Antitumor Agents. 172. Synthesis and Biological Evaluation of Novel Deacetamidothiocolchicin-7-ols and Ester Analogs as Antitubulin Agents†

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A series of novel 7-*O*-substituted deacetamidothiocolchicine derivatives has been synthesized and evaluated for their inhibitory activity against tubulin polymerization, the binding of [^{3}H]-colchicine to tubulin, and the growth of human Burkitt lymphoma cells. Of these new derivatives, thiocolchicone (**8**), wherein an acetamido group in thiocolchicine is replaced by a carbonyl oxygen at C(7), was obtained from deacetylthiocolchicine (**6**) by Schiff's base equilibration and acid hydrolysis. Reduction of thiocolchicone with sodium borohydride yielded the racemic alcohol **9**, the structure of which was verified by X-ray crystallographic analysis. Optically pure alcohols **9a**,**b** were obtained by treatment of **9** with the optically pure reagent (1*S*)-(−)-camphanic chloride followed by chromatographic separation of the camphanate esters and hydrolysis of the diastereomers. X-ray crystallographic analysis established the a*S*,7*S*-configuration of **9a**. Racemic and optically active esters **11**−**15**, **11a**,**b**, **12a**, **14a**, and **15a** were obtained by esterification of the corresponding alcohols. The compounds showing activity equivalent to or greater than (−)-thiocolchicine (**2a**) in all the biological assays were three (−)-a*S*,7*S* optically pure enantiomers: the alcohol **9a**, the acetate **11a** (an oxygen isostere of thiocolchicine), and the isonicotinoate **15a**. In addition, the ketone **8** and two (−)-a*S*,7*S* enantiomers (**12a**, **14a**) had high activity in the biochemical assays with tubulin but reduced antiproliferative activity. In all cases, optically pure isomers with the (−)-a*S*,7*S* configuration exhibited greater biological activity than racemic mixtures or isomers with the (+)-a*R*,7*R* configuration.

Introduction

Colchicine (**1a**) (Figure 1), a well-known alkaloid from *Colchicum autumnale*, interferes with microtubule growth and, therefore, affects mitosis and other microtubule dependent functions.[2] Colchicine is one of the oldest drugs still in use for the treatment of gout and amyloidosis of familial Mediterranean fever.[3] Although colchicine is a potent antimitotic agent, its medicinal uses are limited due to its high toxicity. Virtually all of its therapeutic and toxic effects are believed to be a consequence of its interaction with tubulin. For these reasons, colchicine has become an attractive molecule for the medicinal chemist in pursuit of less toxic and more selective analogs which bind to tubulin.

Structurally, colchicine can be divided into three parts: a trimethoxybenzene A ring moiety, a seven-membered B ring with a side chain at C(7), and a tropolone C ring. Previous studies suggested that the interaction between colchicinoids and tubulin was stereoselective and mainly attributed to the configuration and conformation of the biaryl system composed of the trimethoxyphenyl A ring and tropolonic C ring.[4,5] This has also been confirmed in our recent study reported elsewhere.[6−8] Colchicinoids with an a*S*-configured biaryl system[2,4,9] bind readily to tubulin, while those with an a*R* configuration bind poorly. Moreover, it has been proposed that in addition to the effect of the C(1) substituents on the biaryl configuration, the C(7) side chain could also influence the binding rate of colchicinoids to tubulin as well as the configuration of the colchicinoids through steric effects.[10]

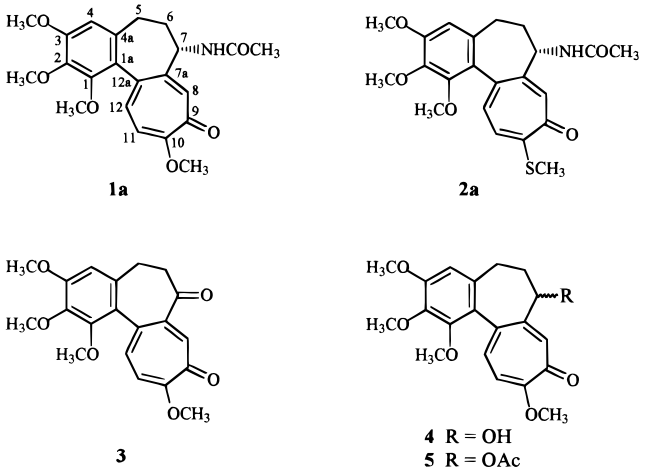

Figure 1. Structures of colchicine, thiocolchicine, and C(7)-oxygenated deacetamidocolchicines.

The features described above provide promise for the rational design of colchicine analogs. Much work has been done with thiocolchicine (**2a**) (Figure 1), which acts similarly to **1a**[11] but is stable toward acid and therefore is a preferred compound in structure−activity studies.[2,12] Interchanging groups in bioactive molecules is a valuable concept to derive new molecules that often show different solubility and bioavailability.[13] Accordingly, in colchicine type compounds, the acetamido group in **2a** can be varied considerably[2,14] and in **1a** can

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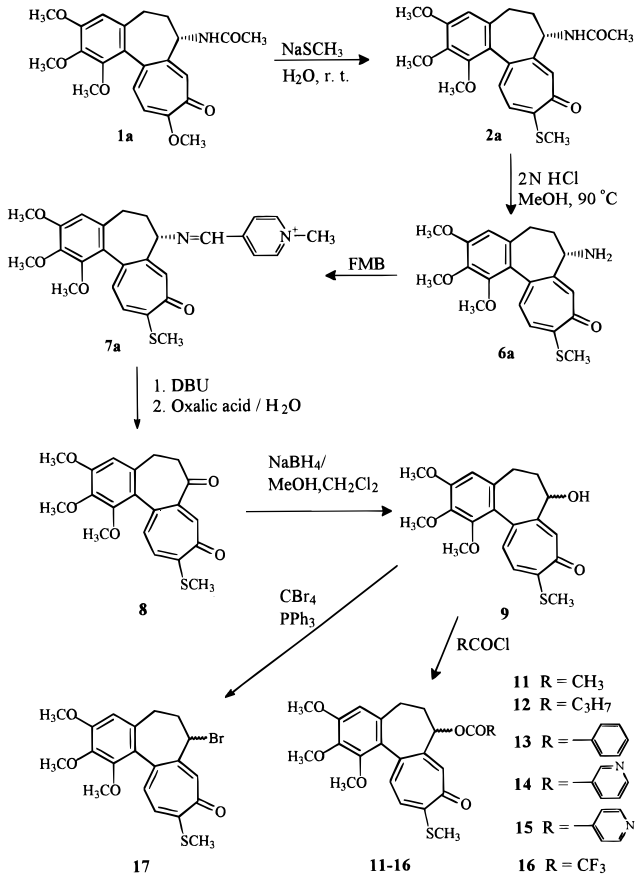
Scheme 1. Synthesis of Thiocolchicone, (±)-Deacetamidothiocolchicin-7-ol, and Its Esters



