl functional group at the C3' position is required for activity, but that the phenyl group can be replacable by large alkyl groups, alkenyl groups or substituted phenyl groups without significantly affecting the activity. We rationalized that the tubulin assembly activity of the docetaxel moiety would be preserved if we were to connect the cyanonilutamide moiety to docetaxel at the C3' position in place of the *tert*-butoxycarbonyl group. Therefore, we chose these three positions as the connecting sites on the paclitaxel or docetaxel moieties.

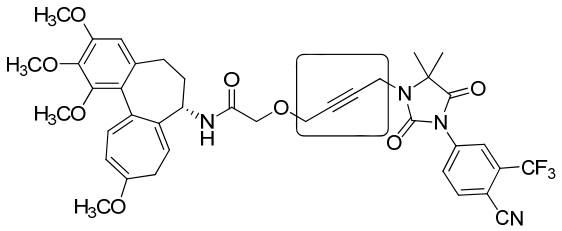


Figure 3.3 The colchicines-cyanonilutamide conjugate

As for the AR ligand, we selected cyanonilutamide as the preferred candidate. Cyanonilutamide is a nonsteroidal antiandrogen and binds to the androgen receptor with an affinity one and a half times higher than nilutamide, which is classified as a pure antiandrogen and is currently in clinical use.[20] More importantly, it was observed that the 3′ position in cyanonilutamide can be modified with a wide variety of substituents without losing binding affinity, and in some cases the binding affinities were even improved.[21] One possible issue we might encounter is that the AR ligand might be

completely buried in a hydrophobic binding pocket when it binds to the AR. A rigid linker would thus be needed to allow the paclitaxel or docetaxel moiety to extend outside the AR binding pocket. According to a finding by Nima Sharifi and coworkers, an alkyne-based linker might be a good choice for us.[22] They designed and synthesized a conjugate of colchicine and cyanonilutamide (Figure 3.3), and they found that the rigid alkyne linker allowed the colchicine moiety to extend outside the binding site on the AR. Moreover, Cogan and Koch found that the connection of cyanonilutamide to an alkyne-based linker preserves much of the AR binding activity of cyanonilutamide.[17,23] In addition, we also designed several docetaxel analogs with the cyanonilutamide moiety linked to the C10 position through poly(ethylene glycol) based linkers of various lengths. When we chose the linkers, one of our concerns was that the solubility of the docetaxel analogs might be reduced by connecting docetaxel to a hydrophobic cyanonilutamide moiety, so we chose ethylene glycol based linkers to compensate for this solubility decrease. We also believed that longer linkers would favor the possibility of the cross-linking between AR and tubulin.

We first designed four AR targeted paclitaxel analogs with cyanonilutamide linked to the C7 position (Figure 3.4). But these analogs were all less active than paclitaxel in the A2780 bioassay, indicating that connecting the cyanonilutamide moiety to paclitaxel at the C7 position does not preserve the antiproliferative activity of paclitaxel. Since the C7 position was not a good option, we designed six AR targeted docetaxel analogs with cyanonilutamide linked to the C10 position through the linkers of various lengths, and one analog with cyanonilutamide linked to the C3′ position (Figure 3.5). The synthesis

and biological evaluations of all these analogs will be discussed in the following two sections.

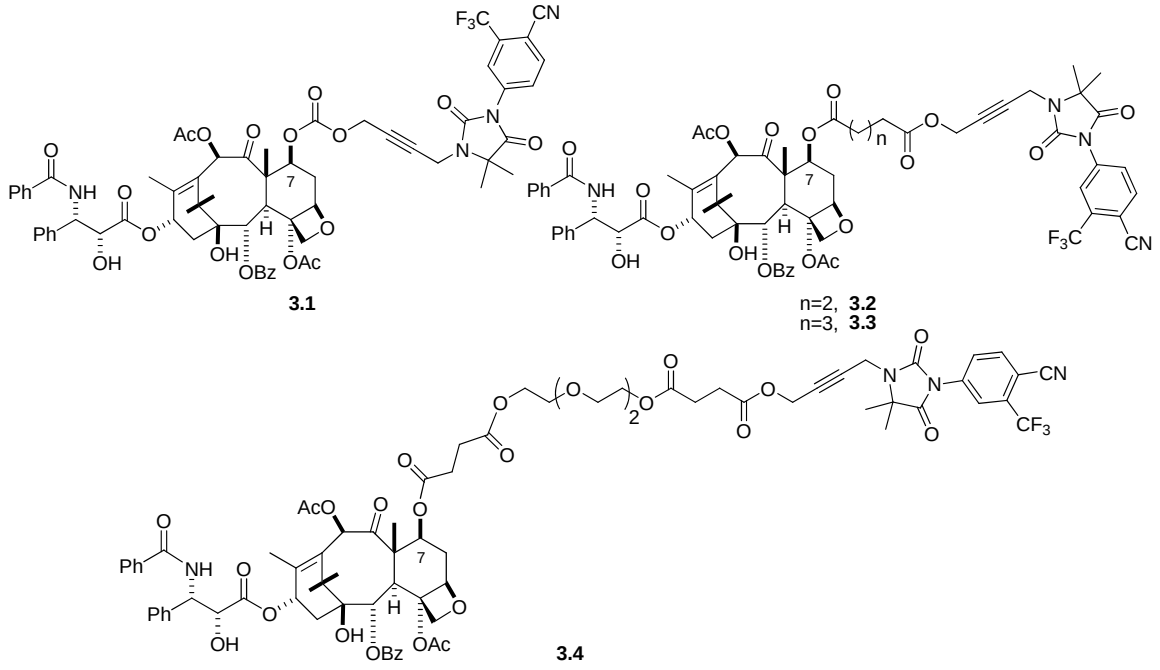


Figure 3.4 Paclitaxel analogs **3.1-3.4**

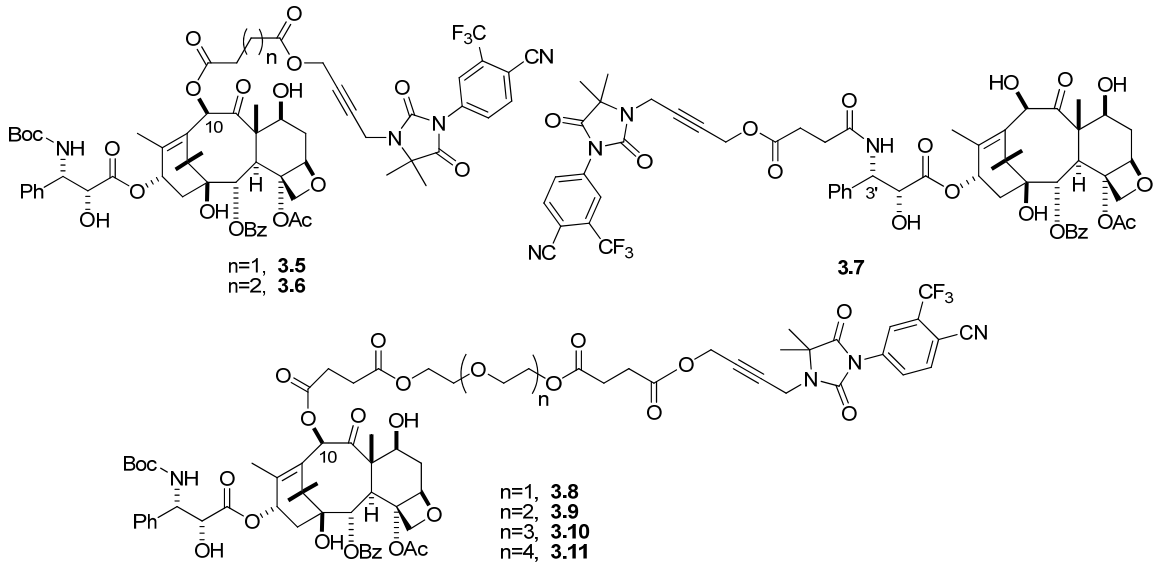
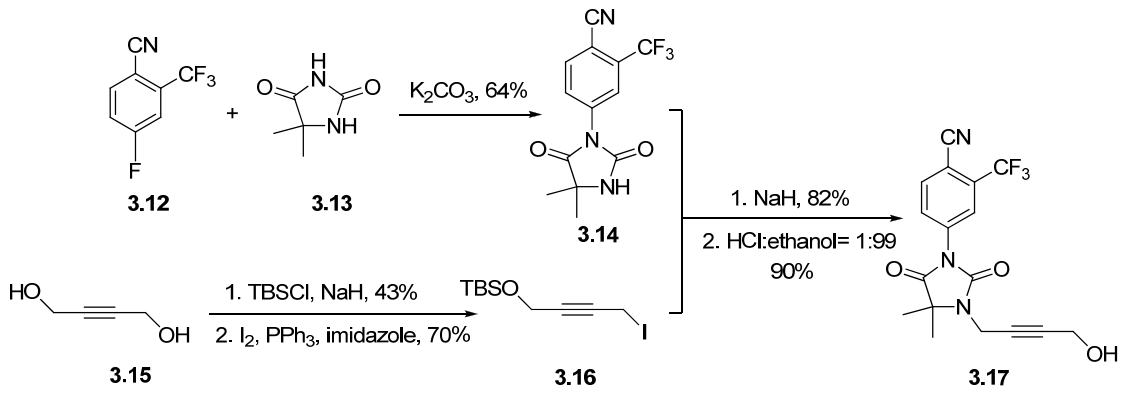


Figure 3.5 Docetaxel analogs **3.5-3.11**

3.2.2 Synthesis of the analogs

3.2.2.1 Synthesis of the cyanonilutamide moiety

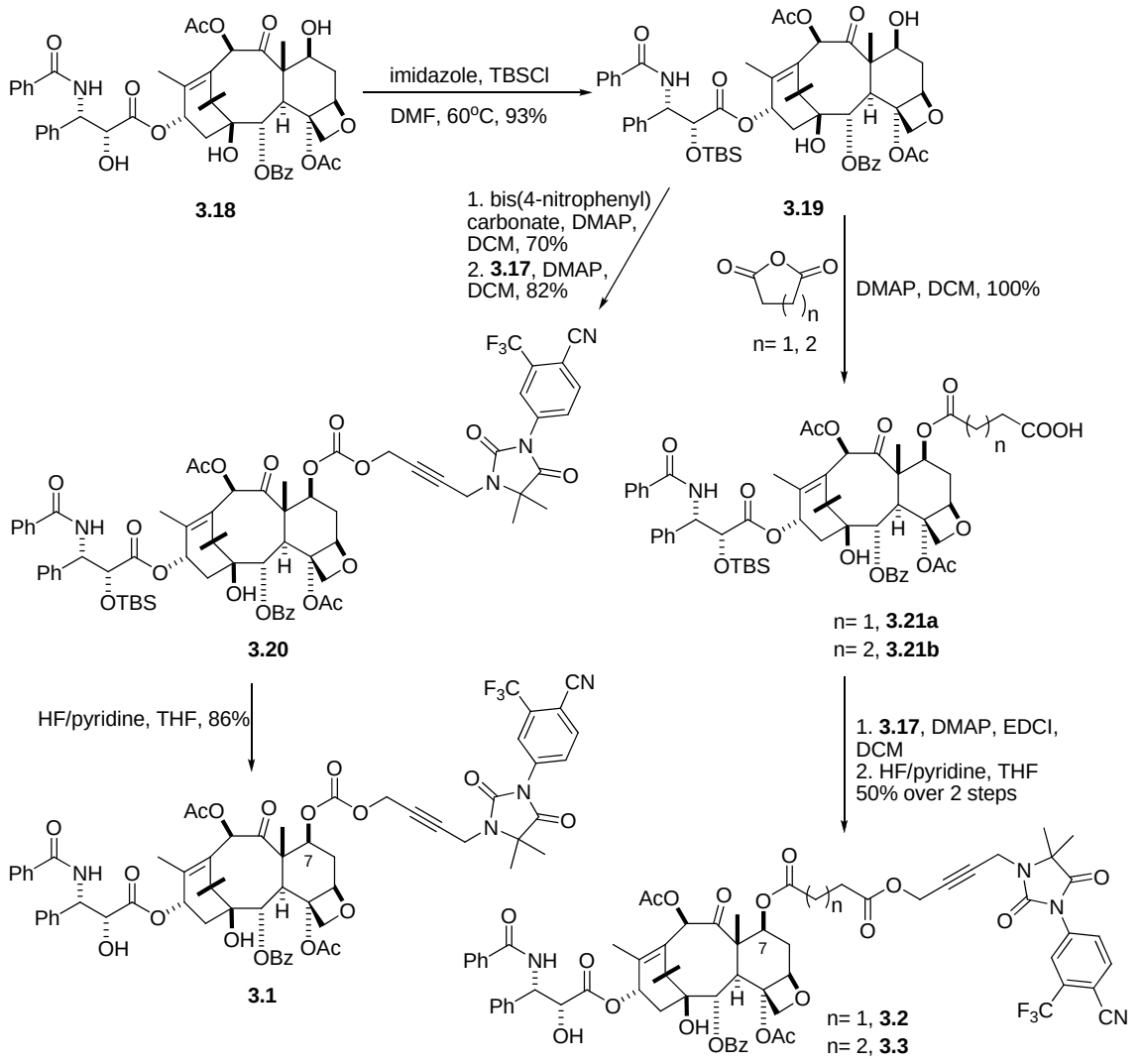
Cyanonilutamide **3.14** and the designed cyanonilutamide moiety **3.17** with an alkyne linker connected at the C3′ position was synthesized through reported methods (Scheme 3.1).



Scheme 3.1 Synthesis of cyanonilutamide moiety **3.17**

3.2.2.2 Synthesis of analogs 3.1-3.3

The syntheses of paclitaxel analogs **3.1, 3.2** and **3.3** are outlined in Scheme 3.2. The syntheses began with paclitaxel, which was first protected as its 2′-TBS ether. The 2'-TBS-paclitaxel was then treated with 4-(dimethylamino)pyridine (DMAP) and linking agents such as bis(4-nitrophenyl) dicarbonate, succinic anhydride, or glutaric anhydride in DCM, and the resulting intermediates were coupled with cyanonilutamide moiety **3.17** upon treatment with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDCI) and DMAP. The removal of the silyl protecting group finally gave analogs **3.1**, **3.2** and **3.3** in good overall yields.

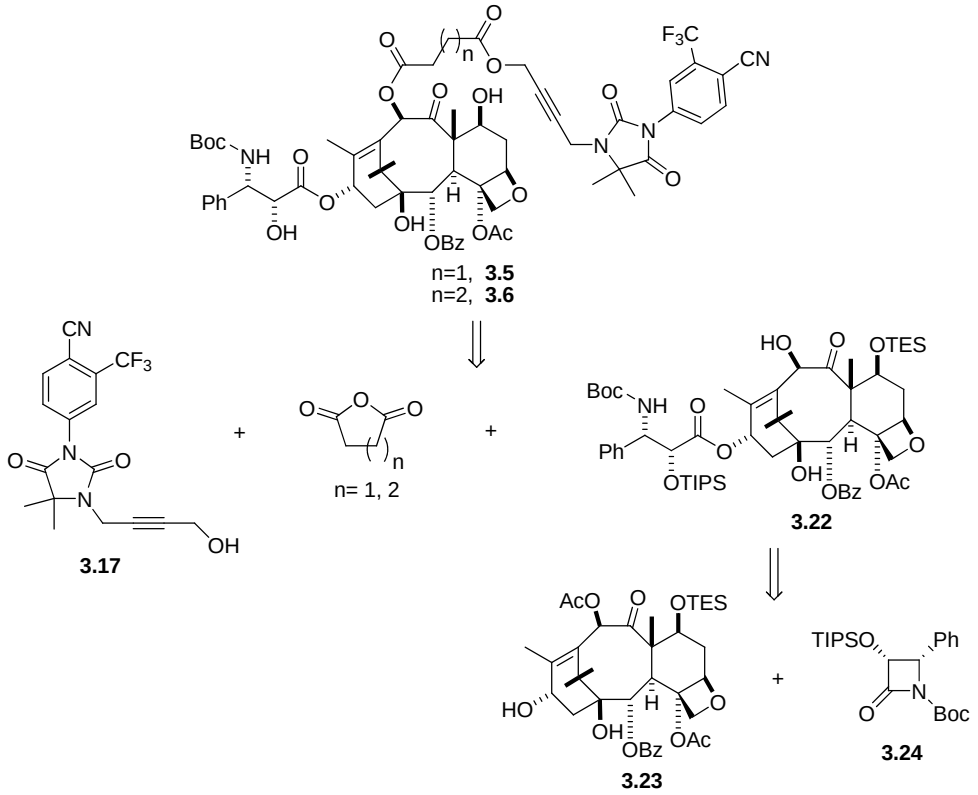


Scheme 3.2 Synthesis of paclitaxel analogs **3.1**-**3.3**

3.2.2.3 Synthesis of docetaxel analogs 3.5 and 3.6

The retrosynthetic analysis of **3.5** and **3.6** is shown in Scheme 3.3. The syntheses of analogs **3.5** and **3.6** began with 10-deacetyl baccatin III **3.25**, which was acetylated at the C10 position and then protected at the C7 position as its TES ether via a one-pot method to give protected baccatin **3.23**. The synthesis of the C13 side chain precursor, β-lactam **3.24**, was carried out by a reported approach, shown in Scheme 3.4. The protected

