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DESIGN AND SYNTHESIS OF 4-CHLOROCOLCHICINE-DERIVED PRODRUG CAPABLE OF FORMING NANOPARTICLES BY SELF-ASSEMBLY

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Abstract – We have designed and synthesized colchicine-derived prodrug **7**, which is composed of a 4-chlorocolchicine derivative, a dipeptide side chain cleavable by cathepsin B, a spacer containing a disulfide bond, and hydrophobic vitamin E. Prodrug **7** was capable of forming nanoparticles by self-assembly. Mean particle diameter evaluated by dynamic light scattering measurement was ca. 205 nm.

Colchicine (**1**) (Figure 1) is the major alkaloid in *Colchicum autumnale* (Liliaceae) and has been used to treat acute gout. It is also known as an antimitotic agent that acts by binding to tubulin.[1] However, no **1**-derived anticancer medicines have been developed so far because **1** produces severe adverse effects and has a narrow range of effective dosages. In the course of our chemical studies of new biologically active compounds originating from plant alkaloids,[2] we found that 4-chlorocolchicine (**2**) exhibits potent cell-growth inhibitory activities against human tumor cells *in vitro* and *in vivo* and has lower toxicity than **1**.[3] An SAR study on 4-chlorocolchicine derivatives[4] revealed that compounds **3** and **4** possessing an α-hydroxyalkanamide side chain at the C-7 position exhibited significant antitumor activity *in vivo* and broad effective dosage ranges.[4a] In order to develop colchicine derivatives possessing selectivity for cancer cells, we have designed and synthesized colchicine-derived prodrugs **5** and **6** having a Phe-Lys dipeptide side chain cleavable by cathepsin B, an enzyme overexpressed in solid tumors, resulting in an approximately 2-fold higher selectivity for tumor cells than normal cells.[3] Based on the above results, we aimed to develop more highly tumor-selective prodrugs in this study.

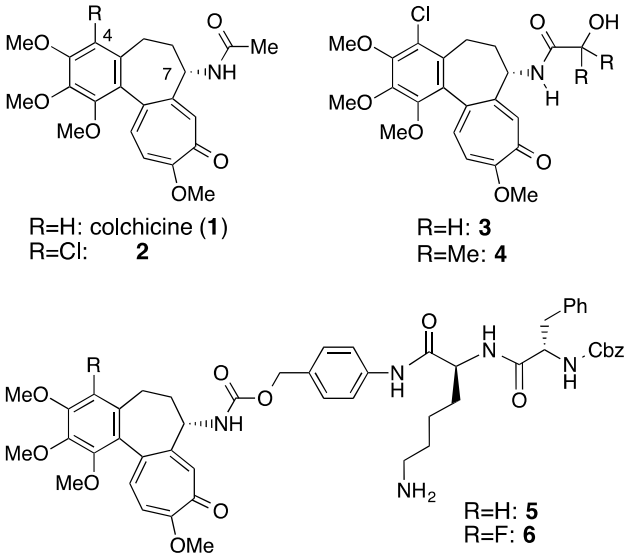


Figure 1. Structures of colchicine (**1**), its derivatives **2**-**4**, and prodrug compounds **5** and **6**

Nanoparticle drug delivery systems have been well studied as one of the efficient strategies for the delivery of anticancer agents with small molecular size. It was demonstrated that nanomedicines preferentially accumulated in tumor tissues through the enhanced permeability and retention (EPR) effect,[5] thus decreasing their adverse effects on normal tissues.