be replaced by a hydrogen atom without loss of tubulin-binding ability.[15] An interesting compound to elaborate further the importance of a C(7) substituent is colchicone (**3**) (Figure 1), a non-nitrogen-containing compound of *Colchicum richterii*,[16] which has shown good inhibition of tubulin polymerization.[17] The alcohol **4** and the acetate **5** (Figure 1) prepared from **3** as racemic mixtures have not been investigated for their interaction with tubulin.[2,16] This fact prompted us to design and synthesize a series of compounds related to **4** and **5** in both racemic and optically active forms and to evaluate their antitubulin action.

This study was carried out in the thiocolchicine (**2a**) series including the synthesis of the racemic compounds **8**−**19** (Schemes 1 and 2) and their antipodal isomers **9a**−**15a**, **10b**, and **11b** (Scheme 3). The inhibitory effects of these compounds on tubulin polymerization, the binding of [^{3}H]colchicine to tubulin, and the growth of human Burkitt lymphoma cells are reported in this paper.

Chemistry

The key compound involved in the synthesis described herein is thiocolchicone (**8**), the thiomethyl ether analog of **3**. Diketone **8** is readily available from deacetylthiocolchicine (**6a**)[15,18,19] by the Schiff's base−equilibration−hydrolysis procedure developed by Rapoport and others.[20−22] Compound **8** is a racemic mixture, suggesting that its enantiomers with a chiral aryltropolone backbone equilibrate quickly. It afforded racemic alcohol **9** on reduction with sodium borohydride. The structure of **9** was verified by X-ray crystallographic

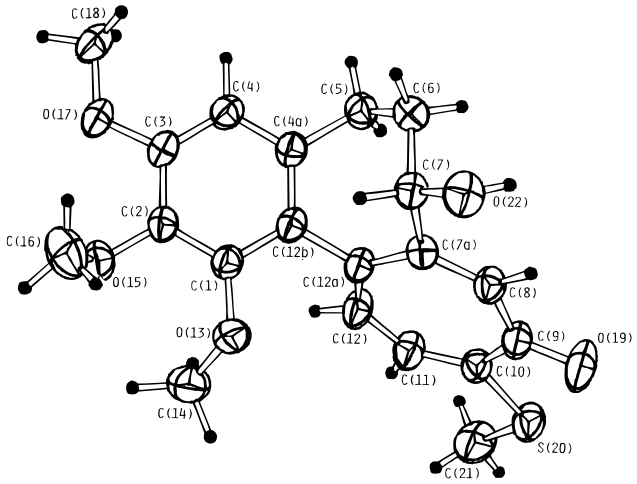
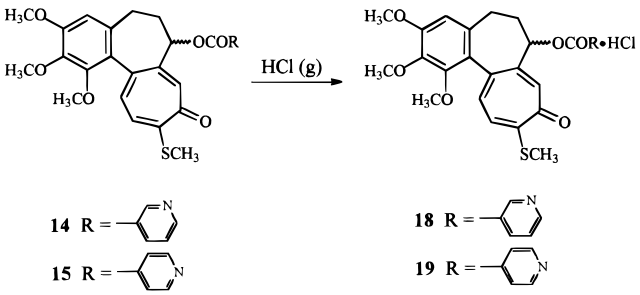


Figure 2. Structure of compound **9** determined by X-ray crystallography. ORTEP diagram (50% probability ellipsoids) showing the crystallographic atom-numbering scheme and solid-state conformation of the a*S*,7*S* enantiomer in crystals of racemic alcohol **9**; small filled circles represent hydrogen atoms.

Scheme 2. Synthesis of Compounds **18** and **19**



analysis (see the Experimental Section). A view of the solid-state conformation of the a*S*,7*S* enantiomer is presented in Figure 2. Esterification of compound **9** by reaction with the corresponding acid chlorides gave the esters **10**−**16**. Compound **17** was obtained by reaction of compound **9** with carbon tetrabromide and triphenylphosphine. Reaction of esters **14** and **15** with HCl formed water-soluble salts **18** and **19** (Scheme 2).

The optically active alcohols of **9** were not successfully obtained either by enantioselective reduction of the 7-ketone group in compound **8** followed by Corey's procedure[23] or by chromatography of racemic **9** on a chiral column. However, synthesis of the desired alcohols was achieved by esterification of **9** with the optically pure reagent (1*S*)-(−)-camphanic chloride as shown in Scheme 3. The two ester diastereomers **10a**,**b** were separated successfully by flash column chromatography on silica gel with **10a** eluting faster than **10b**. Both compounds showed identical NMR spectra but opposite optical rotations. The enantiomers were found to be optically pure by the analysis performed with a chiral column. Acid hydrolysis of **10a**,**b** yielded the crystalline alcohols **9a**,**b**, respectively, with identical NMR and TLC properties but opposite optical rotations. X-ray crystallographic analysis of the biologically active **9a** (see the Experimental Section) showed it to have the a*S*,7*S*-configuration, which is identical with that of natural colchicine. The solid-state conformation is illustrated in Figure 3. Corresponding bond lengths and angles in **9a** and **9** agree well. Noteworthy, however, are the significantly different orientations of their C(1)-meth-