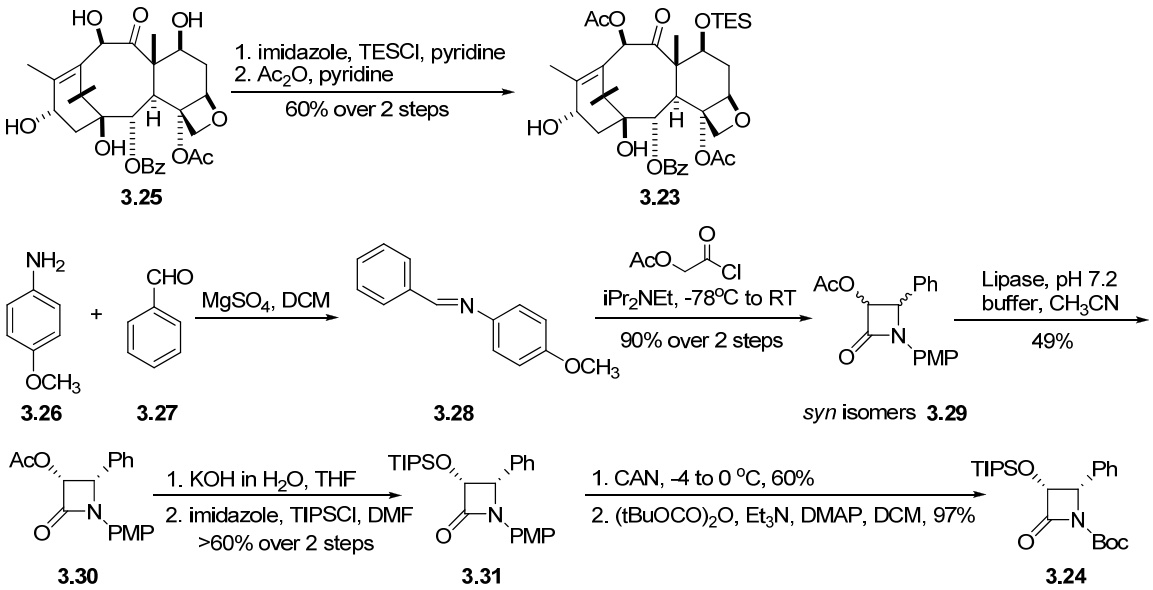
baccatin **3.23** was then coupled with β-lactam **3.24** upon treatment with LiHMDS in THF. Deacetylation at the C10 position by hydrazine then afforded alcohol **3.22** in 60% yield.



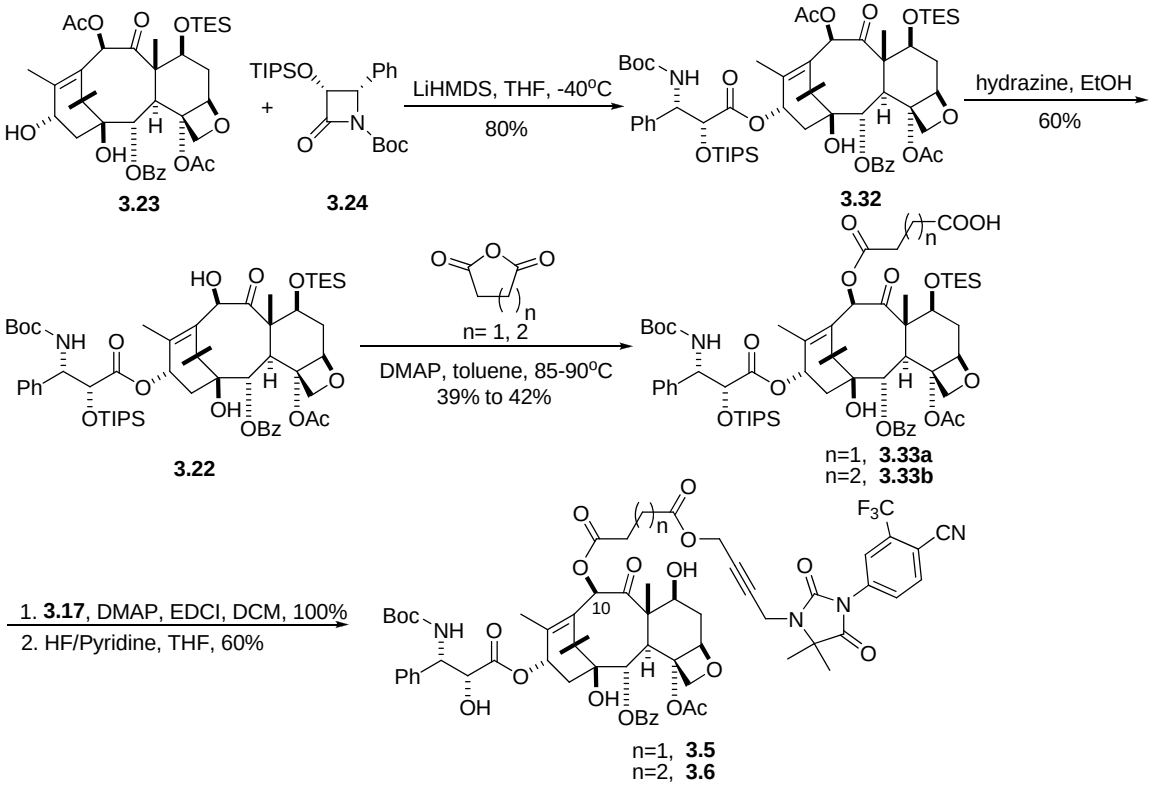
Scheme 3.3 Retrosynthetic analysis of docetaxel analog **3.5** and **3.6**

The subsequent connection to a linking agent at the C10 position was found to be difficult when the C7 position was protected as TES ether. Various linking agents, such as bis(4-nitrophenyl) dicarbonate, carbonyl diimidazole, 4-nitrophenyl chloroformate, succinic anhydride and glutaric anhydride, were evaluated, but only succinic anhydride and glutaric anhydride were successfully connected to the C10 position. So alcohol **3.22** was treated with succinic anhydride or glutaric anhydride and DMAP in toluene at 85-90 ^{o}C and stirred for 5 days to give carboxylic acid **3.33a** or **3.33b** in about 40% yield. After

EDCI coupling with cyanonilutamide moiety **3.17** and global silyl deprotection, docetaxel C10 conjugates **3.5** and **3.6** were prepared (Scheme 3.5).



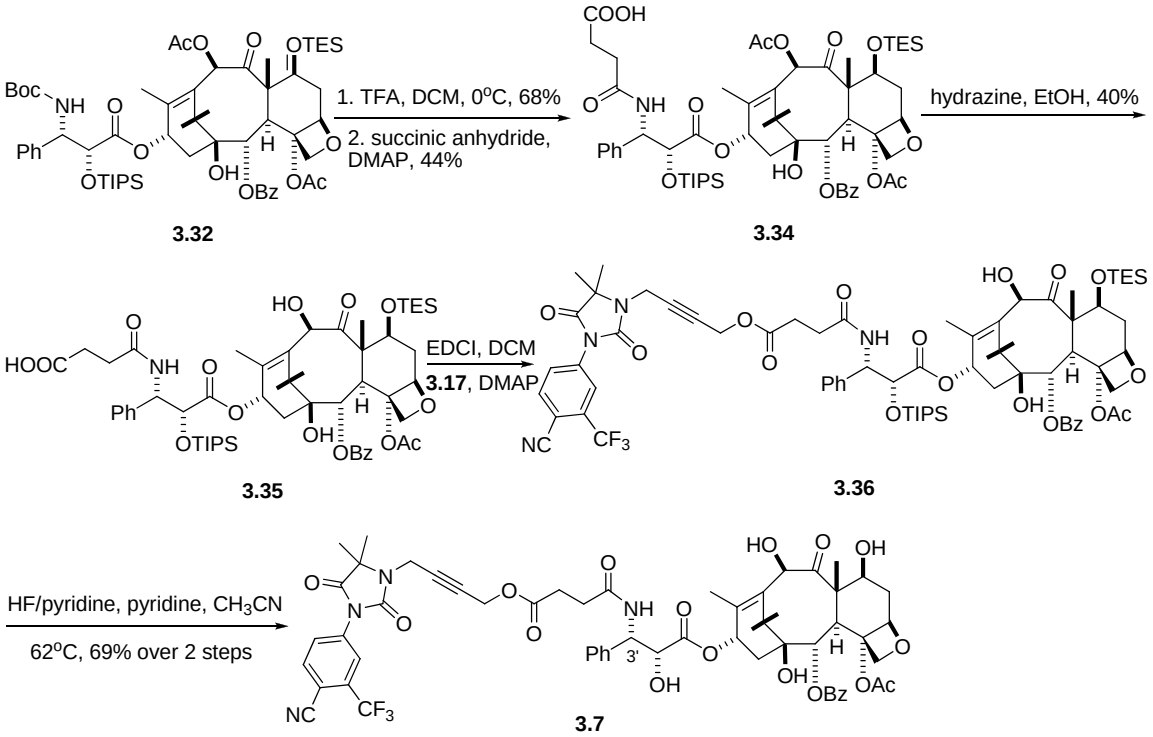
Scheme 3.4 Synthesis of baccatin **3.23** and β-lactam **3.24**



Scheme 3.5 Synthesis of docetaxel analog **3.5** and **3.6**

3.2.2.4 Synthesis of docetaxel analog 3.7

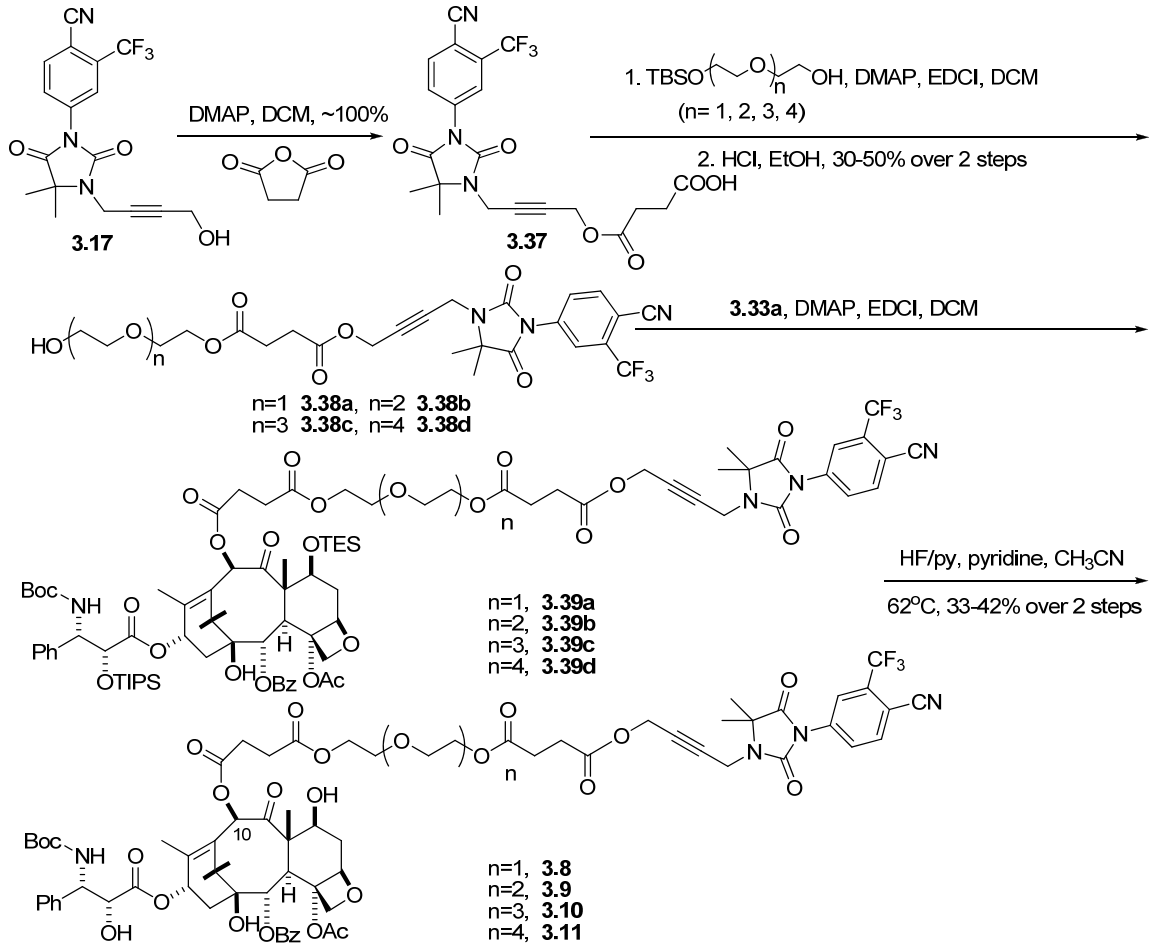
The synthesis of docetaxel analog **3.7** began with docetaxel derivative **3.32** which was treated with TFA in DCM at 0 ^{o}C to remove the *tert*-butoxycarbonyl group (Boc) at the C3' position. Formic acid and acetic acid were also tried as catalysts to remove the Boc group, but only TFA proved to be reliable and to give high yields. The resulting amine was coupled with succinic anhydride and subsequently treated with hydrazine in ethanol to give carboxylic acid **3.35**. An EDCI coupling reaction with cyanonilutamide derivatives **3.17** and global silyl group deprotection finally furnished C3' docetaxel analog **3.7** in 69% yield (Scheme 3.6).



Scheme 3.6 Synthesis of docetaxel analog **3.7**

3.2.2.5 Synthesis of docetaxel analogs 3.8-3.11

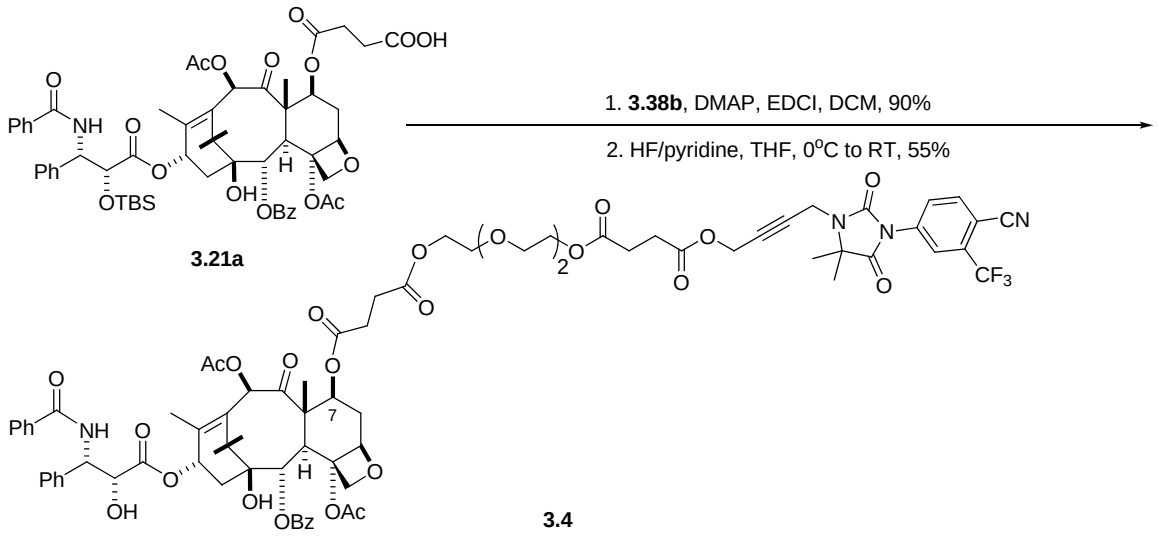
The synthesis of these docetaxel analogs is outlined in Scheme 3.7. The cyanonilutamide moiety **3.17** was first converted quantitatively to carboxylic acid **3.37** upon treatment with succinic anhydride and DMAP in DCM. Then EDCI coupling of acid **3.37** and mono TBS protected ethylene glycol linkers, followed by removal of TBS under acidic conditions, provided alcohol **3.38a-d** in good yields. Subsequently, they were coupled with intermediate **3.33a** by EDCI and DMAP in DCM to give analogs **3.39a-d** respectively, which were treated with HF in pyridine and acetonitrile to afford docetaxel analogs **3.8-3.11**.



Scheme 3.7 Synthesis of docetaxel analogs **3.8-3.11**

3.2.2.6 Synthesis of paclitaxel analog 3.4

Carboxylic acid **3.21a** was connected to cyanonilutamide derivative **3.38b** by an EDCI coupling reaction, and subsequent deprotection of the TBS group produced the C7 paclitaxel conjugate **3.4** in 55% yield (Scheme 3.8).