Recently, Wang et al. developed novel self-assembled disulfide-induced nanomedicines and reported that the insertion of a single disulfide bond into hydrophobic molecules, such as paclitaxel and vitamin E, promoted and stabilized nanomedicines.[6] Disulfide bonds are cleaved by glutathione, the concentration of which is higher in tumor cells than normal cells and blood plasma,[7] thereby releasing the active molecule. In this study, we applied this strategy to colchicine derivatives. We designed prodrug **7**, which has a disulfide bond and a hydrophobic moiety in the molecule, as shown in Figure 2. Prodrug **7** consisting of colchicine-dipeptide **8**, a spacer containing a disulfide bond,[6,8] and vitamin E (**9**) was expected to form a self-assembling nanomedicine. This nanomedicine would accumulate in tumor tissues via the EPR effect and be taken up by tumor cells. In the tumor cells, compound **7** would provide **8** that corresponds to prodrugs **5** and **6** via cleavage of the disulfide bond by glutathione followed by fragmentation of the spacer moiety. Cleavage of the dipeptide unit in **8** by cathepsin B, fragmentation of the *p*-aminobenzyloxycarbonyl (PABC) spacer, and decarboxylation would generate active colchicinoid **10** in tumor cells selectively.

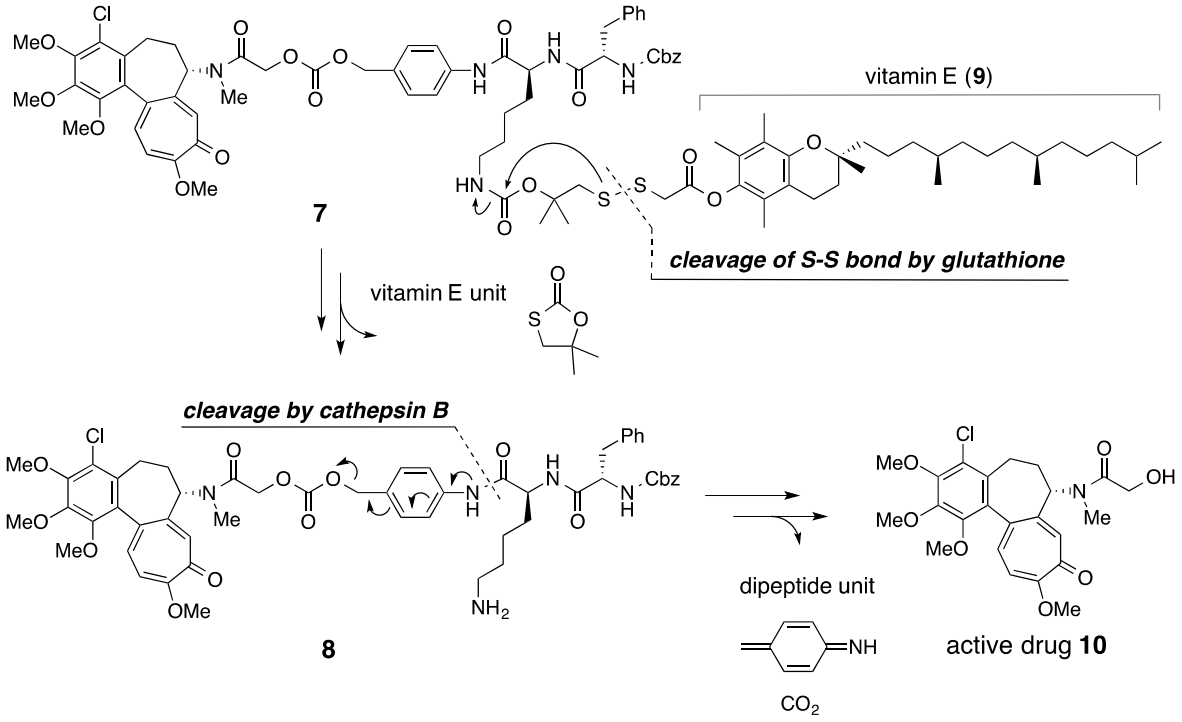
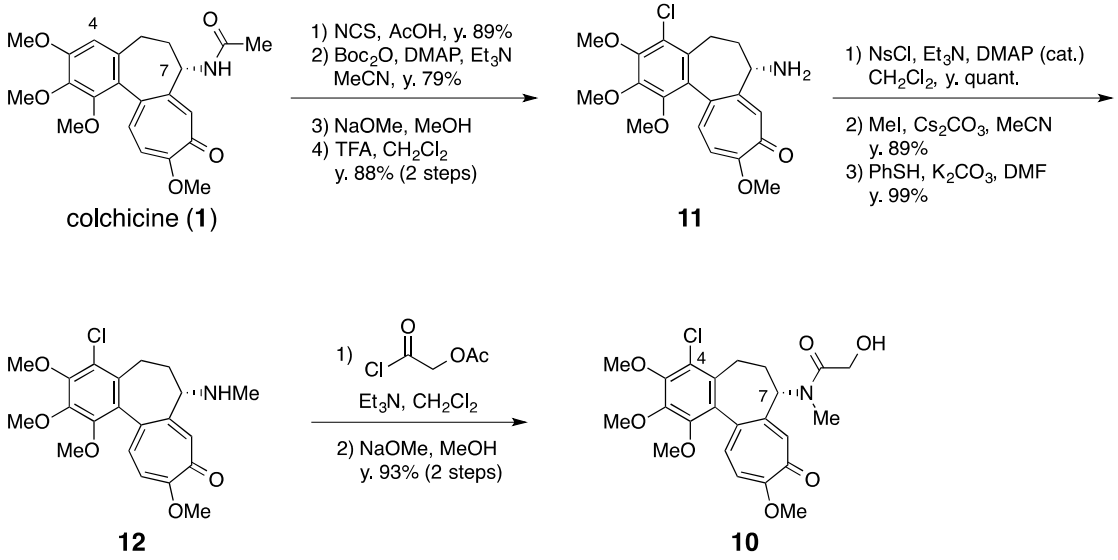