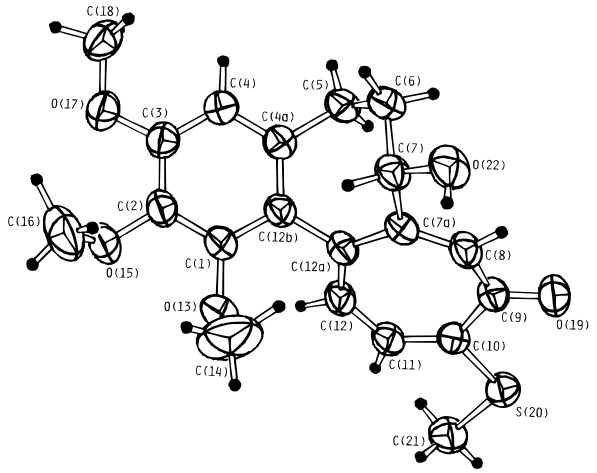
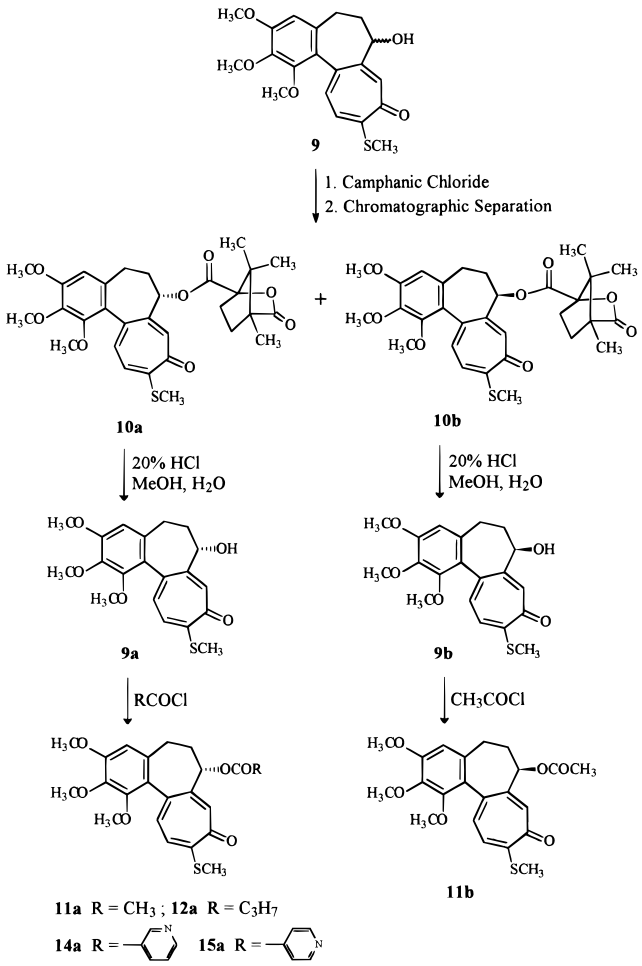


Figure 3. Structure of compound **9a** determined by X-ray crystallography. ORTEP diagram (50% probability ellipsoids) showing the crystallographic atom-numbering scheme and solid-state conformation of compound **9a**; small filled circles represent hydrogen atoms.

Scheme 3. Synthesis of (−)- and (+)-Deacetamidothiocolchicin-7-ols and Their Ester Derivatives



oxymethyl groups due to crystal-packing effects. Thus, the C(2)−C(1)−O(13)−C(14) torsion angle in **9a** is −92.6(3)°, whereas in the a*S*,7*S* enantiomer of **9** it is 57.4(2)°. Nevertheless, the conformations of their central seven-membered rings are still very similar. [Endocyclic torsion angles ω_{ij} (σ 0.2−0.3° in **9a**, 0.1−0.2° in **9**) about the bonds between atoms *i* and *j* in **9a**, with values for the a*S*,7*S* enantiomer of **9** in parentheses, follow (degrees): $\omega_{4a,5}$ −70.1 (−67.5), $\omega_{5,6}$ 45.9 (40.7), $\omega_{6,7}$ 43.4 (49.1), $\omega_{7,7a}$ −79.2 (−75.6), $\omega_{7a,12a}$ 7.3 (−2.0), $\omega_{12a,12b}$ 51.9 (60.1), $\omega_{12b,4a}$ −6.3 (−7.8).]

Table 1. Biological Activities of Thiocolchicine Derivatives

compd	polymerization[a] IC_{50} (μM) ± SD	binding[b] 1:1[d] % inhib	binding[b] 10:1[e] % inhib	lymphoma cell growth[c] IC_{50} (μM) ± SD
1a	1.5 ± 0.3[f]			15[g]
2a	0.65 ± 0.03	45		4.5[h]
6a	1.3 ± 0.08	49		38 ± 1
8	0.76 ± 0.1	70		18 ± 10
9	1.2 ± 0.1			
9a	0.75 ± 0.2	85		2.8 ± 0.2
9b	6.2 ± 0.2	16	73	120 ± 4
10a	1.4 ± 0.4			
10b	17 ± 2			
11	1.3[h]			
11a	0.60 ± 0.07	79		3.4 ± 0.2
11b	2.6 ± 0.5	11	74	40 ± 0.7
12	1.5 ± 0.4			
12a	0.85 ± 0.03	66		29[h]
13	1.4 ± 0.2			
14	0.88 ± 0.05			
14a	0.76 ± 0.04	65		27 ± 0.7
15	1.1 ± 0.3			
15a	0.56 ± 0.08	69		2.8 ± 0.5
17	1.1 ± 0.2			
19	0.97 ± 0.2			

[a] Tubulin polmerization was evaluated as described in ref 10. A minimum of two independent experiments was performed with each compound. The IC_{50} value is defined as the concentration that inhibits by 50% the extent of assembly after 20 min at 30 °C. [b] The binding of [^{3}H]colchicine (5.0 μM) to tubulin (1.0 μM, 0.1 mg/mL) was measured as described in ref 7. Incubation was for 10 min at 37 °C. The values shown in the table represent the averages of two independent experiments, each performed with triplicate samples. [c] Human Burkitt lymphona line CA46 was cultured in 5 mL suspension culture for 24 h at 37 °C under a 5% CO_2 atmosphere as described for another cell line in ref 8. The IC_{50} value represents the concentration that inhibits the increase in cell number relative to the control without drug by 50%. Averages obtained in two independent experiments are presented. [d] Compound (inhibitor) concentration was 5.0 μM. [e] Compound concentration was 50 μM. [f] Data from ref 14. The values obtained for **2a** and **6a** in this earlier study were 0.79 ± 0.09 and 2.2 ± 0.2 μM, respectively. [g] Data from ref 26. [h] The same value was obtained in both experiments.