Scheme 3.8 Synthesis of paclitaxel analog **3.4**

3.2.3 Biological evaluations of all analogs

All these analogs were submitted to our A2780 bioassay for antiproliferative activity. The results are summarized in Table 3.1. We found that C10 docetaxel conjugates **3.5**, **3.6** and **3.8**-**3.10** show better activity than C7 paclitaxel conjugates, and they have comparable activities to paclitaxel and docetaxel. The C3′ docetaxel conjugate **3.7** lost all activity in this assay, and did not preserve as much of the tubulin assembly activity as had

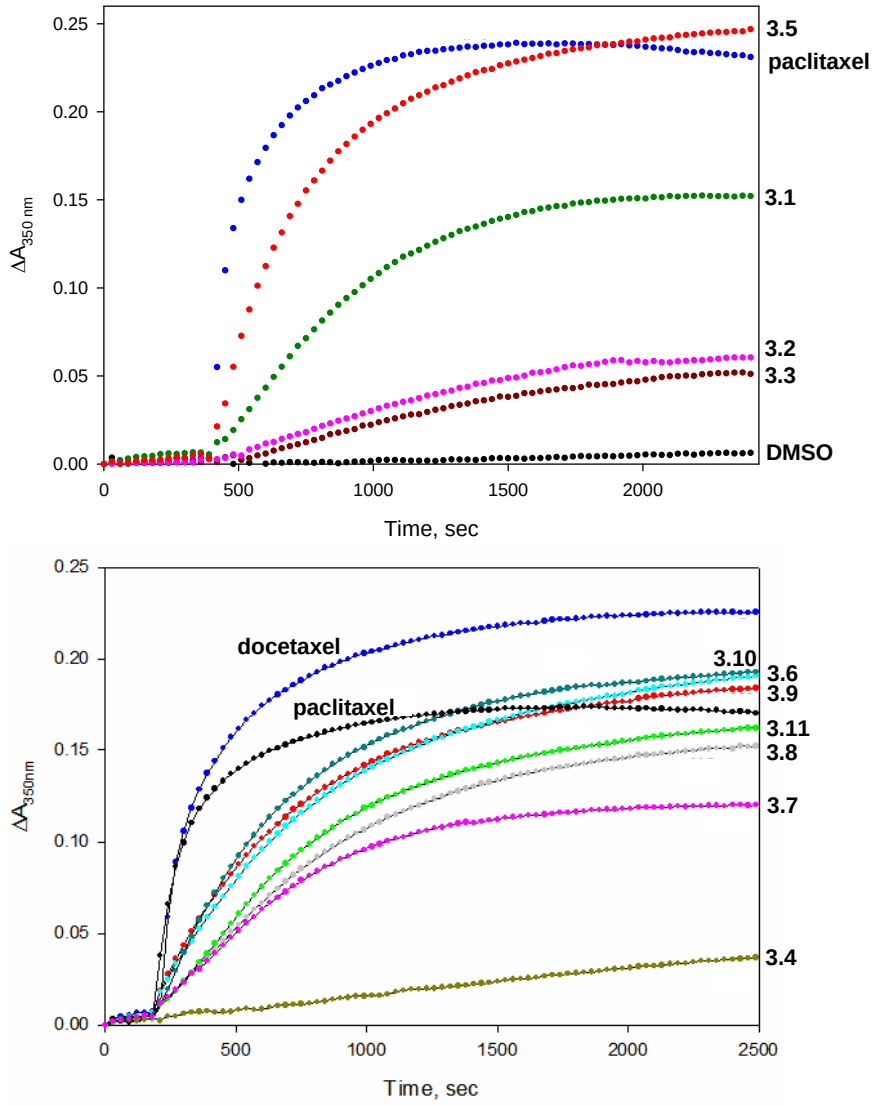
been expected, indicating the limitation of modification at this position. Interestingly, analogs **3.5**, **3.10** and **3.11** are even more active than docetaxel in this assay.

Table 3.1 A2780 bioassay results

Compound	IC_{50} against A2780 (nM)
Paclitaxel	14 ± 2
Docetaxel	8 ± 3
3.14	NA
3.17	NA
3.1	110 ± 90
3.2	320 ± 110
3.3	5400*
3.4	300 ± 100
3.5	2 ± 1
3.6	20*
3.7	NA
3.8	18 ± 8
3.9	17 ± 9
3.10	4 ± 2
3.11	2 ± 1

* Single test

These analogs were then submitted to Susan L. Bane at SUNY at Binghamton for bioassay for tubulin assembly activity (Chart 3.1). In agreement with their antiproliferative activities from the A2780 bioassay, C7 paclitaxel conjugates **3.1-3.4** showed significantly reduced tubulin assembly activities, while the C10 docetaxel conjugates **3.5**, **3.6** and **3.8-3.11** preserved essentially all of the assembly activity of paclitaxel. The C7 paclitaxel conjugate **3.4** with a TEG linker still showed diminished activity like the other C7 paclitaxel analogs without TEG linkers. These results indicate that an ethylene glycol based linker does not help improve the tubulin assembly activity of C7 paclitaxel conjugates, and that the C7 position in paclitaxel is more sensitive to bulky substituents than is the C10 position.

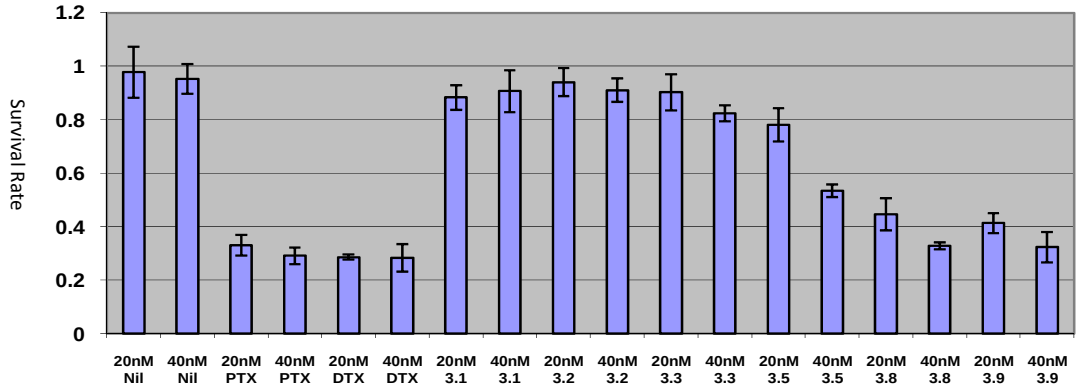


Tubulin assembly by drugs 2mM in concentration

Chart 3.1 Tubulin-assembly activities

The analogs were also submitted to Nima Sharifi at NCI to test their activity against androgen-dependent prostate cancer cells (Chart 3.2) and androgen-independent prostate cancer cells (Chart 3.3). We found that the C10 docetaxel conjugates always showed much better activity in both of the cancer cell lines than the C7 paclitaxel conjugates, which was consistent with the results from the A2780 antiproliferative bioassay and

tubulin assembly assay. In androgen-dependent prostate cancer cells, the C10 docetaxel analogs **3.5**, **3.6**, and **3.8-3.11** show similar activities to those of paclitaxel and docetaxel, while C3' docetaxel analog **3.7** shows essentially no activity. Unfortunately, in an androgen-independent prostate cancer cell line, even the most active analogs **3.8-3.11** are still not as active as docetaxel or paclitaxel. This result may be due to fact that paclitaxel or docetaxel bind to the inner surface of microtubules, which would require a longer linker to allow the cyanonilutamide moiety to reach outside the microtubule tube, making the crosslinking of microtubules and androgen receptor extremely difficult.



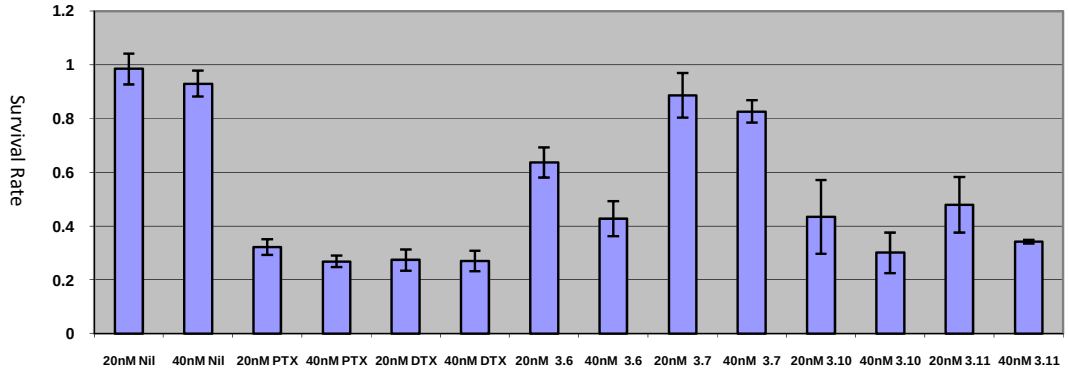
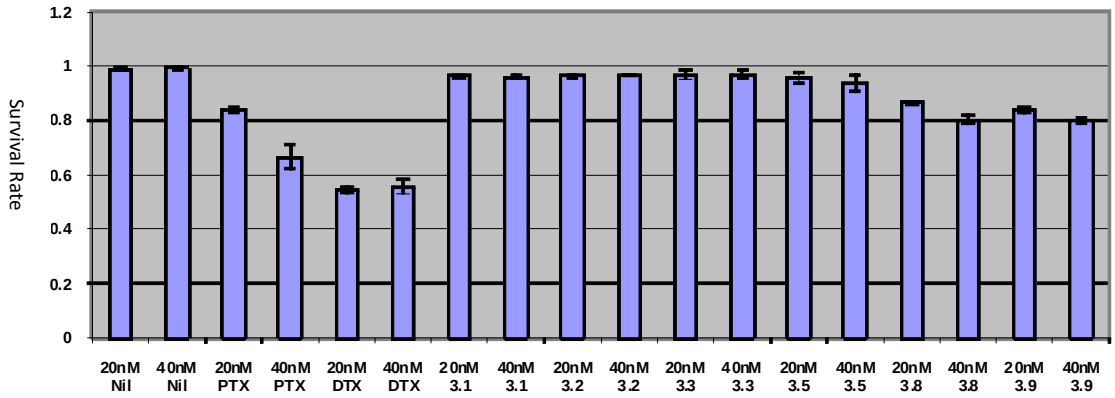


Chart 3.2 Activity in androgen-dependent prostate cancer cells



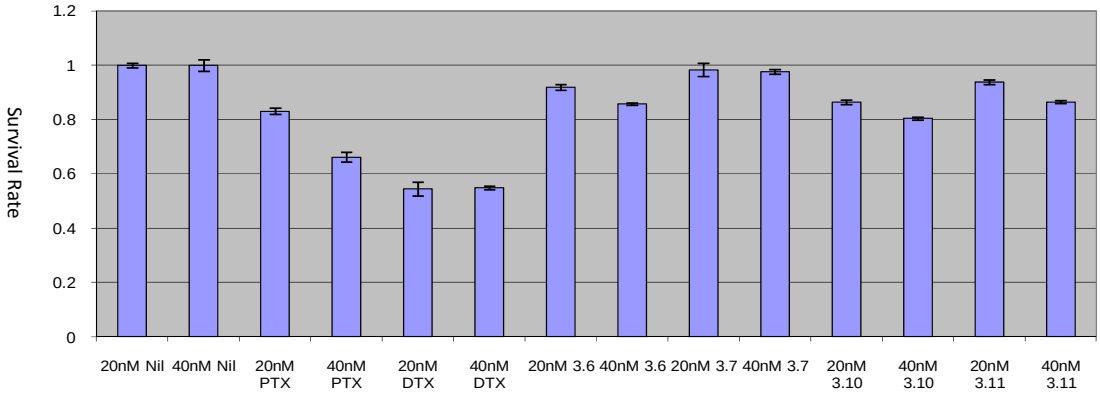


Chart 3.3 Activity in androgen-independent prostate cancer cells

3.3 Conclusions

Eleven paclitaxel or docetaxel analogs with a cyanonilutamide moiety linked at the C7, C10 or C3' position through linkers of various lengths were designed and synthesized. Among them, the C7 paclitaxel analogs all showed significantly reduced activity in the A2780 bioassay and tubulin assembly bioassays compared with paclitaxel, but all C10 docetaxel analogs preserved the tubulin assembly activities or even showed enhanced

activities in comparison with docetaxel. This result suggests that the C7 position in paclitaxel or docetaxel is more sensitive to the influence of bulky substituents on tubulin assembly and antiproliferative activity than the C10 position. Additionally, the C3' docetaxel analog was not as active as we had been expected, indicating the limitation of modifications at this position.

In androgen-dependent prostate cancer cells, the C10 docetaxel analogs displayed similar activity to that of paclitaxel or docetaxel in agreement with the results from the A2780 and tubulin assembly bioassays. However, it was disappointing that none of them were as active as paclitaxel or docetaxel against androgen-independent prostate cancer cells, although the C10 docetaxel analogs did show much better activity than the other analogs. This result may be due to fact that paclitaxel or docetaxel bind to the inner surface of microtubules, which would require a longer linker to allow the cyanonilutamide moiety to reach outside the microtubule tube, making the crosslinking of microtubules and androgen receptor extremely difficult. Therefore, a docetaxel analog with the cyanonilutamide moiety connected to the C10 position through a much longer PEG linker could be the next candidate in further work.

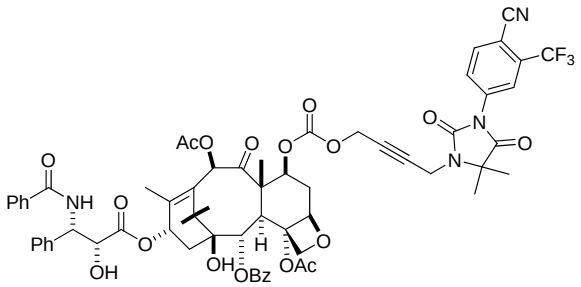
3.4 Experimental section

General experimental methods

All chemicals obtained from commercial sources were used without further purification. The anhydrous reactions were performed under nitrogen or argon. All solvents were of reagent grade or HPLC grade. Tetrahydrofuran (THF) was distilled over

sodium/benzophenone, and dichloromethane (DCM) was distilled over calcium hydride. The reactions were monitored by the analytical thin layer chromatography (TLC) plates (silica gel 60 GF) and analyzed with 254 nm UV light, $KMnO_4$ stain, and/or vanillin/sulfuric acid spray. Preparative thin layer chromatography (PTLC) plates were purchased from Analtech. All NMR spectral data were obtained on JEOL Eclipse spectrometer at 500 MHz, Varian Unity or Varian Inova spectrometer at 400 MHz. Chemical shifts reported as δ-values relative to known solvent residue peaks. High-resolution mass spectra (HRMS) were obtained in the analytical service in the Department of Chemistry at Virginia Tech. Known compounds were prepared through the reported procedures, and the NMR data of these compounds matched the literature values. All compounds were more than 95% pure as judged by the TLC and ^{1}H NMR.