Figure 2

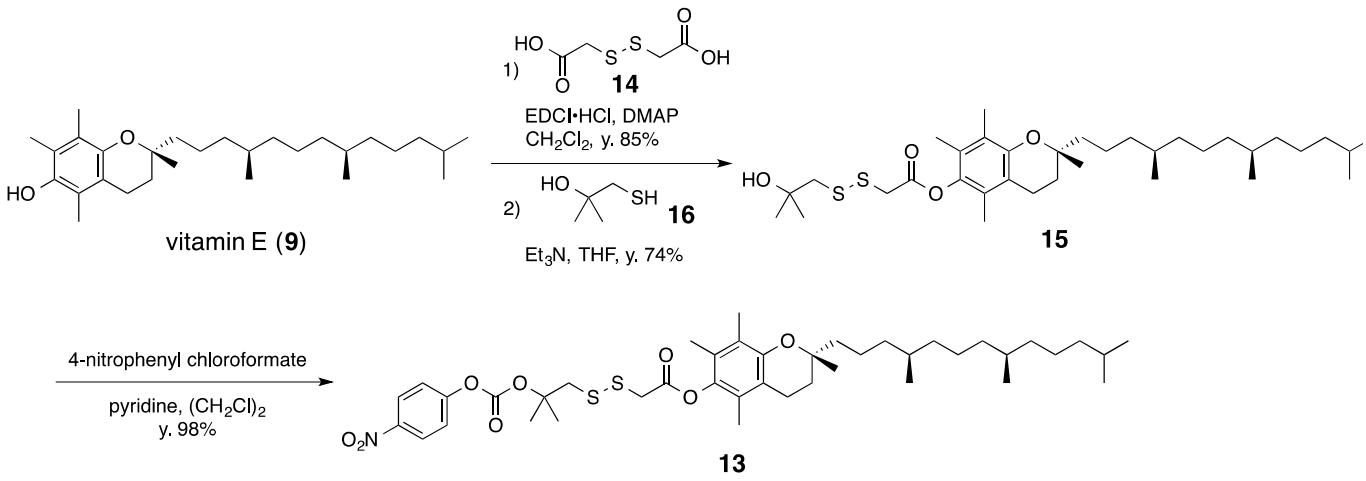
Initially, colchicine derivative **10** was synthesized (Scheme 1) and its cytotoxicity was evaluated. Colchicine (**1**) was converted into 4-chlorodeacetylcolchicine (**11**)[4] in four steps. After protection of the primary amine in **11** by an Ns group, mono-methylation by treatment with MeI and Cs_2CO_3 followed by deprotection of the Ns group gave 4-chloro-*N*-methyldeacetylcolchicine (**12**)[9] in good yield. A hydroxyacetyl group was introduced on the nitrogen atom by treating **12** with 2-acetoxyacetyl chloride in the presence of Et_3N in CH_2Cl_2 and deacetylation by using NaOMe in MeOH to afford colchicine