Esterification of **9a** gave the esters **11a**−**15a** (Scheme 3). The enantiomeric a*R*,7*R* alcohol **9b** and its acetate **11b,** which is the C(7)-oxygen isostere of unnatural (+)-thiocolchicine, also were prepared (Scheme 3).

Biological Results and Discussion

The newly synthesized 7-*O*-substituted deacetamidothiocolchicines were evaluated for inhibition of tubulin polymerization, and the most potent inhibitors of polymerization (the ketone **8** and the 7*S* compounds **9a**, **11a**, **12a**, **14a**, and **15a**), **9b**, and **11b** were examined for inhibitory effects on the binding of [^{3}H]colchicine to tubulin and on the growth of the human Burkitt lymphoma CA46 cell line (Table 1).

Invariably, the racemic mixtures were less active than the 7*S* enantiomers, generally one-half as active, in inhibiting tubulin assembly. Further, when both enantiomers (**9a,b**, **10a,b**, and **11a,b**) were available for study, the 7*R* isomer was invariably less active than the 7*S* isomer. The difference ranged from 4-fold between the acetates **11a**,**b** to 12-fold between the camphanates **10a**,**b**.

Both (−)-thiocolchicine (**2a**) and 7-deacetylthiocolchicine (**6a**) were directly compared with the new compounds. The amine **6a** was one-half as active as **2a** as an inhibitor of tubulin polymerization. The ketone **8** and five 7*S* enantiomers (**9a**, **11a**, **12a**, **14a**, and **15a**) had inhibitory effects (IC_{50} values of, 0.56−0.85 μM) on tubulin polymerization indistinguishable from that of (−)-thiocolchicine (IC_{50} value of 0.65 μM). The alcohol **9a** was significantly more inhibitory than the amine **6a**.

As inhibitors of the binding of [^{3}H]colchicine to tubulin, the ketone **8** and the five 7*S* enantiomers were all more potent than (−)-thiocolchicine and **6a**, with **9a** having the greatest inhibitory effect. The two 7*R* enantiomers **9b** and **11b** were weak inhibitors of [^{3}H]-colchicine binding, with **9b** 5-fold and **11b** 7-fold less active than **9a** and **11a**, respectively.

The strong inhibitory effects of the oxygen isostere analogs on [^{3}H]colchicine binding led us to examine the effects of these compounds on proliferation of a human Burkitt lymphoma line. Three of the five 7*S* enantiomers (**9a**, **11a**, and **15a**) had inhibitory effects that appeared to be slightly greater than that of (−)-thiocolchicine, while the ketone **8**, **12a**, **14a** were 4−6-fold less active. The 7*R* alcohol **9b** was 40-fold less active than its enantiomer **9a** and the 7*R* acetate **11b** 12-fold less active than its enantiomer **11a**.

In summary, this class of colchicinoids merits further study. The oxygen isosteres of (−)-thiocolchicine and **6a** have greater activity than the nitrogen isosteres in selected assays, and three newly synthesized compounds (**9a**, **11a**, and **15a**) have antiproliferative activity with Burkitt lymphoma cells that equals or exceeds that of (−)-thiocolchicine. The apparently universal superiority of the 7*S* enantiomers confirms the postulate that the tubulin-colchicinoids interaction is stereoselective.[4−8] The potent activity of the ketone **8** in the tubulin-based assays probably derives from an increased flexibility in the biaryl system, with a rapid a*R*/a*S* equilibration, suggesting that introduction of the sp^2 center reduces the conformational rigidity of the molecule. As with amide side chain studies modeled on colchicine and thiocolchicine,[2,12,14] a wide variety of C(7) esters had antitubulin activity. Also of potential importance, enhancing the water solubility of racemic **15** by formation of the hydrochloride salt **19** had no negative impact on the biochemical activity, but effects on cell growth have not yet been examined.

Experimental Section

Chemistry. Melting points were measured with a Fisher-Johns melting point apparatus without correction. Optical rotations were determined with a DIP-1000 polarimeter. The proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) spectra were measured on a Bruker AC-300 spectrometer with Me_4Si (TMS) as the internal reference and $CDCl_3$ as solvent. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA. MS was determined by NIH. Thin-layer chromatography (TLC) silica gel plates were purchased from Analtech, Inc. Silica gel (230−400 mesh), from Aldrich, Inc., was used for column chromatography.

Starting Material. Deacetylthiocolchicine (**6a**) was prepared according to the procedure reported by Velluz *et al.*[19] in 74.8% yield: mp 198−200 °C; $[\alpha]^{25}_D$ −160.6° (*c* 0.31, MeOH).