7-((4-(Cyanonilutamide-3'-yl)but-2-ynyloxy)carbonyloxy)-paclitaxel (3.1)

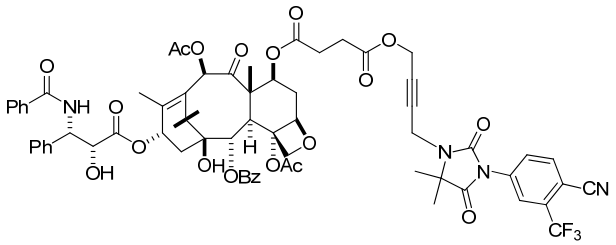


3.1

To a solution of 7-((4-(cyanonilutamide-3'-yl)but-2-ynyloxy)carbonyl)-2'-TBS paclitaxel **3.20** (15.7 mg, 0.012 mmol) in THF (1 mL) was added HF-pyridine (0.2 mL) at 0 ^{o}C. The mixture was stirred at room temperature and the reaction was monitored by TLC. When the reaction was complete, saturated $NaHCO_3$ solution was added to the

reaction mixture. The mixture was then extracted with ethyl acetate (3 x 15 mL), washed with saturated brine, and dried over Na_2SO_4. After filtration and rotary evaporation, the residue was then purified by flash chromatography (50% ethyl acetate in hexane) to afford C7 paclitaxel conjugate **3.1** (12.9 mg, 86.4%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (1H, d, *J* = 2.0 Hz), 8.11 (2H, dd, *J* = 8.0, 1.5Hz), 7.99 (1H, dd, *J* = 8.5, 2.0 Hz), 7.92 (1H, d, *J* = 8.5 Hz), 7.75 (2H, dd, *J* = 8.5, 1.5 Hz), 7.62 (1H, tt, *J* = 7.5, 1.5 Hz), 7.52 – 7.40 (9H, m), 7.35 (1H, tt, *J* = 7.5, 1.5 Hz), 7.01 (1H, d, *J* = 9.0 Hz), 6.28 (1H, s), 6.18 (1H, t, *J* = 9.0 Hz), 5.79 (1H, dd, *J* = 9.0, 2.5 Hz), 5.66 (1H, d, *J* = 7.0 Hz), 5.46 (1H, dd, *J* = 9.0, 2.5 Hz), 4.93 (1H, d, *J* = 7.0 Hz), 4.91 (1H, dt, *J* = 16.0, 2.0 Hz), 4.79 (1H, dd, *J* = 4.0, 2.5 Hz), 4.67 (1H, dt, *J* = 16.0, 2.0 Hz), 4.32 (1H, d, *J* = 8.0 Hz), 4.28 (2H, t, *J* = 2.0 Hz), 4.17 (1H, d, *J* = 8.5 Hz), 3.91 (1H, d, *J* = 6.5 Hz), 3.54 (1H, broad s), 2.58 (1H, ddd, *J* = 15.0, 10.0, 7.5 Hz), 2.38 (3H, s), 2.33 (1H, d, *J* = 9.5 Hz), 2.32 (1H, d, *J* = 9.0 Hz), 2.15 (3H, s), 1.97 (1H, ddd, *J* = 14.5, 10.5, 2.0 Hz), 1.84 (3H, d, *J* = 1.0 Hz), 1.80 (3H, s), 1.71 (1H, broad s), 1.61 (6H, s), 1.21 (3H, s), 1.16 (3H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 201.6, 174.4, 172.7, 170.6, 169.2, 167.1, 166.9, 153.7, 152.4, 140.6, 138.0, 136.3, 135.4, 133.9, 133.7, 133.0, 132.1, 130.3, 129.1, 128.9, 128.8, 128.5, 128.1, 127.2, 127.1, 123.2, 123.2, 115.1, 108.6, 83.8, 81.7, 81.0, 78.6, 78.3, 76.5, 76.1, 75.4, 74.3, 73.2, 72.3, 64.5, 62.2, 56.3, 55.9, 55.0, 47.0, 43.3, 35.6, 33.5, 29.3, 26.6, 23.3, 22.6, 21.0, 20.9, 14.7, 10.7; HRMS (FAB+) calcd for $C_{65}H_{64}F_3N_4O_{18}$ $[M+H]^+$ 1245.4168, found 1245.4193.

7-(4-(4-(Cyanonilutamide-3'-yl)but-2-ynyloxy)-4-oxobutanoyl)paclitaxel (3.2)

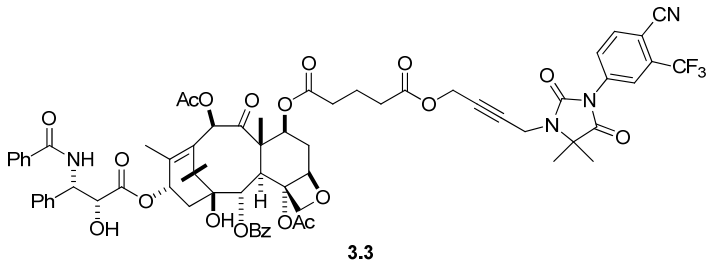


3.2

To a solution of 7-(3-carboxypropanoyl)-2'-TBS paclitaxel **3.21a** (21.4 mg, 0.02 mmol), 3'-(4-hydroxybut-2-ynyl)cyanonilutamide **3.17** (21.4 mg, 0.06 mmol) and DMAP (7 mg, 0.06 mmol) in DCM (1 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDCI) (11.5 mg, 0.06 mmol). The mixture was stirred at room temperature overnight. After the addition of water, the mixture was extracted with ethyl acetate (3 x 15 mL), washed with saturated brine, and dried over Na_2SO_4. Then the mixture was vacuum filtered and rotary evaporated. Without purification, the residue (19.4 mg) was dissolved in THF (1 mL) and HF-pyridine (0.23 mL) was added at 0 ^oC. The mixture was stirred at room temperature until the completion of the reaction (monitored by TLC). Then saturated $NaHCO_3$ solution was added to the reaction mixture. The mixture was then extracted with ethyl acetate (3 x 15 mL), washed with saturated brine, and dried over Na_2SO_4. After vacuum filtration and rotary evaporation, the residue was then purified by flash chromatography (50% ethyl acetate in hexane) to give 7-(4-(4-(cyanonilutamide-3'-yl)but-2-ynyloxy)-4-oxobutanoyl)paclitaxel **3.2** (14 mg, 53.8% for two steps) as a white solid: ^1H NMR (500 MHz, $CDCl_3$) δ 8.12 (1H, d, J = 2.0 Hz), 8.10 (2H, dd, J = 8.0, 1.5Hz), 7.97 (1H, dd, J = 8.0, 2.0 Hz), 7.89 (1H, d, J = 8.0 Hz), 7.76 (2H, dd, J = 8.5, 1.5 Hz), 7.62 (1H, tt, J = 7.5, 1.5 Hz), 7.52 – 7.40 (9H, m), 7.34 (1H, tt, J =

7.0, 1.5 Hz), 7.04 (1H, d, *J* = 9.0 Hz), 6.16 (1H, s), 6.16 (1H, t, *J* = 9.0 Hz), 5.79 (1H, dd, *J* = 9.0, 2.5 Hz), 5.66 (1H, d, *J* = 7.0 Hz), 5.56 (1H, dd, *J* = 10.8, 7.0 Hz), 4.92 (1H, d, *J* = 10.0 Hz), 4.79 (1H, d, *J* = 3.0 Hz), 4.67 (2H, d, *J* = 3.5 Hz), 4.30 (1H, d, *J* = 8.5 Hz), 4.27 (2H, d, *J* = 2.0 Hz), 4.17 (1H, d, *J* = 8.5 Hz), 3.90 (1H, d, *J* = 7.0 Hz), 3.61 (1H, d, *J* = 5.0 Hz), 2.73 – 2.65 (1H, m), 2.66 – 2.54 (4H, m), 2.36 (3H, s), 2.33 (1H, d, *J* = 9.0 Hz), 2.32 (1H, d, *J* = 9.0 Hz), 2.15 (3H, s), 1.86 – 1.79 (1H, m), 1.81 (3H, s), 1.80 (3H, s), 1.61 (6H, d, *J* = 2.5 Hz), 1.20 (3H, s), 1.13 (3H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 202.0, 174.4, 172.6, 171.9, 171.3, 170.6, 169.2, 167.0, 152.5, 140.6, 138.2, 136.4, 135.5, 133.9, 133.8, 133.0, 132.1, 130.4 129.1, 128.9, 128.7, 128.3, 127.3, 123.1, 115.0, 108.6, 83.9, 81.2, 80.9, 78.9, 78.7, 76.6, 75.5, 74.4, 73.3, 72.2, 71.9, 62.3, 56.2, 55.0, 52.1, 47.1, 43.5, 35.8, 33.5, 29.2, 29.1, 28.8, 26.7, 23.2, 22.6, 20.9, 14.8, 10.9; HRMS (FAB+) calcd for $C_{68}H_{68}F_3N_4O_{19}$ $[M+H]^+$ 1301.4430, found 1301.4403.

7-(5-(4-(Cyanonilutamide-3'-yl)but-2-ynyloxy)-5-oxopentanoyl)paclitaxel (3.3)



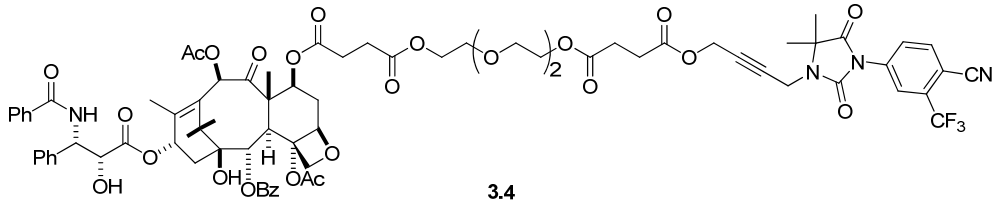
3.3

To a solution of 7-(4-carboxybutanoyl)-2'-TBS paclitaxel **3.21b** (10 mg, 0.01 mmol), 3'-(4-hydroxybut-2-ynyl)cyanonilutamide **3.17** (11 mg, 0.03 mmol) and DMAP (4 mg, 0.03 mmol) in DCM (1 mL) was added EDCI (6 mg, 0.03 mmol). The mixture was stirred at room temperature overnight. After the addition of water, the mixture was

extracted with ethyl acetate (3 x 15 mL), washed with saturated brine, and dried over Na_2SO_4. Then the mixture was filtered, concentrated and vacuum dried. Without purification, the residue (12.3 mg) was dissolved in THF (1 mL) and HF-pyridine (0.2 mL) was added at 0 ^oC. The mixture was stirred at room temperature and the reaction was monitored by TLC. When starting material was completely consumed, saturated $NaHCO_3$ solution was added to the reaction mixture carefully. The mixture was then extracted with ethyl acetate (3 x 15 mL), washed with saturated brine, and dried over Na_2SO_4. After filtration and rotary evaporation, the residue was then purified by flash chromatography (50% ethyl acetate in hexane) to give 7-(4-(4-(cyanonilutamide-3'-yl)but-2-ynyl)-4-oxopentanoyloxy)paclitaxel **3.3** (10.2 mg, 77.6% for two steps) as a white solid: ^1H NMR (500 MHz, $CDCl_3$) δ 8.13 (1H, d, *J* = 2.0 Hz), 8.10 (2H, d, *J* = 8.0 Hz), 7.99 (1H, dd, *J* = 8.5, 2.0 Hz), 7.92 (1H, d, *J* = 8.5 Hz), 7.76 (2H, d, *J* = 8.0 Hz), 7.62 (1H, t, *J* = 7.5 Hz), 7.52 – 7.47 (5H, m), 7.43 – 7.39 (4H, m), 7.34 (1H, t, *J* = 7.0 Hz), 7.05 (1H, d, *J* = 8.5 Hz), 6.19 (1H, s), 6.16 (1H, t, *J* = 9.0 Hz), 5.79 (1H, dd, *J* = 9.0, 2.0 Hz), 5.65 (1H, d, *J* = 6.0 Hz), 5.54 (1H, dd, *J* = 10.0, 8.0 Hz), 4.92 (1H, d, *J* = 8.5 Hz), 4.79 (1H, d, *J* = 3.0 Hz), 4.66 (2H, s), 4.31 (1H, d, *J* = 8.5 Hz), 4.28 (2H, s), 4.17 (1H, d, *J* = 8.5 Hz), 3.90 (1H, d, *J* = 7.5 Hz), 3.61 (1H, d, *J* = 5.0 Hz), 2.57 (1H, ddd, *J* = 14.5, 9.5, 8.0 Hz), 2.45 – 2.36 (4H, m), 2.37 (3H, s), 2.32 (1H, s), 2.31(1H, s), 2.14 (3H, s), 1.94 – 1.87 (2H, m), 1.85 – 1.78 (1H, m), 1.82 (3H, d, *J* = 0.8 Hz), 1.79 (3H, s), 1.61 (6H, s), 1.19 (3H, s), 1.15 (3H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 202.0, 174.4, 172.5, 172.3, 172.0, 170.6, 169.0, 167.1, 152.5, 140.7, 138.2, 136.4, 135.5, 133.9, 133.8, 133.0, 132.0, 130.3, 129.2, 128.9, 128.5, 128.1, 127.2, 123.2, 115.1, 108.6, 84.0, 81.1, 80.9, 78.8, 78.6, 76.6, 75.4, 74.4, 73.3, 72.2, 71.6, 62.2, 56.3, 55.0, 52.0, 47.0, 43.3, 35.6, 33.6, 33.1, 29.3, 26.6, 23.3, 22.7, 20.9, 19.6,

14.8, 10.9; HRMS (FAB+) calcd for $C_{69}H_{70}F_3N_4O_{19}$ $[M+H]^+$ 1315.4586, found 1315.4564.

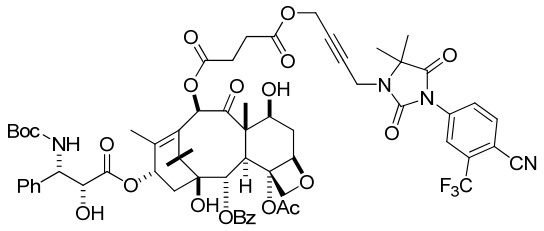
7-(23-(Cyanonilutamide-3'-yl)-4,15,18-trioxo-5,8,11,14,19-pentaoxatricos-21-yneoyl) paclitaxel (3.4)



To a solution of palitaxel analog **3.21a** (24 mg, 0.02 mmol), cyanonilutamide derivative **3.38b** (20 mg, 0.034 mmol) and DMAP (8 mg, 0.06 mmol) in DCM (0.6 mL) was added EDCI (12 mg, 0.06 mmol). The mixture was stirred at room temperature overnight. After the addition of water, the mixture was extracted with ethyl acetate (3 x 10 mL), washed with saturated brine, and dried over Na_2SO_4. Then the mixture was vacuum filtered and rotary evaporated. After purification by flash chromatography (60% ethyl acetate in hexane), the product (14 mg, 0.0085 mmol) was dissolved in THF (0.6 mL) and HF-pyridine (0.14 mL) was added at 0 ^oC. The mixture was stirred at room temperature until the completion of the reaction (monitored by TLC). Then saturated $NaHCO_3$ solution was added to the reaction mixture. The mixture was then extracted with ethyl acetate (3 x 10 mL), washed with saturated brine, and dried over Na_2SO_4. After vacuum filtration and rotary evaporation, the residue was then purified by flash chromatography (70% ethyl acetate in hexane) to give the C7 paclitaxel conjugate **3.4** (7 mg, 22.8% for two steps) as a white solid: ^1H NMR (500 MHz, $CDCl_3$) δ 8.13 (1H, d, J =

2.0 Hz), 8.10 (2H, dd, *J* = 8.0, 1.5Hz), 7.98 (1H, dd, *J* = 8.5, 2.0 Hz), 7.91 (1H, d, *J* = 8.5 Hz), 7.76 (2H, dd, *J* = 8.0, 1.5 Hz), 7.61 (1H, tt, *J* = 7.5, 1.5 Hz), 7.52 – 7.46 (5H, m), 7.43 – 7.38 (4H, m), 7.34 (1H, tt, *J* = 7.5, 2.0 Hz), 7.08 (1H, d, *J* = 9.0 Hz), 6.17 (1H, s), 6.16 (1H, dt, *J* = 9.0, 1.5 Hz), 5.79 (1H, dd, *J* = 8.5, 2.5 Hz), 5.65 (1H, d, *J* = 7.0 Hz), 5.55 (1H, dd, *J* = 10.5, 7.5 Hz), 4.92 (1H, dd, *J* = 9.0, 1.0 Hz), 4.79 (1H, d, *J* = 2.5 Hz), 4.68 (2H, d, *J* = 2.0 Hz), 4.30 (1H, d, *J* = 9.0 Hz), 4.27 (2H, d, *J* = 2.0 Hz), 4.25 – 4.21 (4H, m), 4.17 (1H, d, *J* = 9.0 Hz), 3.89 (1H, d, *J* = 7.0 Hz), 3.70 – 3.67 (4H, m), 3.64 (4H, s), 2.71 – 2.54 (9H, m), 2.36 (3H, s), 2.35 – 2.28 (2H, m), 2.15 (3H, s), 1.86 – 1.79 (1H, m), 1.81 (3H, d, *J* = 1.0 Hz), 1.79 (3H, s), 1.61 (6H, d, *J* = 2.5 Hz), 1.19 (3H, s), 1.15 (3H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 201.9, 174.4, 172.6, 172.5, 172.2, 171.6, 170.4, 169.1, 167.1, 167.0, 152.4, 140.5, 138.1, 136.3, 135.4, 133.9, 133.8, 133.0, 132.0, 130.2, 129.1, 128.8, 128.4, 128.1, 127.2, 127.1, 123.2, 115.1, 108.6, 84.0, 81.1, 78.6, 76.5, 75.4, 74.3, 73.3, 72.2, 71.6, 70.6, 69.2, 69.1, 64.0, 63.8, 62.2, 56.2, 55.0, 52.3, 47.1, 43.3, 35.6, 33.4, 29.2, 28.9, 28.8, 26.6, 23.3, 22.6, 20.9, 14.7, 10.9; HRMS (MALDI+) calcd for $C_{78}H_{83}F_3N_4O_{25}Na$ $[M+Na]^+$ 1555.5196, found 1555.4937.