Scheme 1

derivative **10**. As was expected, compound **10** showed significant cytotoxic activity against several human cancer cell lines: IC_{50} 0.045 μM (A549 human lung adenocarcinoma), 0.019 μM (HT29 human colon adenocarcinoma), and 0.041 μM (HCT116 human colorectal carcinoma).[10,11]

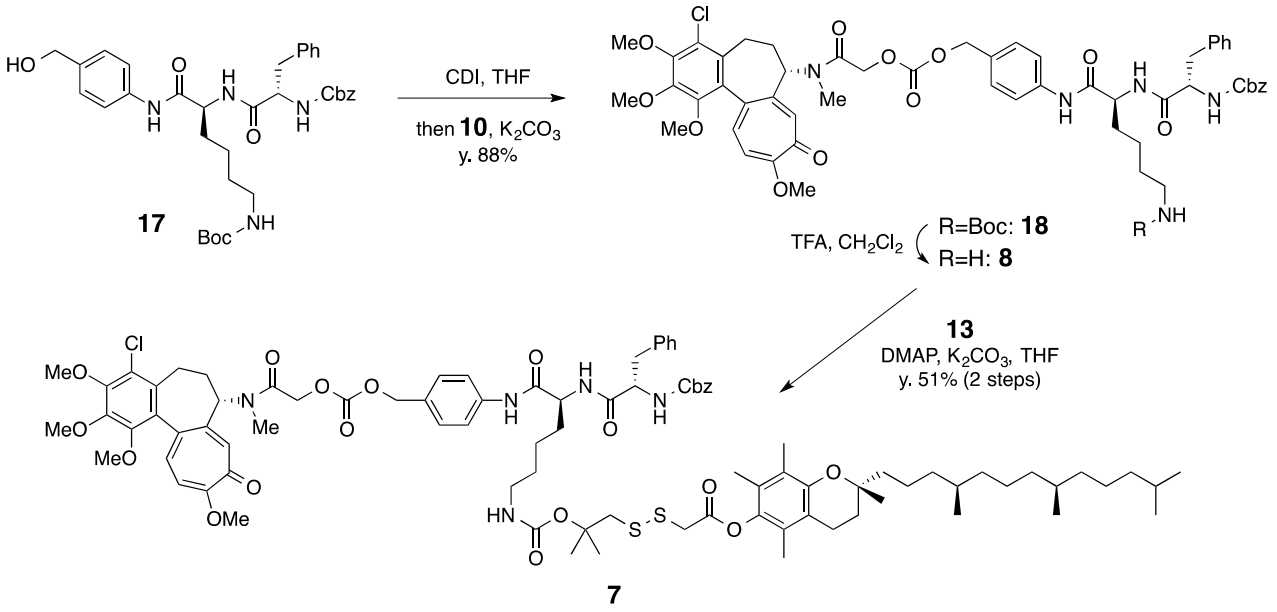
Then, vitamin E derivative **13** incorporating a disulfide unit was prepared (Scheme 2). Dithiodiglycolic acid (**14**) was treated with vitamin E (**9**) (2.5 equiv.), EDCI (2.2 equiv.), and DMAP (0.1 equiv.) in CH_2Cl_2, and **15** was obtained by thiol-disulfide exchange using dimethyl derivative of 2-mercaptoethanol **16**[12] (1-mercapto-2-methyl-2-propanol). For the coupling with the dipeptide unit, **15** was converted into carbonate **13** by reacting with *p*-nitrophenyl chloroformate in the presence of pyridine in $(CH_2Cl)_2$.