Thiocolchicone (8). Deacetylthiocolchicine (**6a**) (882.1 mg, 2.365 mmol) was dissolved in a 1:1 mixture of CH_2Cl_2/DMF. 4-Formyl-1-methylpyridinium benzenesulfonate (FMB) (851 mg, 3.05 mmol) was added to the solution, and the resulting mixture was stirred under N_2 at room temperature for 10 h. To this mixture was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) dropwise (0.8 mL) to afford a deep-purple solution. After stirring at room temperature for 0.5 h, oxalic acid-saturated aqueous solution (40 mL) was added, and vigorous stirring was continued at room temperature overnight. The reaction mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were concentrated to an orange solid, which was crystallized from ethyl acetate and recrystallized from CH_2Cl_2/MeOH to yield **8**: 675.8 mg (76.6%); mp 233−235 °C; $[\alpha]^{25}_D$ 0° (*c* 0.32, MeOH); ^{1}H NMR ($CDCl_3$) δ 2.48 (s, 3H, SCH_3-10), 2.68−3.20 (m, 4H, H-5,6), 3.59 (s, 3H, OCH_3-1), 3.89 (s, 6H, OCH_3-2,3), 6.57 (s, 1H, H-4), 6.97 (s, 1H, H-8) 7.10 (d, J = 10.37 Hz, 1H, H-11), 7.25 (d, J = 10.4 Hz, 1H, H-12); ^{13}C NMR ($CDCl_3$) δ 15.2 (SCH_3), 29.3 (C-5), 47.4 (C-6), 56.0 (OCH_3-3), 61.1 (OCH_3-1), 61.2 (OCH_3-2), 107.1 (C-4), 124.6 (C-1a), 126.4 (C-11), 130.0 (C-8), 133.8 (C-4a), 135.5 (C-12a), 135.9 (C-12), 141.7 (C-2), 149.8 (C-1), 151.9 (C-7a), 154.0 (C-3), 160.1 (C-10), 182.3 (C-9), 205.6 (C-7). Anal. ($C_{20}H_{20}O_5S$) C, H, S.

(±)-Deacetamidothiocolchicin-7-ol (9). To a solution of thiocolchicone (**8**) (171.6 mg, 0.46 mmol) in a mixture of CH_2Cl_2/MeOH was added $NaBH_4$ (58.6 mg, 1.45 mmol), and the solution was stirred at −78−0 °C overnight. The reaction mixture was acidified with 50% acetic acid and then extracted with CH_2Cl_2 (4 × 10 mL). The combined organic phases were washed with saturated NaCl, dried over Na_2SO_4, and concentrated to give a residue (170.5 mg), which was crystallized from Et_2O/MeOH to afford pure **9**: 138.0 mg (80% yield); mp 224−226 °C; $[\alpha]^{25}_D$ 0° (*c* 0.21, MeOH); ^{1}H NMR ($CDCl_3$) δ 2.47 (s, 3H, SCH_3-10), 2.80−3.0 (m, 4H, H-5,6), 3.64 (s, 3H, OCH_3-1), 3.90 (s, 6H, OCH_3-2,3), 4.53 (m, 1H, H-7), 6.56 (s, 1H, H-4), 7.16 (d, J = 10.6 Hz, 1H, H-11), 7.32 (d, J = 10.5 Hz, 1H, H-12), 7.93 (s, 1H, H-8); ^{13}C NMR ($CDCl_3$) δ 15.1 (SCH_3), 29.9 (C-5), 39.0 (C-6), 56.1 (OCH_3-3), 61.2 (OCH_3-2,3), 71.2 (C-7), 107.2 (C-4), 125.0 (C-1a), 126.6 (C-11), 128.9 (C-8), 134.8 (C-4a), 135.3 (C-12a), 137.7 (C-12), 141.2 (C-2), 150.8 (C-7a), 153.6 (C-1), 153.8 (C-3), 158.2 (C-10), 182.2 (C-10); CIMS m/z 374 (M^+). Anal. ($C_{20}H_{22}O_5S$) C, H, S.

General Procedure for Synthesizing (±)-7-*O*-Acyldeacetamidothiocolchicines 10−16 and Their Optically Active Isomers 11a−15a and 11b. To a solution of the corresponding alcohol **9**, **9a**, or **9b** in dry pyridine was added an appropriate acyl chloride or anhydride (1.5−2.0 equiv) at 0 °C or room temperature. The mixture was allowed to stand overnight, and the volatiles were evaporated *in vacuo*. The residue was diluted with water and extracted three times with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on a preparative TLC plate or flash column or purified by crystallization.

(±)-7-*O*-Acetyldeacetamidothiocolchicine (11): yield 98% (starting with 66.8 mg of **9**); crystallization from Et_2O gave yellow needles; mp 185−186 °C; $[\alpha]^{25}_D$ 0° (*c* 0.24, MeOH); ^{1}H NMR ($CDCl_3$) δ 2.13 (s, 3H, acetyl CH_3), 2.45 (s, 3H, SCH_3-10), 2.3−2.6 (m, 4H, H-5.6), 3.63 (s, 3H, OCH_3-1), 3.92 (s, 3H, OCH_3-2), 3.94 (s, 3H, OCH_3-3), 5.34 (dd, J = 6.24, 11.0 Hz, 1H, H-7), 6.56 (s, 1H, H-4), 7.07 (d, J = 10.5 Hz, 1H, H-11), 7.29 (d, J = 10.5 Hz, 1H, H-12), 7.36 (s, 1H, H-8); CIMS m/z 416 (M^+). Anal. ($C_{22}H_{24}O_6S$) C, H, S.

(±)-7-*O*-Butyryldeacetamidothiocolchicine (12): yield 94.1% (starting with 27.3 mg of **9**); amorphous; $[\alpha]^{25}_D$ 0° (*c* 0.36, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.94 (t, J = 14.8 Hz, 3H, butyryl CH_3), 1.65 (m, 2H, butyryl CH_2), 1.95−2.6 (m, 6H, H-5,6, butyryl CH_2), 2.45 (s, 3H, SCH_3), 3.65 (s, 3H, OCH_3-1), 3.92 (s, 3H, OCH_3-3), 3.94 (s, 3H, OCH_3-2), 5.34 (dd, J = 6.38, 11.0 Hz, 1H, H-7), 6.56 (s, 1H, H-4), 7.06 (d, J = 10.4 Hz, 1H, H-11), 7.28 (d, J = 10.3 Hz, 1H, H-12), 7.32 (s, 1H, H-8); CIMS m/z 445 ($M + H)^+$. Anal. ($C_{24}H_{28}O_6S$) C, H, S.