10-(4-(4-(Cyanonilutamide-3'-yl)but-2-ynyloxy)-4-oxobutanoyl) docetaxel (3.5)

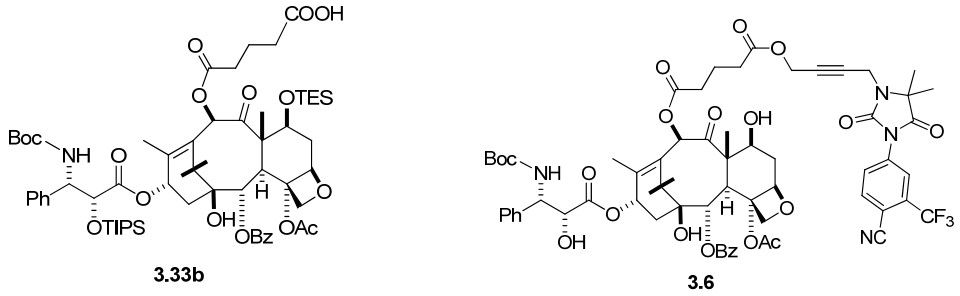


3.5

To a solution of 7-TES-10-(3-carboxypropanoyl)-2'-TIPS docetaxel **3.33a** (22 mg, 0.019 mmol), 3'-(4-hydroxybut-2-ynyl)-cyanonilutamide **3.17** (22.3 mg, 0.057 mmol) and DMAP (7 mg, 0.057 mmol) in DCM (1 mL) was added EDCI (11 mg, 0.057 mmol). The mixture was stirred at room temperature overnight. After the addition of water, the mixture was extracted with ethyl acetate (3 x 15 mL), washed with saturated brine, and dried over Na_2SO_4. Then the mixture was vacuum filtered and rotary evaporated. Without purification, the residue (28 mg) was dissolved in THF (1 mL) and HF-pyridine (0.3 mL) was added at 0 ^{o}C. The mixture was stirred at room temperature until the completion of the reaction (monitored by TLC). Then saturated $NaHCO_3$ solution was added to the reaction mixture. The mixture was extracted with ethyl acetate (3 x 15 mL), washed with saturated brine, and dried over Na_2SO_4. After vacuum filtration and rotary evaporation, the residue was then purified by flash chromatography (50% ethyl acetate in hexane) to give 10-(4-(4-(cyanonilutamide-3'-yl)but-2-ynyloxy)-4-oxobutanoyl) docetaxel **3.5** (13.3 mg, 55.8% for two steps) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (1H, d, *J* = 2.0 Hz), 8.10 (2H, d, *J* = 7.5 Hz), 7.99 (1H, dd, *J* = 8.0, 2.0 Hz), 7.90 (1H, d, *J* = 8.0 Hz), 7.61 (1H, tt, *J* = 7.5, 1.0 Hz), 7.50 (2H, t, *J* = 8.0 Hz), 7.42 – 7.35 (4H, m), 7.32 (1H, tt, *J* = 7.0, 1.0 Hz), 6.29 (1H, s), 6.22 (1H, t, *J* = 8.5 Hz), 5.65 (1H, d, *J* = 7.0 Hz), 5.35 (1H, d, *J* = 9.5 Hz), 5.25 (1H, d, *J* = 8.0 Hz), 4.93 (1H, dd, *J* = 9.0, 1.5 Hz), 4.71 (2H, s), 4.61 (1H, d, *J* = 1.0 Hz), 4.38 (1H, dd, *J* = 11.0, 6.5 Hz), 4.29 (1H, d, *J* = 8.0 Hz), 4.29 (2H, s), 4.15 (1H, d, *J* = 8.0 Hz), 3.78 (1H, d, *J* = 7.0 Hz), 3.32 (1H, s), 2.91 – 2.83 (2H, m), 2.83 – 2.66 (2H, m), 2.51 (1H, ddd, *J* = 15.5, 10.0, 7.0 Hz), 2.43 (1H, broad s), 2.37 (3H, s), 2.35 – 2.20 (2H, m), 1.89 – 1.81 (1H, m), 1.84 (3H, s), 1.66 (3H, s), 1.62 (6H, s), 1.33 (9H, s), 1.25 (3H, s), 1.13 (3H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 203.5, 174.5, 172.4,

171.3, 170.2, 167.2, 155.5, 152.4, 142.6, 138.3, 136.3, 135.4, 133.9, 132.9, 130.3, 129.1, 129.0, 128.8, 128.2, 128.1, 126.8, 123.2, 115.0, 108.8, 84.5, 81.2, 80.4, 79.2, 78.6, 76.5, 76.0, 75.0, 73.9, 72.5, 72.3, 62.3, 58.8, 56.3, 52.5, 45.7, 43.3, 35.8, 35.6, 29.2, 29.0, 28.9, 28.3, 26.9, 23.3, 22.8, 22.1, 15.0, 9.7; HRMS (FAB+) calcd for $C_{64}H_{70}F_3N_4O_{19}$ $[M+H]^+$ 1255.4586, found 1255.4573.

10-(5-(4-(Cyanonilutamide-3'-yl)but-2-ynyloxy)-5-oxopentanoyl) docetaxel (3.6)

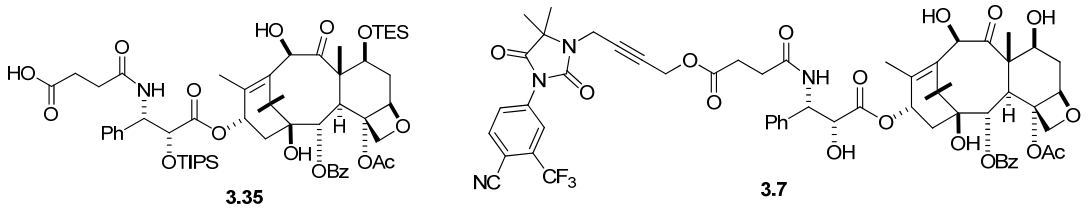


To a solution of docetaxel derivative **3.22** (87 mg, 0.082 mmol) in toluene (5 mL) was added glutaric anhydride (28 mg, 0.25 mmol) and DMAP (30 mg, 0.25 mmol). The mixture was stirred at 85-90 ^{o}C for 5 days. After it had cooled to room temperature, the reaction mixture was diluted with ethyl acetate and aqueous HCl solution (0.2%) was added carefully. The aqueous layer was then extracted with ethyl acetate (3 x 20 mL). The combined organic phase was washed with saturated brine and dried over Na_2SO_4. After vacuum filtration and rotary evaporation, the residue was purified by flash chromatography (40% ethyl acetate in hexane) to afford carboxylic acid **3.33b** as a white solid (38 mg, 39%).

To a solution of docetaxel analog **3.33b** (38 mg, 0.032 mmol), cynanolutamide analog **3.17** (34 mg, 0.096 mmol) and DMAP (12 mg, 0.096 mmol) in DCM (1.7 mL) was added EDCI (19 mg, 0.096 mmol). The mixture was stirred at room temperature overnight. After the addition of water, the mixture was extracted with ethyl acetate (3 x 15 mL), washed with saturated brine, and dried over Na_2SO_4. The mixture was vacuum filtered and rotary evaporated. Without purification, the residue was dissolved in pyridine (1.6 mL) and acetonitrile (0.8 mL). HF-pyridine (0.23 mL) was added to the solution at room temperature. The mixture was stirred at 62 ^{o}C until the completion of the reaction (monitored by TLC). Then saturated $NaHCO_3$ solution was added to the reaction mixture dropwise. The mixture was extracted with ethyl acetate (3 x 20 mL), washed with saturated brine, and dried over Na_2SO_4. After vacuum filtration and rotary evaporation, the residue was then purified by flash chromatography (50% ethyl acetate in hexane) to give C10 docetaxel analog **3.6** (20.4 mg, 53.6% for two steps) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (1H, d, *J* = 2.0 Hz), 8.10 (2H, d, *J* = 7.5 Hz), 7.99 (1H, dd, *J* = 8.5, 2.0 Hz), 7.90 (1H, d, *J* = 8.5 Hz), 7.61 (1H, tt, *J* = 7.5, 1.5 Hz), 7.50 (2H, t, J = 8.0 Hz), 7.42 – 7.35 (4H, m), 7.32 (1H, tt, *J* = 7.0, 1.5 Hz), 6.30 (1H, s), 6.23 (1H, t, *J* = 3.5 Hz), 5.66 (1H, d, *J* = 7.5 Hz), 5.35 (1H, d, *J* = 9.5 Hz), 5.25 (1H, d, *J* = 8.0 Hz), 4.94 (1H, dd, *J* = 9.5, 2.0 Hz), 4.69 (2H, t, *J* = 2.0 Hz), 4.62 (1H, s), 4.43 – 4.37 (1H, m), 4.30 (1H, d, *J* = 9.0 Hz), 4.29 (2H, t, *J* = 2 Hz), 4.16 (1H, d, *J* = 9.0 Hz), 3.79 (1H, d, *J* = 7.0 Hz), 3.34 (1H, d, *J* = 3.5 Hz), 2.65 – 2.50 (4H, m), 2.46 (1H, d, *J* =4.5 Hz), 2.37 (3H, s), 2.34 – 2.22 (2H, m), 2.04 (2H, tt, *J* = 7, 7 Hz), 1.90 – 1.83 (1H, m), 1.84 (3H, d, *J* = 1.0 Hz), 1.66 (3H, s), 1.63 (6H, s), 1.33 (9H, s), 1.25 (3H, s), 1.13 (3H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 203.7, 174.4, 173.0, 172.2, 170.3, 167.1, 155.5, 152.4, 142.3, 138.2, 136.3,

135.4, 133.9, 133.8, 132.9, 130.3, 129.2, 129.0, 128.8, 128.2, 128.1, 126.8, 123.2, 123.1, 115.0, 108.6, 84.4, 81.1, 81.0, 79.1, 78.7, 76.5, 75.7, 75.0, 73.8, 72.4, 72.3, 62.2, 58.7, 56.3, 52.1, 45.7, 43.3, 35.7, 35.5, 33.1, 32.8, 29.2, 28.2, 26.8, 23.3, 22.7, 22.0, 20.0, 14.9, 9.7; HRMS (FAB+) calcd for $C_{65}H_{72}F_3N_4O_{19}$ $[M+H]^+$ 1269.4743, found 1269.4652.

3'-(4-(4-(Cyanonilutamide-3'-yl)but-2-ynyloxy)-4-oxobutanoyl)-10-deacetyl-3′-debenzoyl paclitaxel (3.7)



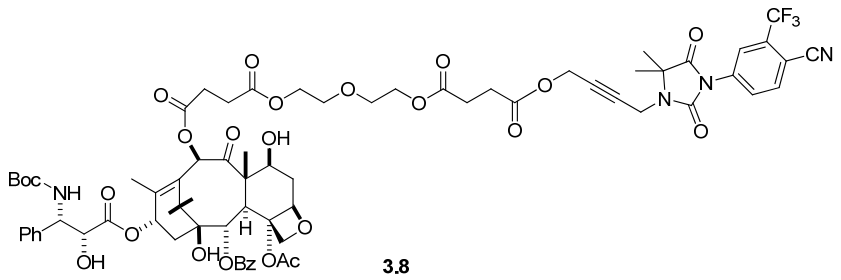
Paclitaxel derivative **3.34** (31 mg, 0.028 mmol) was dissolved in ethanol (2.6 mL) and hydrazine monohydrate (0.29 mL) was added. The mixture was stirred at room temperature overnight. After the addition of saturated NH_4Cl solution, the mixture was extracted with ethyl acetate (3 x 25 mL), washed with saturated brine, and dried over Na_2SO_4, followed by vacuum filtration and rotary evaporation. The residue was then purified by flash chromatography (50% ethyl acetate in hexane) to give docetaxel analog **3.35** (12 mg, 40%) as a white solid.

To a solution of compound **3.35** (12 mg, 0.011 mmol), cyanonilutamide analog **3.17** (12 mg, 0.034 mmol) and DMAP (4.2 mg, 0.034 mmol) in DCM (0.6 mL) was added EDCI (6.6 mg, 0.034 mmol). The mixture was stirred at room temperature overnight. After the addition of water, the mixture was extracted with ethyl acetate (3 x 15 mL),

washed with saturated brine, and dried over Na_2SO_4. Then the mixture was vacuum filtered and rotary evaporated. After purification by flash chromatography (50% ethyl acetate in hexane), the product (15 mg, 0.011 mmol) was dissolved in pyridine (0.6 mL) and acetonitrile (0.3 mL). HF-pyridine (0.081 mL) was added to the solution at room temperature. The mixture was stirred at 62 ^{o}C until the completion of the reaction (monitored by TLC). Then saturated $NaHCO_3$ solution was added to the reaction mixture dropwise. The mixture was extracted with ethyl acetate (3 x 20 mL), washed with saturated brine, and dried over Na_2SO_4. After vacuum filtration and rotary evaporation, the residue was then purified by flash chromatography (100% ethyl acetate) to give C3' docetaxel conjugate **3.7** (8.7 mg, 68.5% for two steps) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (d, 1H, *J* = 2.5 Hz), 8.09 (2H, dd, *J* = 8.5, 1.0 Hz), 7.96 (1H, dd, *J* = 8.5, 2.5 Hz), 7.89 (1H, d, *J* = 8.5 Hz), 7.60 (1H, tt, *J* = 7.5, 1.5 Hz), 7.50 (2H, t, *J* = 7.5 Hz), 7.41 – 7.33 (4H, m), 7.32 (1H, tt, *J* = 6.5, 2.0 Hz), 6.52 (1H, d, *J* = 9.0 Hz), 6.22 (1H, dt, *J* = 9.0, 1.5 Hz), 5.67 (1H, d, *J* = 7.0 Hz), 5.56 (1H, dd, *J* = 9.0, 2.5 Hz), 5.19 (1H, s), 4.91 (1H, dd, *J* = 9.5, 2.0 Hz), 4.65 (1H, d, *J* = 2.0 Hz), 4.53 (1H, dt, *J* = 16.0, 2.0 Hz), 4.32 (1H, dt, *J* = 16.0, 2.0 Hz), 4.27 (1H, d, *J* = 9.0 Hz), 4.23 – 4.20 (3H, m), 4.20 (1H, d, *J* = 8.5 Hz), 3.88 (1H, d, *J* = 6.5 Hz), 3.49 (1H, broad s), 2.71 – 2.64 (1H, m), 2.59 – 2.42 (4H, m), 2.34 (3H, s), 2.26 (2H, dd, *J* = 9.0, 6.5 Hz), 2.11 (1H, broad s), 1.87 – 1.79 (1H, m), 1.83 (3H, d, *J* = 0.5 Hz), 1.74 (3H, s), 1.57 (6H, s), 1.24 (3H, s), 1.12 (3H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 211.4, 174.3, 172.8, 172.4, 170.8, 170.3, 166.9, 152.4, 138.3, 138.0, 136.3, 136.2, 135.4, 133.8, 130.3, 129.4, 129.0, 128.8, 128.3, 128.1, 126.9, 123.2, 123.1, 115.0, 108.6, 84.2, 81.2, 81.1, 78.7, 78.3, 76.7, 74.9, 74.6, 73.2, 72.8, 72.1, 62.2,

57.7, 54.6, 52.4, 46.5, 43.2, 37.0, 36.0, 30.4, 29.1, 29.0, 26.6, 23.2, 22.7, 20.9, 14.4, 10.0; HRMS (FAB+) calcd for $C_{59}H_{62}F_3N_4O_{17}$ $[M+H]^+$ 1155.4062, found 1155.5883.