Scheme 2

Next, the coupling of Phe-Lys dipeptide unit **17**[13] and colchicine derivative **10** was carried out by using CDI to furnish **18** (Scheme 3). After removal of the Boc group on the primary amine in **18**, resultant amine **19** was coupled with carbonate **13** in the presence of DMAP and K_2CO_3 in THF to yield prodrug compound **7**. Having **7** in hand, an *in vitro* release experiment using glutathione was conducted. Treatment of **7** with glutathione in 0.5 M phosphate buffer (pH 7.2) and acetone yielded **8**, as expected in Figure 2.

The cytotoxic activities of compound **7** and HCl salt of **8** against three human cancer cell lines (A549, HT29, and HCT116) were evaluated. The IC_{50} values of **7** were 3.8 μM (A549), 3.6 μM (HT29), and 3.7 μM (HCT116), whereas those of HCl salt of **8** were 0.20 μM (A549), 0.14 μM (HT29), and 0.18 μM (HCT116), respectively.[11]



Scheme 3

Next, the formation of nanoparticles of compound **7** by antisolvent method was investigated. An ethanol solution of **7** was added dropwise into water over a period of 5 min under sonication, and the sonication was continued further for 1 min to obtain a bluish solution with the final concentration of 0.05 mg/mL. The particle size distribution profile obtained by dynamic light scattering (DLS) measurement demonstrated the formation of nanoparticles with unimodal size distribution and the mean particle size of 205 nm[14] (Figure 3a). Nanoparticle morphology was evaluated by negative-stained field emission (FE)-transmission electron microscopy (TEM) using phosphotungstic acid solution. The negative-stained FE-TEM image revealed spherical nanoparticles (Figure 3b).

As expected, newly designed and synthesized compound **7** had the capability to form nanoparticles. *In vivo* experiments of the anticancer activity of prodrug **7** will be carried out in due course.

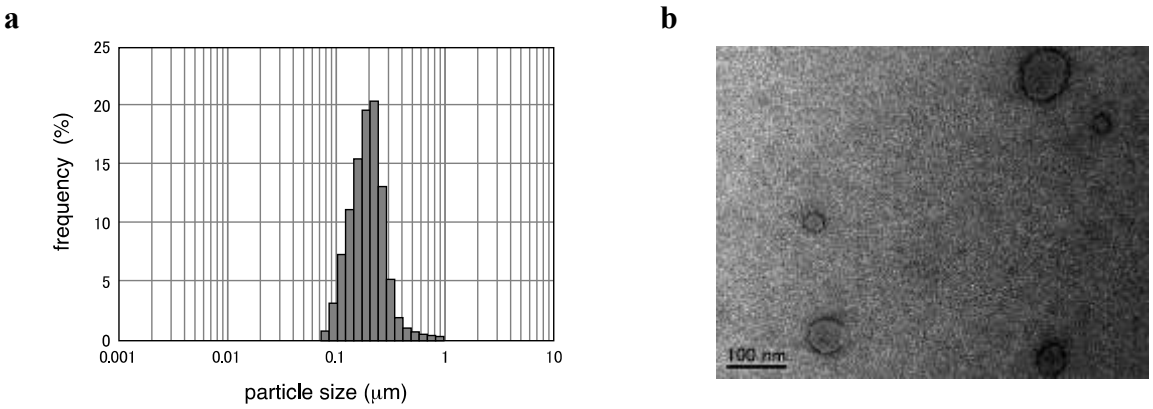


Figure 3. Particle size distribution (a) and negative-stained FE-TEM image (b) of nanoparticles of prodrug **7** in water

ACKNOWLEDGEMENTS

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