(±)-7-*O*-Benzoyldeacetamidothiocolchicine (13): yield 88% (starting with 45.3 mg of **9**); amorphous; $[\alpha]^{25}_D$ 0° (*c* 0.52, MeOH); ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3H, SCH_3-10), 2.13−2.65 (m, 4H, H-5,6), 3.72 (s, 3H, OCH_3-2), 3.93 (s, 3H, OCH_3-3), 3.96 (s, 3H, OCH_3-2), 5.59 (dd, J = 4.86, 11.4 Hz, 1H, H-7), 6.59 (S, 1H, H-4), 7.10 (d, J = 10.5, 1H, H-11), 7.35 (d, J = 10.4 Hz, 1H, H-12), 7.48 (s, 1H, H-8), 7.41−7.52 (m, 2H,

benzoyl H-3,5), 7.57−7.67 (m, 1H, benzoyl H-4), 8.05−8.15 (m, 2H, benzoyl H-2,6); CIMS *m*/*z* 479 (M + H)$^+$. Anal. ($C_{27}H_{26}O_6S$) C, H, S.

(±)-7-*O*-Nicotinoyldeacetamidothiocolchicine (14): yield 96% (starting with 44.7 mg of **9**); amorphous; $[\alpha]^{25}_D$ 0° (*c* 0.33, MeOH); ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H, SCH_3-10), 2.05−2.60 (m, 4H, H-5,6), 3.62 (s, 3H, OCH_3-1), 3.84 (s, 3H, OCH_3-3), 3.87 (s, 3H, OCH_3-2), 5.50 (dd, *J* = 5.1, 11.3 Hz, 1H, H-7), 6.51 (s, 1H, H-4), 6.98 (d, *J* = 10.4 Hz, 1H, H-11), 7.22 (d, *J* = 10.1 Hz, 1H, H-12), 7.21 (s, 1H, H-8), 7.38 (s, 1H, nicotinoyl H-5), 8.26 (d, 1H, nicotinoyl H-4), 8.75 (s, 1H, nicotinoyl H-6), 9.20 (s, 1H, nicotinoyl H-2); CIMS *m*/*z* 480 (M + H)$^+$. Anal. ($C_{26}H_{25}NO_6S$) C, H, S.

(±)-7-*O*-Isonicotinoyldeacetamidothiocolchicine (15): yield 76.8% (starting with 69.7 mg of **9**); crystallization from Et_2O/MeOH afforded yellow plates; mp 198−200 °C; $[\alpha]^{25}_D$ 0° (*c* 0.43, MeOH); ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3H, SCH_3-10), 2.50−2.72 (m, 4H, H-5,6), 3.72 (s, 3H, OCH_3-1), 3.95 (s, 6H, OCH_3-2,3), 5.60 (dd, *J* = 5.7, 11.0 Hz, 1H, H-7), 6.60 (s, 1H, H-4), 7.08 (d, *J* = 10.5 Hz, 1H, H-11), 7.33 (d, *J* = 10.5 Hz, 1H, H-12), 7.29 (s, 1H, H-8), 8.01 (s, 2H, isonicotinoyl H-3,5), 8.85 (s, 2H, isonicotinoyl H-2,6); CIMS *m*/*z* 479 (M + H)$^+$. Anal. ($C_{26}H_{25}NO_6S$) C, H, S.

(±)-7-*O*-(Trifluoroacetyl)deacetamidothiocolchicine (16): yield 55% (starting with 33.5 mg of **9**); crystallization from Et_2O/hexane gave yellow prisms; mp 212−214 °C; $[\alpha]^{25}_D$ 0° (*c* 0.44, MeOH); ^{1}H NMR ($CDCl_3$) δ 2.51 (s, 3H, SCH_3-10), 3.65 (s, 3H, OCH_3-1), 3.93 (s, 6H, OCH_3-2,3), 4.54 (dd, *J* = 6.4, 10.3 Hz, 1H, H-7), 6.58 (s, 1H, H-4), 7.35 (d, *J* = 10.5 Hz, 1H, H-11), 7.49 (d, *J* = 10.5 Hz, 1H, H-12), 8.11 (s, 1H, H-8). Anal. ($C_{22}H_{21}O_6SF_3$) C, H, S.

(±)-7-Bromodeactamidothiocolchicine (17). A mixture of **9** (45.5 mg, 0.122 mmol) and CBr_4 (60.4 mg, 0.18 mmol) in CH_2Cl_2 (5 mL) was stirred under N_2 at room temperature. To this solution was added PPh_3 (38.3 mg, 0.146 mmol) in CH_2Cl_2 (1 mL). This mixture was refluxed overnight under N_2. The reaction mixture was concentrated, and the residue was chromatographed with preparative TLC using hexane/ethyl acetate/MeOH (6:3:1) as eluant: yield 47%; amorphous; $[\alpha]^{25}_D$ 0° (*c* 0.315, MeOH); ^{1}H NMR ($CDCl_3$) δ 2.48 (s, 3H, SCH_3-10), 2.3−2.6 (m, 4H, H-5,6), 3.71 (s, 3H, OCH_3-1), 3.92 (s, 6H, OCH_3-2,3), 4.80 (dd, *J* = 6.0, 11.4 Hz, 1H, H-7), 6.53 (s, 1H, H-4), 7.04 (d, *J* = 10.4 Hz, 1H, H-11), 7.20 (d, *J* = 10.4 Hz, 1H, H-12), 7.80 (s, 1H, H-8); ^{13}C NMR ($CDCl_3$) δ 15.2 (SCH_3-10), 32.1 (C-5), 43.1 (C-6), 52.1 (C-7), 56.1 (OCH_3-1), 61.1 (OCH_3-3), 61.3 (OCH_3-2), 107.4 (C-4), 125.9 (C-11), 126.5 (C-1a), 133.6 (C-8), 133.9 (C-4a), 134.0 (C-12a), 136.5 (C-12), 141.5 (C-2), 146.9 (C-7a), 150.8 (C-1), 153.9 (C-3), 158.6 (C-10), 182.3 (C-9); CIMS *m*/*z* 439 (M + 2)$^+$. Anal. ($C_{20}H_{21}O_4SBr$) C, H, S, Br.

(±)-7-*O*-Nicotinoyldeacetamidothiocolchicine Hydrochloride (18) and (±)-7- Isonicotinoyldeacetamidothiocolchicine Hydrochloride (19). A solution of **14** (22.3 mg) or **15** (26.5 mg) in CH_2Cl_2, respectively, was allowed to react with HCl(g) for 1 min with TLC monitoring. The reaction mixture was concentrated to give pure powders.