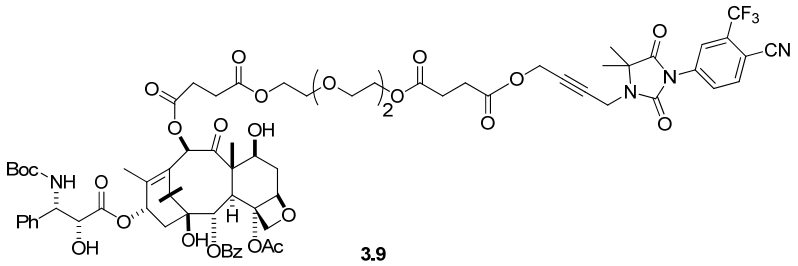
10-(20-(Cyanonilutamide-3'-yl)-1,4,12,15-tetraoxo-5,8,11,16-tetraoxaicos-18-yneoyl) docetaxel (3.8)



To a solution of docetaxel analog **3.33a** (20 mg, 0.017 mmol), compound **3.38a** (11 mg, 0.0204 mmol) and DMAP (6.7 mg, 0.051 mmol) in DCM (0.5 mL) was added EDCI (10 mg, 0.051 mmol). The mixture was stirred at room temperature overnight. After the addition of water, the mixture was extracted with ethyl acetate (3 x 10 mL), washed with saturated brine, and dried over Na_2SO_4. Then the mixture was vacuum filtered and rotary evaporated. After purified by flash chromatography (40% ethyl acetate in hexane), the product (14.5 mg, 0.0085 mmol) was dissolved in pyridine (0.5 mL) and acetonitrile (0.25 mL). HF-pyridine (0.063 mL) was added to the solution at room temperature. The mixture was stirred at 62 ^oC until the completion of the reaction (monitored by TLC). Then saturated $NaHCO_3$ solution was added to the reaction mixture dropwise. The mixture was extracted with ethyl acetate (3 x 15 mL), washed with saturated brine, and dried over Na_2SO_4. After vacuum filtration and rotary evaporation, the residue was then purified by flash chromatography (70% ethyl acetate in hexane) to give C10 docetaxel

analog **3.8** (10.2 mg, 40.8% for two steps) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (1H, d, *J* = 1.5 Hz), 8.10 (2H, d, *J* = 7.5 Hz), 7.99 (1H, dd, *J* = 8.5, 2.0 Hz), 7.91 (1H, d, *J* = 8.5 Hz), 7.61 (1H, tt, *J* = 7.5, 1.5 Hz), 7.49 (2H, t, *J* = 7.5 Hz), 7.42 – 7.35 (4H, m), 7.32 (1H, tt, *J* = 7.0, 1.5 Hz), 6.30 (1H, s), 6.22 (1H, t, *J* = 8.5 Hz), 5.65 (1H, d, *J* = 7.0 Hz), 5.36 (1H, d, *J* = 10.0 Hz), 5.25 (1H, d, *J* = 9.0 Hz), 4.93 (1H, dd, *J* = 9.5, 2.0 Hz), 4.69 (2H, t, *J* = 2.0 Hz), 4.61 (1H, m), 4.39 (1H, dd, *J* = 11.0, 7.0 Hz), 4.29 (1H, d, *J* = 8.5 Hz), 4.27 (2H, t, *J* = 2.5 Hz), 4.27 – 4.23 (4H, m), 4.16 (1H, d, *J* = 8.5 Hz), 3.78 (1H, d, *J* = 7.5 Hz), 3.71 – 3.67 (4H, m), 3.34 (1H, broad s), 2.85 (2H, dt, *J* = 6.5, 2.5 Hz), 2.81 – 2.68 (2H, m), 2.68 – 2.65 (4H, m), 2.53 (1H, ddd, *J* = 15.0, 9.5, 6.5 Hz), 2.47 (1H, broad s), 2.37 (3H, s), 2.37 – 2.22 (2H, m), 1.89 – 1.82 (1H, m), 1.84 (3H, s), 1.72 (1H, broad s), 1.66 (3H, s), 1.61 (6H, s), 1.33 (9H, s), 1.25 (3H, s), 1.13 (3H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 203.6, 174.4, 173.1, 172.5, 172.2, 172.0, 171.6, 170.3, 167.1, 155.5, 152.4, 142.7, 138.4, 136.3, 135.4, 133.8, 133.0, 130.3, 129.2, 129.0, 128.8, 128.2, 128.1, 126.8, 123.2, 115.0, 108.6, 84.5, 81.2, 81.1, 80.4, 79.1, 78.6, 76.5, 75.9, 75.0, 73.7, 72.5, 72.2, 69.0, 63.9, 62.2, 58.6, 56.3, 52.3, 45.7, 43.3, 35.8, 35.6, 29.2, 29.1, 29.0, 28.9, 28.8, 28.2, 26.8, 23.2, 22.7, 22.0, 14.9, 9.6; HRMS (FAB+) calcd for $C_{72}H_{82}F_3N_4O_{24}$ $[M+H]^+$ 1443.5271, found 1443.5204.

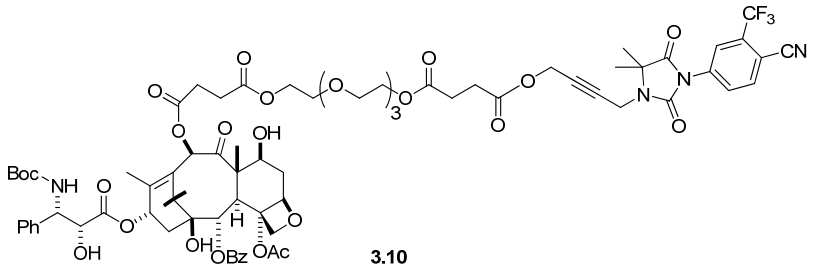
10-(23-(Cyanonilutamide-3'-yl)-1,4,15,18-tetraoxo-5,8,11,14,19-pentaoxatricos-21-yneoyl)docetaxel (3.9)

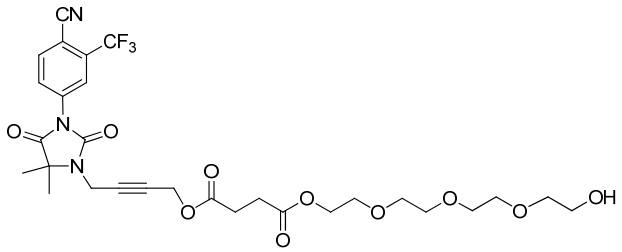


To a solution of docetaxel analog **3.33a** (20 mg, 0.017 mmol), compound **3.38b** (15 mg, 0.026 mmol) and DMAP (6.2 mg, 0.051 mmol) in DCM (0.8 mL) was added EDCI (10 mg, 0.051 mmol). The mixture was stirred at room temperature overnight. After the addition of water, the mixture was extracted with ethyl acetate (3 x 15 mL), washed with saturated brine, and dried over Na_2SO_4. Then the mixture was vacuum filtered and rotary evaporated. After purification by flash chromatography (50% ethyl acetate in hexane), the product (12 mg, 0.007 mmol) was dissolved in pyridine (0.4 mL) and acetonitrile (0.2 mL). HF-pyridine (0.052 mL) was added to the solution at room temperature. The mixture was stirred at 62 ^{o}C until the completion of the reaction (monitored by TLC). Then saturated $NaHCO_3$ solution was added to the reaction mixture dropwise. The mixture was extracted with ethyl acetate (3 x 15 mL), washed with saturated brine, and dried over Na_2SO_4. After vacuum filtration and rotary evaporation, the residue was then purified by flash chromatography (70% ethyl acetate in hexane) to give C10 docetaxel conjugate **3.9** (8.2 mg, 34.5% for two steps) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (1H, d, *J* = 2.0 Hz), 8.10 (2H, d, *J* = 7.0 Hz), 7.99 (1H, dd, *J* = 8.5, 2.0 Hz), 7.91 (1H, d, *J* = 8.5 Hz), 7.61 (1H, tt, *J* = 7.0, 1.5 Hz), 7.49 (2H, t, J = 7.5 Hz), 7.42 – 7.35

(4H, m), 7.32 (1H, tt, *J* = 7.0, 1.5 Hz), 6.29 (1H, s), 6.22 (1H, t, *J* = 9.0 Hz), 5.65 (1H, d, *J* = 7.0 Hz), 5.37 (1H, d, *J* = 9.0 Hz), 5.25 (1H, d, *J* = 8.5 Hz), 4.93 (1H, dd, *J* = 9.0, 1.5 Hz), 4.68 (2H, t, *J* = 2.0 Hz), 4.61 (1H, broad s), 4.38 (1H, dd, *J* =4.5, 3.5 Hz), 4.29 (1H, d, *J* = 8.5 Hz), 4.27 (2H, t, *J* = 2.0 Hz), 4.27 – 4.22 (4H, m), 4.16 (1H, d, *J* = 8.5 Hz), 3.78 (1H, d, *J* = 7.0 Hz), 3.71 – 3.67 (4H, m), 3.65 (4H, s), 3.36 (1H, broad s), 2.85 (2H, dt, *J* = 7.0, 2.0 Hz), 2.81 – 2.67 (2H, m), 2.68 – 2.63 (4H, m), 2.52 (1H, ddd, *J* = 15.0, 10.0, 6.5 Hz), 2.48 (1H, broad s), 2.37 (3H, s), 2.28 (2H, m), 1.90 – 1.81 (1H, m), 1.84 (3H, s), 1.73 (1H, broad s), 1.66 (3H, s), 1.61 (6H, s), 1.33 (9H, s), 1.25 (3H, s), 1.13 (3H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 203.7, 174.4, 173.1, 172.6, 172.2, 172.1, 171.6, 170.3, 167.1, 155.5, 152.4, 142.6, 138.4, 136.3, 135.4, 133.8, 133.0, 130.3, 129.2, 129.0, 128.8, 128.2, 128.1, 126.8, 123.2, 115.0, 108.6, 84.5, 81.2, 81.1, 80.4, 79.1, 78.6, 76.5, 75.9, 75.0, 73.7, 72.5, 72.2, 70.6, 69.2, 64.0, 62.2, 58.6, 56,3, 52.3, 45.7, 43.2, 35.7, 35.6, 29.2, 29.1, 28.9, 28.8, 28.2, 26.8, 23.3, 22.7, 22.0, 14.9, 9.6; HRMS (FAB+) calcd for $C_{74}H_{86}F_3N_4O_{25}$ $[M+H]^+$ 1487.5533, found 1487.5585.

10-(26-(Cyanonilutamide-3'-yl)-1,4,18,21-tetraoxo-5,8,11,14,17,22-hexaoxahexacos-24-yneyl)docetaxel (3.10)





3.38c

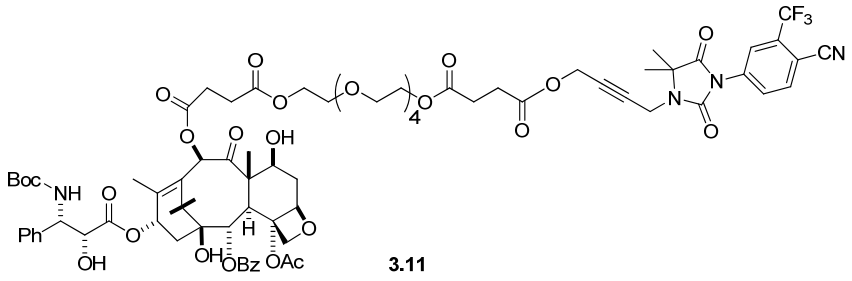
Tetra(ethylene glycol) (1 mL, 6 mmol) and pyridine (0.47 mL, 5.8 mmol) were dissolved in DCM (4.6 mL) and a solution of TBSCl (0.873 g, 5.8 mmol) in DCM (2.3 mL) was added at 0 ^{o}C. The mixture was stirred at room temperature overnight. The reaction solution was concentrated and purified by flash chromatography (50% ethyl acetate in hexane) to give TBS ether as a colorless oil (717 mg, 40%).

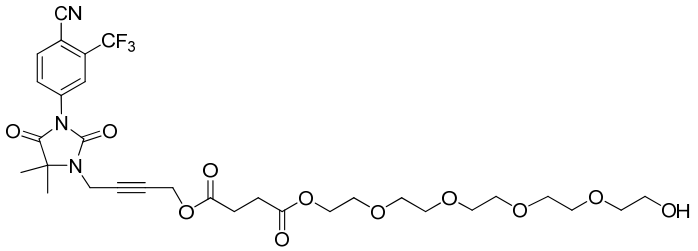
To a solution of tetra(ethylene glycol) TBS ether (70 mg, 0.22 mmol), cyanonilutamide analog **3.37** (34 mg, 0.075 mmol) and DMAP (28 mg, 0.22 mmol) in DCM (4 mL) was added EDCI (44 mg, 0.22 mmol). The mixture was stirred at room temperature overnight. After the addition of water, the mixture was extracted with ethyl acetate (3 x 20 mL), washed with saturated brine, and dried over Na_2SO_4. Then the mixture was vacuum filtered and rotary evaporated. Without purification, the residue was dissolved in a solution of HCl in ethanol (1%, 7.2 mL) at room temperature. After stirring for 15 minutes, the mixture was treated with saturated $NaHCO_3$ solution carefully and extracted with ethyl acetate (3 x 20 mL). The combined organic phase was then washed with saturated brine, dried over Na_2SO_4, vacuum filtered, and rotary evaporated. The residue was purified by flash chromatography (5% methanol in DCM) to afford cyanonilutamide derivative **3.38c** (19.5 mg, 40.6% for two steps) as a white solid.

To a solution of docetaxel analog **3.33a** (22 mg, 0.019 mmol), cyanonilutamide derivative **3.38c** (10 mg, 0.0156 mmol) and DMAP (6 mg, 0.047 mmol) in DCM (0.5 mL) was added EDCI (10 mg, 0.05 mmol). The mixture was stirred at room temperature overnight. After the addition of water, the mixture was extracted with ethyl acetate (3 x 15 mL), washed with saturated brine, and dried over Na_2SO_4. Then the mixture was vacuum filtered and rotary evaporated. After purified by flash chromatography (60% ethyl acetate in hexane), the product (16 mg, 0.009 mmol) was dissolved in pyridine (0.5 mL) and acetonitrile (0.25 mL). HF-pyridine (0.068 mL) was added to the solution at room temperature. The mixture was stirred at 62 ^{o}C until the completion of the reaction (monitored by TLC). Then saturated $NaHCO_3$ solution was added to the reaction mixture dropwise. The mixture was extracted with ethyl acetate (3 x 15 mL), washed with saturated brine, and dried over Na_2SO_4. After vacuum filtration and rotary evaporation, the residue was then purified by flash chromatography (70% ethyl acetate in hexane) to give C10 docetaxel conjugate **3.10** (8.4 mg, 35.2% for two steps) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (1H, d, *J* = 1.5 Hz), 8.09 (2H, d, *J* = 8.0 Hz), 7.98 (1H, dd, *J* = 8.5, 1.5 Hz), 7.91 (1H, d, *J* = 8.5 Hz), 7.60 (1H, t, *J* = 7.5 Hz), 7.49 (2H, t, J = 7.5 Hz), 7.41 – 7.35 (4H, m), 7.31 (1H, tt, *J* = 7.0, 1.5 Hz), 6.29 (1H, s), 6.22 (1H, t, *J* = 8.5 Hz), 5.65 (1H, d, *J* = 7.0 Hz), 5.37 (1H, d, *J* = 9.5 Hz), 5.25 (1H, d, *J* = 9.0 Hz), 4.93 (1H, dd, *J* = 9.0, 1.5 Hz), 4.68 (2H, t, *J* = 2.0 Hz), 4.61 (1H, broad s), 4.38 (1H, dd, *J* =10.5, 7.0 Hz), 4.29 (1H, d, *J* = 8.0 Hz), 4.27 (2H, t, *J* = 2.0 Hz), 4.27 – 4.21 (4H, m), 4.15 (1H, d, *J* = 8.0 Hz), 3.78 (1H, d, *J* = 7.5 Hz), 3.71 – 3.67 (4H, m), 3.65 (4H, s), 3.65 (4H, s), 2.85 (2H, dt, *J* = 7.5, 1.5 Hz), 2.81 – 2.67 (2H, m), 2.68 – 2.64 (4H, m), 2.53 (1H, ddd, *J* = 15.0, 9.5, 7.0 Hz), 2.47 (1H, broad s), 2.37 (3H, s), 2.36 – 2.21 (2H, m), 1.89 – 1.82

(1H, m), 1.84 (3H, s), 1.70 (1H, broad s), 1.65 (3H, s), 1.61 (6H, s), 1.32 (9H, s), 1.24 (3H, s), 1.13 (3H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 203.6, 174.4, 173.1, 172.6, 172.2, 172.1, 171.6, 170.3, 167.2, 155.5, 152.4, 142.6, 138.3, 136.3, 135.4, 133.8, 133.0, 130.3, 129.2, 129.0, 128.8, 128.2, 128.1, 126.8, 123.2, 123.1, 115.0, 108.6, 84.5, 81.1, 80.3, 79.1, 78.6, 76.5, 75.9, 75.0, 73.7, 72.5, 72.2, 70.7, 69.1, 64.0, 62.2, 58.6, 56.2, 52.3, 45.7, 43.3, 35.7, 35.5, 29.2, 29.1, 28.9, 28.8, 28.2, 26.8, 23.3, 22.7, 22.0, 14.9, 9.6; HRMS (FAB+) calcd for $C_{76}H_{90}F_3N_4O_{26}$ $[M+H]^+$ 1531.5795, found 1531.5819.

10-(29-(Cyanonilutamide-3'-yl)-4,21,24-trioxo-5,8,11,14,17,20,25-heptaoxanonacos-27-yneoyl)docetaxel (3.11)





Penta(ethylene glycol) (2 mL, 10 mmol) and pyridine (0.767 mL, 9.5 mmol) were dissolved in DCM (7.5 mL) and a solution of TBSCl (1.43 g, 9.5 mmol) in DCM (3.8 mL) was added at 0 ^{o}C. The mixture was stirred at room temperature overnight. The reaction

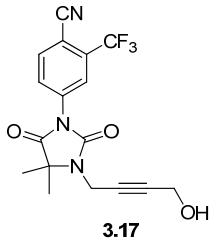
solution was concentrated and purified by flash chromatography (70% ethyl acetate in hexane) to give the TBS ether as a colorless oil (1.426 mg, 43%).