(±)-7-O-Nicotinoyldeacetamidothiocolchicine Hydrochloride (18): yield 98.5%, $[\alpha]^{25}_D$ 0° (*c* 0.35, H_2O); ^{1}H NMR ($CDCl_3$) spectrum was identical with that of **14**. Anal. ($C_{26}H_{26}NO_6SCl$) C, H, N, S.

(±)-7-*O*-Isonicotinoyldeacetamidothiocolchicine hydrochloride (19): yield 100.2%; $[\alpha]^{25}_D$ 0° (*c* 0.20, H_2O); ^{1}H NMR ($CDCl_3$) spectrum was identical with that of **15**. Anal. ($C_{26}H_{26}NO_6SCl$) C, H, N, S.

(−)-7-*O*-Camphanoyldeacetamidothiocolchicine (10a): yield 44% (starting with 27.6 mg of **9**); resolved from a (±)-camphanoyldeacetamidothiocolchicine (**10**) mixture by flash column chromatography using EtOAc/hexane (6:4) as eluant; amorphous; $[\alpha]^{25}_D$ −167 ° (*c* 0.30, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.96 (s, 3H, camphanoyl CH_3), 1.12 (s, 6H, camphanoyl CH_3), 1.58−2.20 (m, 4H, camphanoyl H-4,5), 3.68 (s, 3H, OCH_3-1), 3.93 (s, 3H, OCH_3-3), 3.95 (s, 3H, OCH_3-2), 5.43 (dd, *J* = 6.0, 10.9 Hz, 1H, H-7), 6.58 (s, 1H, H-4), 7.08 (d, *J* = 10.5 Hz, 1H, H-11), 7.31 (d, *J* = 10.5 Hz, 1H, H-12), 8.31 (s, 1H, H-8); CIMS *m*/*z* 555 (M + H)$^+$. Anal. ($C_{30}H_{34}O_8S$) C, H, S.

(+)-7-*O*-Camphanoyldeacetamidothiocolchicine (10b): yield 46% (starting with 27.6 mg of **9**); resolved from a (±)-camphanoyldeacetamidothiocolchicine (**10**) mixture by flash column chromatography after the **10a** fraction using EtOAc/hexane (6:4) as eluant; amorphous; $[\alpha]^{25}_D$ +188° (*c* 0.35, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.03, 1.09, 1.13 (s, each 3H, camphanoyl CH_3), 2.47 (s, 3H, SCH_3-10), 3.67 (s, 3H, OCH_3-1), 3.93 (s, 3H, OCH_3-3), 3.95 (s, 3H, OCH_3-2), 5.48 (dd, *J* = 6.5, 10.8 Hz, 1H, H-7), 6.58 (s, 1H, H-4), 7.11 (d, *J* = 10.5 Hz, 1H, H-11), 7.33 (d, *J* = 10.5 Hz, 1H, H-12), 7.32 (s, 1H, H-8); CIMS *m*/*z* 555 (M + H)$^+$. Anal. ($C_{30}H_{34}O_8S$) C, H, S.

(−)-Deacetamidothiocolchicin-7-ol (9a). To a solution of **10a** (35.4 mg, 0.064 mmol) in MeOH (5 mL) was added 20% HCl (3 mL). This solution was refluxed for 4 h with TLC monitoring. After the reaction mixture was cooled, it was neutralized with saturated $NaHCO_3$ to a pH of 7 and extracted with $CHCl_3$ (3 × 10 mL). The combined organic layer was washed with saturated NaCl and concentrated to give crude **9a.** Crystallization of **9a** afforded pure light-yellow prisms: yield 64%; mp 228−229 °C; $[\alpha]^{25}_D$ −187° (*c* 0.16, MeOH); ^{1}H NMR spectrum ($CDCl_3$) was identical with that of **9**. Anal. ($C_{20}H_{22}O_5S$) C, H, S.

(+)-Deacetamidothiocolchicin-7-ol (9b): preparation procedure same as for **9a**, starting with 21 mg (0.038 mmol) of **10b**; crystallization afforded light-yellow prisms; yield 70.5%; mp 221 °C; $[\alpha]^{25}_D$ +186° (*c* 0.2, MeOH); ^{1}H NMR ($CDCl_3$) spectrum was identical with that of **9**. Anal. ($C_{20}H_{22}O_5S$) C, H, S.

(−)-7-*O*-Acetyldeacetamidothiocolchicine (11a): yield 79.5% (starting with 6.0 mg of **9a**); amorphous; $[\alpha]^{25}_D$ −185° (*c* 0.51, MeOH); ^{1}H NMR ($CDCl_3$) spectrum was identical with that of **11**. Anal. ($C_{22}H_{24}O_6S$) C, H, S.

(+)-7-*O*-Acetyldeacetamidothiocolchicine (11b): yield 81.5% (starting with 6.2 mg of **9b**); amorphous; $[\alpha]^{25}_D$ +202° (*c* 0.41, MeOH); ^{1}H NMR ($CDCl_3$) spectrum was identical with that of **11**. Anal. ($C_{22}H_{24}O_6S$) C, H, S.

(−)-7-*O*-Butyryldeacetamidothiocolchicine (12a): yield 36% (starting with 3.3 mg of **9a**); amorphous; $[\alpha]^{25}_D$ −172° (*c* 0.53, MeOH); ^{1}H NMR ($CDCl_3$) spectrum was identical with that of **12**. Anal. ($C_{24}H_{28}O_6S$) C, H, S.

(−)-Nicotinoyldeacetamidothiocolchicine (14a): yield 80% (starting with 5.6 mg of **9a**); amorphous; $[\alpha]^{25}_D$ −72.5° (*c* 0.59, MeOH); ^{1}H NMR ($CDCl_3$) spectrum was identical with that of **14**. Anal. ($C_{26}H_{25}NO_6S$) C, H, S.

(−)-Isonicotinoyldeacetamidothiocolchicine (15a): yield 76% (starting with 4.4 mg of **9a**); amorphous; $[\alpha]^{25}_D$ −90° (*c* 0.45, MeOH); ^{1}H NMR ($CDCl_3$) spectrum was identical with that of **15**. Anal. ($C_{26}H_{25}NO_6S$) C, H, S.