To a solution of penta(ethylene glycol) TBS ether (90 mg, 0.257 mmol), cyanonilutamide analog **3.37** (39 mg, 0.086 mmol) and DMAP (32 mg, 0.257 mmol) in DCM (4.6 mL) was added EDCI (50 mg, 0.257 mmol). The mixture was stirred at room temperature overnight. After the addition of water, the mixture was extracted with ethyl acetate (3 x 20 mL), washed with saturated brine, and dried over Na_2SO_4. Then the mixture was vacuum filtered and rotary evaporated. Without purification, the residue was dissolved in a solution of HCl in ethanol (1%, 8.2 mL) at room temperature. After stirring for 15 minutes, the mixture was treated with saturated $NaHCO_3$ solution carefully and extracted with ethyl acetate (3 x 25 mL). The combined organic phase was then washed with saturated brine, dried over Na_2SO_4, vacuum filtered, and rotary evaporated. The residue was purified by flash chromatography (5% methanol in DCM) to afford cyanonilutamide derivative **3.38d** (18.8 mg, 31.9% for two steps) as a white solid.

To a solution of docetaxel analog **3.33a** (23 mg, 0.02 mmol), cyanonilutamide derivative **3.38d** (11 mg, 0.0161 mmol) and DMAP (6 mg, 0.05 mmol) in DCM (0.5 mL) was added EDCI (10 mg, 0.05 mmol). The mixture was stirred at room temperature overnight. After the addition of water, the mixture was extracted with ethyl acetate (3 x 15 mL), washed with saturated brine, and dried over Na_2SO_4. Then the mixture was vacuum filtered and rotary evaporated. After purification by flash chromatography (70% ethyl acetate in hexane), the product (17 mg, 0.0092 mmol) was dissolved in pyridine (0.52 mL) and acetonitrile (0.26 mL). HF-pyridine (0.071 mL) was added to the solution at room temperature. The mixture was stirred at 62 ^{o}C until the completion of the reaction

(monitored by TLC). Then saturated $NaHCO_3$ solution was added to the reaction mixture dropwise. The mixture was extracted with ethyl acetate (3 x 15 mL), washed with saturated brine, and dried over Na_2SO_4. After vacuum filtration and rotary evaporation, the residue was then purified by flash chromatography (70% ethyl acetate in hexane) to give C10 docetaxel conjugate **3.11** (9.5 mg, 37.5% for two steps) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (1H, d, *J* = 2.0 Hz), 8.09 (2H, d, *J* = 8.0 Hz), 7.98 (1H, dd, *J* = 8.5, 2.0 Hz), 7.91 (1H, d, *J* = 8.5 Hz), 7.60 (1H, tt, *J* = 7.5, 1.5 Hz), 7.49 (2H, t, J = 8.0 Hz), 7.42 – 7.35 (4H, m), 7.31 (1H, tt, *J* = 7.5, 1.5 Hz), 6.29 (1H, s), 6.22 (1H, t, *J* = 8.0 Hz), 5.65 (1H, d, *J* = 6.5 Hz), 5.38 (1H, d, *J* = 8.5 Hz), 5.25 (1H, d, *J* = 8.0 Hz), 4.93 (1H, dd, *J* = 9.5, 1.5 Hz), 4.68 (2H, t, *J* = 2.0 Hz), 4.62 (1H, broad s), 4.38 (1H, dd, *J* =11.0, 7.0 Hz), 4.29 (1H, d, *J* = 8.5 Hz), 4.27 (2H, t, *J* = 2.0 Hz), 4.26 – 4.21 (4H, m), 4.15 (1H, d, *J* = 8.0 Hz), 3.78 (1H, d, *J* = 7.0 Hz), 3.71 – 3.67 (4H, m), 3.65 (4H, s), 3.65 (4H, s), 3.64 (4H, s), 2.84 (2H, dt, *J* = 6.5, 1.5 Hz), 2.80 – 2.67 (2H, m), 2.66 – 2.63 (4H, m), 2.52 (1H, ddd, *J* = 14.5, 9.0, 6.0 Hz), 2.48 (1H, broad s), 2.37 (3H, s), 2.22 – 2.36 (2H, m), 1.90 – 1.83 (1H, m), 1.84 (3H, s), 1.71 (1H, broad s), 1.65 (3H, s), 1.61 (6H, s), 1.32 (9H, s), 1.24 (3H, s), 1.13 (3H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 203.6, 174.4, 173.1, 172.6, 172.2, 172.1, 171.6, 170.3, 167.1, 155.5, 152.4, 142.7, 138.4, 136.3, 135.4, 133.8, 132.9, 130.3, 129.2, 129.0, 128.8, 128.2, 128.1, 126.8, 123.2, 123.1, 115.1, 108.7, 84.5, 81.1, 80.3, 79.1, 78.6, 76.5, 75.9. 75.0, 73.7, 72.4, 72.2, 70.7, 70.6, 69.1, 64.1, 64.0, 62.2, 60.5, 58.6, 56.2, 52.3, 45.7, 43.3, 35.7, 35.6, 29.2, 29.1, 28.9, 28.8, 28.2, 26.8, 23.3, 22.7, 22.0, 14.9, 9.6; HRMS (FAB+) calcd for $C_{78}H_{94}F_3N_4O_{27}$ $[M+H]^+$ 1575.6058, found 1575.5980.

3'-(4-Hydroxybut-2-ynyl)cyanonilutamide (3.17)

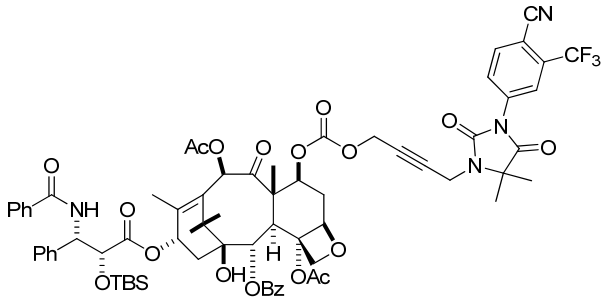


3.17

To a solution of cyanonilutamide **3.14**[23] (825 mg, 2.78 mmol) in DMF (8.5 mL) was added NaH (60% in mineral oil, 134 mg, 3.34 mmol) under argon at room temperature. After stirring for 15 minutes at ambient temperature, the mixture was treated dropwise with a solution of *tert*-butyl(4-iodobut-2-ynyloxy)dimethylsilane (1.01 g, 3.34 mmol) in anhydrous DMF (8.5 mL). The mixture was stirred at room temperature for 3 hours. Then the solution was poured into saturated brine and extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were washed with water and saturated brine, dried over Na_2SO_4, filtered, and concentrated. After purification by flash chromatography (30% ethyl acetate in hexane), the product (885 mg, 1.88 mmol) was dissolved in a solution of HCl in ethanol (1%, 60 mL) at room temperature. After stirring for 15 minutes, the mixture was treated carefully with saturated $NaHCO_3$ solution and extracted with ethyl acetate (3 x 150 mL). The combined organic phases were then washed with saturated brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (50% ethyl acetate in hexane) to afford 3'-(4-hydroxybut-2-ynyl)-cyanonilutamide **3.17** as a white solid (543 mg, 81.4%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (1H, d, J = 2.1 Hz), 7.99 (1H, dd, J = 8.4, 2.1 Hz), 7.92 (1H, d, J = 8.4 Hz), 4.27 (4H, m), 1.62 (6H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 174.4, 152.5, 136.3, 135.4, 133.7, 128.0,

123.1, 120.9, 115.0, 108.6, 82.6, 80.0, 62.2, 51.1, 29.3, 23.3; HRMS (ESI+) calcd for $C_{17}H_{18}F_3N_4O_3$ $[M+NH_4]^+$ 383.1331, found 383.1311.

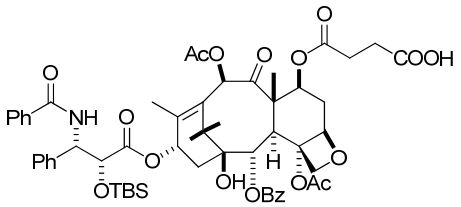
7-((4-(Cyanonilutamide-3'-yl)but-2-ynyloxy)carbonyl)-2'-TBS paclitaxel (3.20)



Bis(4-nitrophenyl) carbonate (20 mg, 0.063 mmol) and *N*,*N*-dimethylaminopyridine (DMAP) (11 mg, 0.063 mmol) were added to a solution of 2'-TBS paclitaxel **3.19**[24] (20 mg, 0.21 mmol) in DCM (0.1 mL). The mixture was stirred at room temperature overnight. After addition of water, the mixture was extracted with ethyl acetate (3 x 10 mL), washed with saturated brine, and dried over Na_2SO_4. The extract was vaccum filtered and concentrated. After purification by flash chromatography (30% ethyl acetate in hexane), the product (16 mg, 0.014 mmol) was dissolved in DCM (0.1 mL), and 3'-(4-hydroxybut-2-ynyl)-cyanonilutamide **3.17** (15 mg, 0.042 mmol) and DMAP (5 mg, 0.042 mmol) were added. The mixture was stirred at room temperature overnight. After the addition of water, the mixture was extracted with ethyl acetate (3 x 10 mL), washed with saturated brine, and dried over Na_2SO_4, followed by vacuum filtration and rotary evaporation. The residue was then purified by flash chromatography (50% ethyl acetate in hexane) to afford 7-((4-(cyanonilutamide-3'-yl)but-2-ynyloxy)carbonyl)-2'-TBS

paclitaxel **3.20** as a white solid (15.7 mg, 82%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (3H, m), 7.99 (1H, dd, *J* = 9.0, 2.2 Hz), 7.91 (1H, d, *J* = 8.4 Hz), 7.74 (2H, dd, *J* = 8.1, 1.4 Hz), 7.60 (1H, t, *J* = 7.4 Hz), 7.50 (3H, dd, *J* = 14.2, 7.7 Hz), 7.44 – 7.36 (4H, m), 7.34 – 7.30 (3H, m), 7.08 (1H, d, *J* = 9.0 Hz), 6.31 (1H, s), 6.25 (1H, t, *J* = 9.0 Hz), 5.73 (1H, d, *J* = 9.0 Hz), 5.69 (1H, d, *J* = 6.9 Hz), 5.51 (1H, dd, *J* = 10.6, 7.1 Hz), 4.97 (1H, d, *J* = 8.4 Hz), 4.91 (1H, d, *J* = 15.8 Hz), 4.67 (2H, m), 4.34 (1H, d, *J* = 8.6 Hz), 4.28 (2H, d, *J* = 1.4 Hz), 4.19 (1H, d, *J* = 8.5 Hz), 3.95 (1H, d, *J* = 6.9 Hz), 2.64 – 2.54 (1H, m), 2.57 (3H, s), 2.40 (1H, dd, *J* = 15.3, 9.6 Hz), 2.20 – 2.12 (1H, m), 2.14 (3H, s), 2.03 – 1.95 (1H, m), 1.97(3H, s) 1.81 (3H, s), 1.61 (6H, s), 1.21 (3H, s), 1.15 (3H, s), 0.79 (9H, s), -0.04 (3H, s), -0.30 (3H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 201.6, 174.4, 171.6, 170.2, 169.2, 167.21, 167.0, 153.7, 152.4, 141.1, 138.3, 136.3, 135.4, 134.1, 133.7, 132.8, 132.0, 130.3, 129.1, 128.9, 128.9, 128.1, 128.1, 127.1, 126.5, 123.2, 123.2, 115.6, 115.1, 108.6, 83.9, 81.6, 81.0, 78.8, 78.4, 76.5, 76.0, 75.4, 75.2, 74.5, 71.4, 64.5, 62.2, 56.1, 55.9, 55.8, 46.9, 43.4, 35.7, 33.4, 29.2, 26.5, 25.6, 23.3, 23.1, 21.6, 20.9, 18.2, 14.7, 10.8, -5.1, -5.7; HRMS (FAB+) calcd for $C_{71}H_{78}F_3N_4O_{18}Si$ $[M+H]^+$ 1359.5033, found 1359.5117.

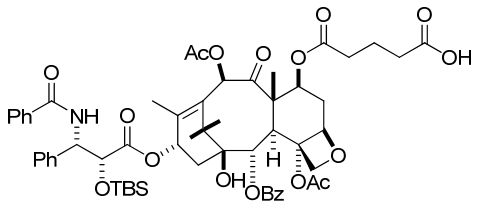
7-(3-Carboxypropanoyl)-2'-TBS paclitaxel (3.21a)



3.21a

To 2'-TBS paclitaxel **3.19** (18.7 mg, 0.02 mmol) in DCM (0.2 mL) was added succinic anhydride (7.1 mg, 0.06 mmol) and DMAP (7.8 mg, 0.06 mmol). The mixture was stirred at room temperature overnight. After the addition of water, the mixture was extracted with ethyl acetate (3 x 10 mL), washed with saturated brine, and dried over Na_2SO_4. After vacuum filtration and rotary evaporation, the residue was then purified by flash chromatography (60% ethyl acetate in hexane) to afford 7-(3-carboxypropanoyl)-2'-TBS paclitaxel **3.21a** as a white solid (21.4 mg, 100%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (2H, d, *J* = 8.1 Hz), 7.74 (2H, d, *J* = 7.8 Hz), 7.61 (1H, t, *J* = 7.5 Hz), 7.54 – 7.47 (3H, m), 7.44 – 7.36 (4H, m), 7.33 – 7.30 (3H, m), 7.08 (1H, d, *J* = 8.9 Hz), 6.25 (1H, t, *J* = 9.2 Hz), 6.22 (1H, s), 5.72 (1H, dd, *J* = 9.1, 1.7 Hz), 5.69 (1H, d, *J* = 7 Hz), 5.62 (1H, dd, *J* = 10.5, 7.2 Hz), 4.97 (1H, d, *J* = 9.5 Hz), 4.66 (1H, d, *J* = 2.2 Hz), 4.33 (1H, d, *J* = 8.5 Hz), 4.20 (1H, d, *J* = 8.5 Hz), 3.95 (1H, d, *J* = 6.9 Hz), 2.72 – 2.64 (1H, m), 2.64 – 2.57 (4H, m), 2.56 (3H, s), 2.40 (1H, dd, *J* = 15.4, 9.6 Hz), 2.17 (1H, d, *J* = 9.2 Hz), 2.14 (3H, s), 1.96 (3H, s), 1.85 (1H, t, *J* = 13.0 Hz), 1.80 (3H, s), 1.20 (3H, s), 1.15 (3H, s), 0.79 (9H, s), -0.04(3H, s), -0.31 (3H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 202.0, 171.6, 171.2, 170.0, 169.3, 167.1, 167.0, 141.1, 138.3, 134.2, 133.8, 132.7, 131.9, 130.3, 129.2, 128.9, 128.8, 128.1, 127.1, 126.5, 88.6, 84.1, 81.1, 78.7, 77.3, 75.4, 75.2, 74.6, 71.8, 71.4, 56.1, 55.7, 46.9, 43.4, 35.7, 33.3, 26.5, 25.6, 23.1, 21.5, 20.8, 18.2, 14.7, 10.9, -5.1, -5.7.; HRMS (APCI+) calcd for $C_{57}H_{69}NO_{17}SiNa$ $[M+Na]^+$ 1090.4233, found 1090.4229.

7-(4-Carboxybutanoyl)-2'-TBS paclitaxel (3.21b)

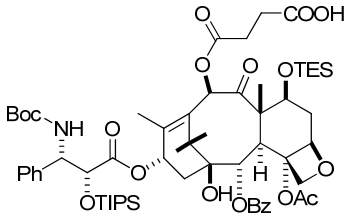


3.21b

To 2'-TBS paclitaxel **3.19** (18 mg, 0.019 mmol) in DCM (1 mL) was added glutaric anhydride (6.5 mg, 0.057 mmol) and DMAP (7 mg, 0.057 mmol). The mixture was stirred at room temperature for 2 days. After the addition of water, the mixture was extracted with ethyl acetate (3 x 15 mL), washed with saturated brine, and dried over $NaSO_4$, followed by vacuum filtration and rotary evaporation. The residue was then purified by flash chromatography (60% ethyl acetate in hexane) to afford 7-(4-carboxybutanoyl)-2'-TBS paclitaxel **3.21b** as a white solid (10 mg, 48.7%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (2H, dd, *J* = 8.5, 1.4 Hz), 7.74 (2H, dd, *J* = 8.5, 1.4 Hz), 7.61 (1H, tt, *J* = 7.4, 1.9 Hz), 7.54 – 7.47 (3H, m), 7.44 – 7.36 (4H, m), 7.34 – 7.29 (3H, m), 7.09 (1H, d, *J* = 8.9 Hz), 6.26 (1H, s), 6.25 (1H, t, *J* = 9.7 Hz), 5.73 (1H, dd, *J* = 8.9, 1.8 Hz), 5.69 (1H, d, *J* = 7 Hz), 5.61 (1H, dd, *J* = 10.6, 7.1 Hz), 4.97 (1H, dd, *J* = 9.3, 1.2 Hz), 4.67 (1H, d, *J* = 2.1 Hz), 4.33 (1H, d, *J* = 8.5 Hz), 4.21 (1H, d, *J* = 8.5Hz), 3.96 (1H, d, *J* = 7.0 Hz), 2.62 – 2.55 (1H, m), 2.57 (3H, s), 2.45 – 2.26 (5H, m), 2.19 – 2.15 (1H, m), 2.14 (3H, s), 1.97 (3H, d, *J* = 1.1 Hz), 1.93 – 1.81 (3H, m), 1.20 (3H, s), 1.15 (3H, s), 0.79 (9H, s), -0.03 (3H, s), -0.31 (3H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 202.1, 176.9, 172.2, 171.6, 170.0, 169.2, 167.2, 167.0, 141.0, 138.3, 134.2, 133.8, 132.8, 131.9, 130.3, 129.2, 128.9, 128.9, 128.8, 128.1, 127.1, 126.5, 84.1, 81.1, 78.7, 77.3, 76.5, 75.3, 75.2, 74.6, 71.4, 56.1, 55.8, 46.9, 43.4, 35.7, 33.5, 33.0, 32.7, 26.5, 25.6, 23.1, 21.5, 20.8, 19.5,

18.2, 14.7, 11.0, -5.1, -5.7; HRMS (APCI+) calcd for $C_{58}H_{71}NO_{17}SiNa$ $[M+Na]^+$ 1104.4389, found 1104.4384.

7-(Triethylsilyl)-10-(3-carboxypropanoyl)-2'-(triisopropylsilyl)docetaxel (3.33a)



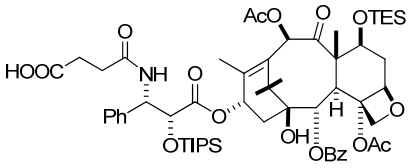
3.33a

To a solution of 7-TES-10-acetyl-2'-TIPS docetaxel **3.32**[24] (310 mg, 0.277 mmol) in ethanol (20 mL) was added hydrazine monohydrate (4.8 mL). The mixture was stirred at room temperature overnight. After the addition of saturated NH_4Cl solution, the mixture was extracted with ethyl acetate (3 x 100 mL), washed with saturated brine, and dried over Na_2SO_4, followed by vacuum filtration and rotary evaporation. The residue was then purified by flash chromatography (15% ethyl acetate in hexane) to afford 7-TES-2'-TIPS docetaxel **3.22** as a white solid (253 mg, 84.8%).

To a solution of 7-TES-2'-TIPS docetaxel **3.22** (36 mg, 0.033 mmol) in toluene (2 mL) was added succinic anhydride (15 mg, 0.1 mmol) and DMAP (13 mg, 0.1 mmol). The mixture was stirred at 85-90 ^oC for 24 hours. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate and aqueous HCl solution (0.2%) was added carefully. The aqueous layer was then extracted with ethyl acetate (3 x 30 mL). The combined organic phase was washed with saturated brine and dried over Na_2SO_4. After vacuum filtration and rotary evaporation, the residue was purified by flash

chromatography (60% ethyl acetate in hexane) to afford 7-TES-10-(3-carboxypropanoyl)-2'-TIPS docetaxel **3.33a** as a white solid (16 mg, 41%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (2H, d, *J* = 7.5 Hz), 7.57 (1H, t, *J* = 7.5 Hz), 7.47 (2H, t, *J* = 7.9 Hz), 7.36 (2H, t, *J* = 7.8 Hz), 7.30 – 7.25 (3H, m), 6.48 (1H, s), 6.25 (1H, t, *J* = 8.9 Hz), 5.69 (1H, d, *J* = 7.1 Hz), 5.40 (1H, d, *J* = 9.8 Hz), 5.30 (1H, d, *J* = 9.8 Hz), 4.94 (1H, dd, *J* = 9.4, 1.6 Hz), 4.80 (1H, s), 4.47 (1H, dd, *J* = 10.7, 6.7 Hz), 4.31 (1H, d, *J* = 8.3 Hz), 4.17 (1H, d, *J* = 8.5 Hz), 3.83 (1H, d, *J* = 7.1 Hz), 2.89 – 2.79 (1H, m), 2.79 – 2.71 (3H, m), 2.56 – 2.49 (1H, m), 2.51 (3H, s), 2.35 (1H, dd, *J* = 15.5, 9.3 Hz), 2.24 – 2.16 (1H, m), 2.00 (3H, s), 1.89 (1H, ddd, *J* = 13.9, 10.5, 2.1 Hz), 1.68 (3H, s), 1.67 (1H, broad s), 1.29 (9H, s), 1.21 (6H, s), 0.94 – 0.84 (30H, m), 0.63 – 0.50 (6H, m); ^{13}C NMR (500 MHz, $CDCl_3$) δ 201.7, 175.4, 171.8, 170.4, 167.2, 155.4, 141.0, 133.7, 133.2, 130.3, 129.3, 128.8, 128.7, 127.8, 126.5, 84.3, 81.2, 80.3, 79.0, 77.3, 76.6, 76.0, 75.4, 75.0, 72.3, 71.6, 58.5, 46.8, 43.4, 37.3, 35.5, 29.1, 28.7, 28.2, 26.5, 23.1, 21.6, 17.9, 17.9, 14.4, 12.6, 10.2, 6.9, 5.4; HRMS (APCI+) calcd for $C_{62}H_{91}NO_{17}Si_2Na$ $[M+Na]^+$ 1200.5723, found 1200.5710.

7-(Triethylsilyl)-2′-(triisopropylsilyl)-3′-debenzoyl-3'-(3-carboxypropanoyl) paclitaxel (3.34)

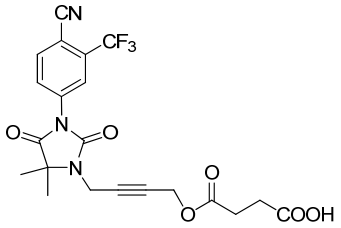


3.34

To a solution of docetaxel derivative **3.32** (105 mg, 0.094 mmol) in DCM (1 mL) was added TFA (0.48 mL) dropwise at 0 ^{o}C. The mixture was stirred at 0 ^{o}C until the completion of the reaction (monitored by TLC). After careful addition of saturated $NaHCO_3$ solution, the mixture was extracted with ethyl acetate (3 x 20 mL), washed with saturated brine, dried over Na_2SO_4, vacuum filtered and rotary evaporated. The residue was then purified by flash chromatography (40% ethyl acetate in hexane) to give primary amine as a white solid (65 mg, 68%). This amine (65 mg, 0.064 mmol) was dissolved in DCM (1 mL). Succinic anhydride (20 mg, 0.192 mmol) and DMAP (24 mg, 0.192 mmol) was added successively. The mixture was then stirred at room temperature overnight. After addition of water, the mixture was extracted with ethyl acetate (3 x 20 mL), washed with saturated brine, dried over Na_2SO_4, vacuum filtered and rotary evaporated. The residue was then purified by flash chromatography (40% ethyl acetate in hexane) to give docetaxel derivative **3.34** (31 mg, 43.8%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (2H, d, *J* = 7.5 Hz), 7.54 (1H, t, *J* = 7.3 Hz), 7.46 (2H, t, *J* = 7.7 Hz), 7.35 (2H, t, *J* = 7.6 Hz), 7.28 (1H, d, *J* = 7.3 Hz), 7.24 (3H, d, *J* = 7.8 Hz), 6.42 (1H, s), 6.41 (1H, d, *J* = 10.0 Hz), 6.33 (1H, t, *J* = 9.0 Hz), 5.69 (1H, d, *J* = 7.2 Hz), 5.56 (1H, d, *J* = 9.0 Hz), 4.91 (1H, d, *J* = 8.5 Hz), 4.82 (1H, d, *J* = 1.8 Hz), 4.46 (1H, dd, *J* = 10.5, 6.7 Hz), 4.23 (2H, dd, *J* = 17.9, 8.5 Hz), 3.80 (1H, d, *J* = 7.1 Hz), 2.56 – 2.44 (3H, m), 2.49 (3H, s), 2.42 – 2.35 (1H, m), 2.32 – 2.23 (2H, m), 2.20 – 2.13 (1H, m), 2.17 (3H, s), 2.01 (3H, s), 1.92 – 1.84 (1H, m), 1.69 (3H, s), 1.23 (3H, s), 1.21 (3H, s), 0.89 (30H, m), 0.80 – 0.46 (6H, m); ^{13}C NMR (500 MHz, $CDCl_3$) δ 201.8, 175.4, 171.6, 171.2, 170.2, 169.5, 167.0, 140.2, 138.1, 133.7, 133.5, 130.3, 129.5, 128.8, 128.0, 126.42, 84.3, 81.2, 79.1, 76.6, 75.6, 75.1, 75.0, 72.3, 71.5, 58.4, 55.7, 46.7, 43.5, 37.2, 35.7, 30.1, 28.3, 26.8, 23.1, 22.0, 21.0, 17.9, 17.9,

14.2, 12.5, 10.3, 6.8, 5.4; HRMS (ESI+) calcd for $C_{59}H_{86}NO_{16}Si_2$ $[M+H]^+$ 1120.5485, found 1120.5470.

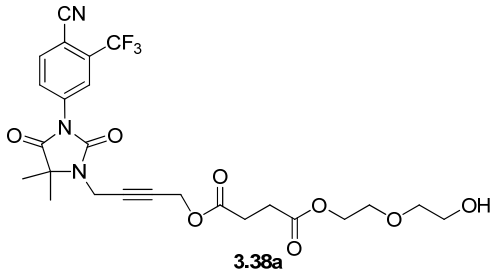
3'-(4-(3-Carboxypropanoyloxy)but-2-ynyl) cyanonilutamide (3.37)



3.37

To a solution of 3'-(4-hydroxybut-2-ynyl)cyanonilutamide **3.17** (61mg, 0.167 mmol) in DCM (1.6 mL) was added succinic anhydride (50 mg, 0.5 mmol) and DMAP (62 mg, 0.5 mmol). The mixture was stirred at room temperature overnight. After the addition of water, the mixture was extracted with ethyl acetate (3 x 15 mL), washed with saturated brine, and dried over Na_2SO_4. After filtration and rotary evaporation, the residue was then purified by flash chromatography (70% ethyl acetate in hexane) to afford 3'-(4-(3-carboxypropanoyloxy)but-2-ynyl)cyanonilutamide **3.37** as a white solid (70 mg, 90%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (1H, d, *J* = 2.1 Hz), 7.98 (1H, dd, *J* = 8.4, 2.1 Hz), 7.92 (1H, d, *J* = 8.4 Hz), 4.69 (2H, t, *J* = 1.9 Hz), 4.26 (2H, t, *J* = 1.9 Hz), 2.70 – 2.61 (4H, m), 1.61 (6H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 176.6, 174.4, 171.4, 152.7, 136.3, 135.4, 133.6, 128.2, 123.3, 123.3, 115.0, 108.7, 81.1, 78.5, 62.3, 52.3, 29.2, 28.7, 28.7, 23.2; HRMS (APCI+) calcd for $C_{21}H_{19}F_3N_3O_6$ $[M+H]^+$ 466.1226, found 466.1243.

3'-(4-(4-(2-(2-Hydroxyethoxy)ethoxy)-4-oxobutanoyloxy)but-2-ynyl)cyanonilut-amide (3.38a)

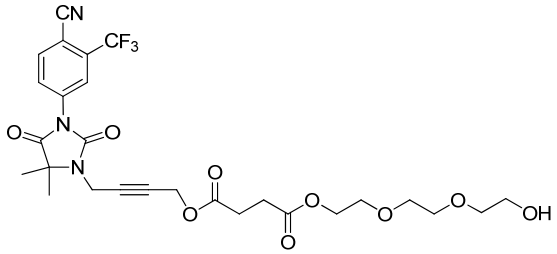


Pyridine (0.85 mL, 11 mmol) and di(ethylene glycol) (1 mL, 0.01 mol) were dissolved in DCM (8 mL) and a solution of *tert*-butyldimethylsilyl Chloride (TBSCl) (1.58 g, 10.5 mmol) in DCM (4 mL) was added at 0 ^{o}C. The mixture was stirred at room temperature overnight. The reaction solution was concentrated and purified by flash chromatography (50% ethyl acetate in hexane) to give TBS ether as a colorless oil (900 mg, 37.2%).

To a solution of di(ethylene glycol) TBS ether (44 mg, 0.2 mmol), 3'-(4-(3-carboxypropanoyloxy)but-2-ynyl)cyanonilutamide **3.37** (31 mg, 0.067 mmol) and DMAP (25 mg, 0.2 mmol) in DCM (4 mL) was added EDCI (40 mg, 0.2 mmol). The mixture was stirred at room temperature overnight. After the addition of water, the mixture was extracted with ethyl acetate (3 x 20 mL), washed with saturated brine, and dried over Na_2SO_4. Then the mixture was filtered and rotary evaporated. Without purification, the residue (25 mg) was dissolved in a solution of HCl in ethanol (1%, 1.2 mL) at room temperature. After stirring for 15 minutes, the mixture was treated with saturated $NaHCO_3$ solution carefully and extracted with ethyl acetate (3 x 15 mL). The combined organic phase was then washed with saturated brine, dried over Na_2SO_4, vacuum filtered,

and rotary evaporated. The residue was purified by flash chromatography (50% ethyl acetate in hexane) to afford 3'-(4-(4-(2-(2-hydroxyethoxy)ethoxy)-4-oxobutanoyloxy)but-2-yny)cyanonilutamide **3.38a** (19 mg, 51.3% for two steps) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (1H, d, *J* = 1.8 Hz), 8.00 (1H, dd, *J* = 8.2, 1.8 Hz), 7.93 (1H, d, *J* = 8.2 Hz), 4.70 (2H, t, *J* = 2.0 Hz), 4.29 – 4.25 (2H, m), 3.76 – 3.68 (4H, m), 3.62 – 3.58 (2H, m), 2.67 (4H, s), 1.62 (6H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 174.4, 172.1, 171.7, 152.4, 136.3, 135.4, 133.9, 133.6, 128.1, 123.2, 123.2, 115.0, 108.6, 100.0, 81.1, 78.5, 72.4, 69.1, 63.8, 62.2, 61.8, 52.4, 29.2, 28.9, 28.9, 23.3; HRMS (ESI+) calcd for $C_{25}H_{26}F_3N_3O_8$ M^+ 553.1672, found 553.1684.

3'-(1-Hydroxy-10,13-dioxo-3,6,9,14-tetraoxaoctadec-16-yn-18-yl)cyanonilutamide (3.38b)



3.38b

Tri(ethylene glycol) (1 mL, 8 mmol) and pyridine (0.61 mL, 7.5 mmol) were dissolved in DCM (6 mL) and a solution of TBSCl (1.1 g, 7.5 mmol) in DCM (3 mL) was added at 0 ^{o}C. The mixture was stirred at room temperature overnight. The reaction solution was concentrated and purified by flash chromatography (50% ethyl acetate in hexane) to give TBS ether as a colorless oil (800 mg, 38.9%).

To a solution of tri(ethylene glycol) TBS ether (50 mg, 0.181 mmol), 3'-(4-(3-carboxypropanoyloxy)but-2-ynyl)cyanonilutamide **3.37** (28 mg, 0.061 mmol) and DMAP (25 mg, 0.2 mmol) in DCM (4 mL) was added EDCI (40 mg, 0.2 mmol). The mixture was stirred at room temperature overnight. After the addition of water, the mixture was extracted with ethyl acetate (3 x 20 mL), washed with saturated brine, and dried over Na_2SO_4. Then the mixture was vacuum filtered and rotary evaporated. Without purification, the residue (20 mg) was dissolved in a solution of HCl in ethanol (1%, 0.9 mL) at room temperature. After stirring for 15 minutes, the mixture was treated with saturated $NaHCO_3$ solution carefully and extracted with ethyl acetate (3 x 15 mL). The combined organic phase was then washed with saturated brine, dried over Na_2SO_4, vacuum filtered, and rotary evaporated. The residue was purified by flash chromatography (60% ethyl acetate in hexane) to afford 3'-(1-hydroxy-10,13-dioxo-3,6,9,14-tetraoxaoctadec-16-yn-18-yl)cyanonilutamide **3.38b** (11 mg, 30.2% for two steps) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (1H, d, *J* = 1.5 Hz), 8.00 (1H, dd, *J* = 8.6, 1.9 Hz), 7.93 (1H, d, *J* = 8.4 Hz), 4.70 (2H, t, *J* = 1.8 Hz), 4.28 (2H, t, *J* = 1.8 Hz), 4.23 (4H, t, *J* = 4.7 Hz), 3.75 – 3.64 (8H, m), 3.64 – 3.59 (2H, m), 2.69 – 2.65 (4H, m), 1.62 (6H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 174.4, 172.2, 171.6, 152.4, 136.3, 135.4, 133.9, 133.6, 128.8, 123.2, 123.2, 115.0, 108.6, 100.0, 81.06, 78.6, 72.5, 70.7, 70.4, 69.1, 63.9, 62.2, 61.9, 52.3, 29.2, 28.9, 28.8, 23.3; HRMS (ESI+) calcd for $C_{27}H_{30}F_3N_3O_9$ M^+ 597.1934, found 597.1954.

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