X-ray Crystal Structure Analyses of Racemic Alcohol 9 and a *S,7S* Alcohol 9a. Crystal data for **9**: $C_{20}H_{22}O_5S$, *M* = 374.46, triclinic, space group $P\bar{1}$(C_i^1); *a* = 11.326(1) Å, *b* = 12.897(1) Å, *c* = 7.171(1) Å; α = 103.36(1)°, β = 99.89(1)°, γ = 109.48(1)°; *V* = 924.8(5) Å^3, *Z* = 2, D_{calcd} = 1.345 g cm^{-3}, μ(Cu Kα radiation, λ = 1.5418 Å) = 17.5 cm^{-1}; crystal dimension: 0.28 × 0.34 × 0.40 mm. Crystal data for **9a**: $C_{20}H_{22}O_5S$, *M* = 374.46, orthorhombic, space group $P2_12_12_1$(D_2^4); *a* = 8.369(1) Å, *b* = 29.984(2) Å, *c* = 7.401(1) Å; *V* = 1875.2(6) Å^3, *Z* = 4, D_{calcd} = 1.339 g cm^{-3}, μ(Cu Kα radiation) = 17.4 cm^{-1}; crystal dimensions 0.20 × 0.25 × 0.60 mm.

Crystals of **9a** have a strong tendency to grow as twins. A fragment cut from a large crystal was shown to be single by photographic methods. Oscillation and Weissenberg photographs furnished preliminary unit cell and space group information for **9** and **9a**. Intensity data (+*h*,±*k*, ±*l*, 4110 reflections for **9**, +*h*, +*k*, +*l*, 2218 reflections for **9a**; θ_{max} = 75°) were recorded on an Enraf-Nonius CAD-4 diffractometer [Cu Kα radiation, graphite monochromator; ω−2θ scans, scanwidth (0.80 + 0.14 tan θ)°]; the intensities of four reference reflections, remeasured every 2 h, showed no significant variation (<1.0%) throughout. Refined unit cell parameters were derived in each case from the diffractometer setting angles for 25 reflections (36° < θ < 40°) widely separated in reciprocal space. Intensity data were corrected for the usual Lorentz and polarization effects; empirical absorption corrections, based on the ϕ dependency of the intensities of several reflections with χ *ca.* 90°, were also applied [T_{max}:T_{min} (relative) = 1.00:0.76 for **9** and 1.00:0.83 for **9a**]. Equivalent reflections for **9** were averaged [R_{merge} (on *I*) = 0.021] to yield 3795

independent values. Totals of 3471 and 2043 reflections with $I > 3.0\sigma(I)$ for **9** and **9a**, respectively, were retained for the structure analyses and refinements. Laue symmetry indicated that crystals of **9** were triclinic, space group $P1$ or $P\bar{1}$; the latter was assumed at the outset, and this choice was shown to be correct by the structure solution and refinement. Crystals of **9a** belong to the orthorhombic system, space group $P2_12_12_1$, as defined uniquely by the systematic absences: $h00$ when $h \neq 2n$, $0k0$ when $k \neq 2n$, and $00l$ when $l \neq 2n$.

Both crystal structures was solved by direct methods (MULTAN11/82). Initial coordinates for all non-hydrogen atoms were derived from an *E*-map. Positional and thermal parameters of these atoms (first isotropic and then anisotropic) were adjusted by means of several rounds of full-matrix least-squares calculations during which $\Sigma w\Delta^2[w = 1/\sigma^2(|F_o|), \Delta = (|F_o| - |F|_c)]$ was minimized. Hydrogen atoms were located in difference Fourier syntheses, and their positional and isotropic thermal parameters were also refined during the subsequent cycles; an extinction correction (g) was included as a variable in the later iterations. The parameter refinements converged at R $(=\Sigma||F_o| - |F_c||/\Sigma|F_o|) = 0.040$ $\{R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2} = 0.074$, GOF $= [\Sigma w\Delta^2/(N_{\text{observations}} - N_{\text{parameters}})]^{1/2} = 2.1$, $g = 8.0(6) \times 10^{-6}$ for **9**$\}$ and $R = 0.034$ ($R_w = 0.50$) for **9a**. The absolute stereochemistry of **9a** was established by introducing the imaginary contributions to the anomalous dispersion corrections into the structure factor calculations. For parameters corresponding to the a*S*,7*S* stereochemistry, R was 0.0328 while R_w was 0.0483, whereas when those of the mirror image were used, the corresponding values were $R' = 0.0405$ and $R'_w = 0.0610$. These differences are significant[24] at the 0.005 level when R'_w/R_w (0.0610/0.0483 = 1.2629) equals to or exceeds 1.0024 and indicate that the absolute stereochemistry is correctly represented as shown. Confirmation of this assignment was derived by comparison of the magnitudes of the relative intensities for Friedel pairs of 39 enantiomer-sensitive reflections with $I > 20\sigma(I)$ and $\Delta I > 20\%$; the measured differences in all cases were in the same sense as those calculated. Continuation of the least-squares refinement of parameters for the a*S*,7*S* enantiomer led to convergence at $R = 0.032$ [$R_w = 0.047$, GOF $= 1.71$, $g = 2.0(2) \times 10^{-6}$]. Final difference Fourier syntheses for **9** and **9a** contained no usual features.

Crystallographic calculations were performed on PDP11/44 and MicroVAX computers by use of the Enraf-Nonius Structure Determination Package (SDP). For all structure factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from ref 25.

Biological Assays. The tubulin polymerization,[14] The [^{3}H]-colchicine binding,[11] and the cell growth[12] assays were all performed as described previously. The human Burkitt lymphoma CA46 line was provided by Dr. P. O'Connor, National Cancer Institute.

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Supporting Information Available: Tables of fractional atomic coordinates and temperature factor parameters, bond lengths, bond angles, and torsion angles for **9** and **9a** and a table of 39 enantiomer-sensitive reflections for **9a** (18 pages). Ordering information can be found on any current masthead page.

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