# Novel 3-*O*-Glycosyl-3-demethylthiocolchicines as Ligands for Glycine and γ-Aminobutyric Acid Receptors

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New 3-*O*-glycosyl-3-demethylthiocolchicines containing natural and unnatural sugar moieties were prepared and tested on γ-aminobutyric acid (GABA) and strychnine-sensitive glycine receptors present in rat brain and spinal cord. Two different synthetic approaches were used with the readily available 3-*O*-demethylthiocolchicine (**1b**) and thiocolchicoside (**2a**). Glycosyl compounds **2a**−**g** were obtained from **1b** and 1-fluorosugars **4**. 6′-Heterosubstituted glycosyl compounds **6**−**12** and the 6′-desoxy derivative **2h** were prepared from **2a**.

## Introduction

Thiocolchicoside (**2a**, Scheme 1) is a semisynthetic compound obtained from natural colchicine, the main alkaloid of *Colchicum autumnale*. In contrast to the parent thiocolchicine (**1a**), which is characterized by antimitotic activity,[1] **2a** is a muscle relaxing agent and an anti-inflammatory drug[1] whose action is due, at least in part, to the activation of γ-aminobutyric acid (GABA) receptors[1] and to the inhibition of the tonic seizures induced by picrotoxin. Recently, it has been proposed that the activity of **2a** may also be due to interaction with strychnine-sensitive glycine receptors.[1] **2a** generates a variety of metabolites,[2] and two of them, the glucoronide[3] and **1b**, are reported to contribute to the pharmacological activity of **2a**, albeit the drug is much more potent in vitro. Furthermore, it is not clear if the glucuronide is generated by glucuronization of **1b** or oxidation of **2a** itself. However, the rate of formation of the metabolites seems to be crucial for the half-life of the drug and its overall activity. Till now, few examples of thiocolchicoside analogues have been reported.[1−8] For this reason, we recently[1] started a program for the structural modification of **2a** aimed at modulating the cleavability of the glycosidic bond and the susceptibility of the glycosidic residue to oxidation. Our aim was to modulate the glycoside residue diversity preserving the potency of the parent drug. A first approach had been oriented to obtain analogues of **2a**, compounds **3**, by coupling amino compound **1c** and the corresponding glycosyl derivatives (Figure 1). The displacement assay of [$^3$H]strychnine and [$^3$H]muscimol in rat spinal cord (sc) and cerebral cortex (cx) showed mild but correlated changes to the activity of the parent compound. We here report the findings obtained by coupling **1b** and a few natural or unnatural sugars.

Two synthetic strategies were used with the readily available **1b** and **2a**. The reaction of **1b** with sugars **4a**−**g** gave the 3-*O*-glycosyl derivatives **2a**−**g** (Scheme 1). A series of 6′-heterosubstituted compounds **6**−**12** and the 6′-desoxy derivative **2h** were prepared by taking advantage of the selective reactivity of the 6′ hydroxy group of **2a** (Scheme 2). Compounds **2a**−**h**, **6**, **8a**, **9**, **10**, and **12** were tested for their interaction with [$^3$H]-strychnine and [$^3$H]muscimol binding sites in rat spinal cord and cerebral cortex.

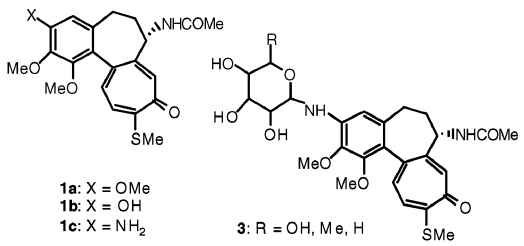


**Figure 1.** Thiocolchicine derivatives.

## Chemistry

Methods for the glycosylation of phenol derivatives with activated sugars were studied,[9−11] but some of them cannot be applied to complex compounds where other functional groups may be degraded. In particular, the reaction of **1b** and acetobromoglucose gives **2a** in poor yield (22%).[12] Our efforts on the glycosidation reaction of **1b** led to a significant improvement of the glycosidation cited above with control of the stereochemistry. According to our knowledge,[9] we performed the reaction of 1,2,3,4,6-*O*-pentaacetyl-β-D-glucopyranose with the *O*-stannyl derivative of **1b** but the reaction failed. We then switched to the coupling of **1b** with glycosyl fluorides **4**, known to be easy to handle and usually used to promote high diastereoselection.[13,14] The reaction of glucopyranosyl fluoride **4a** with **1b** in MeCN in the presence of $BF_3$·$Et_2O$ and of 1,1,3,3-tetramethylguanidine (TMG)[13] gave **5a** (97%) as a single β-diastereomer (Scheme 1). The deprotection of the hydroxy groups was done with NaOH in EtOH, affording pure thiocolchicoside (β-**2a**) (Scheme 1). Under "one-pot" conditions, β-**2a** was obtained from **1b** in 97% yield. This synthetic protocol allowed us to prepare a series of thiocolchicine derivatives **2** (Scheme 1) by reacting **1b** with glycosyl fluorides **4**: two hexoses (D-mannose (**4b**) and D-galactose (**4c**)), the 6-deoxyhexose-L-rhamnose (**4d**), and three pentoses (D-xylose (**4e**), D-arabinose (**4f**), D-lyxose (**4g**)). Single β-diastereomers **5b**−**e** were obtained from glycopyranosyl fluorides **4b**−**e**. In the case of **4f** and **4g**, a mixture of epimers **5f** (β/α, 1 : 6) and **5g** (β/α, 1 : 3) was formed,

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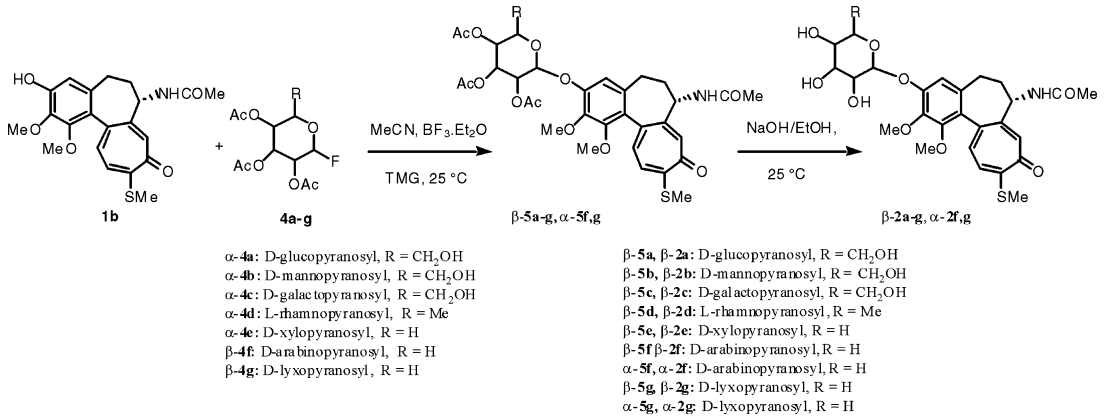
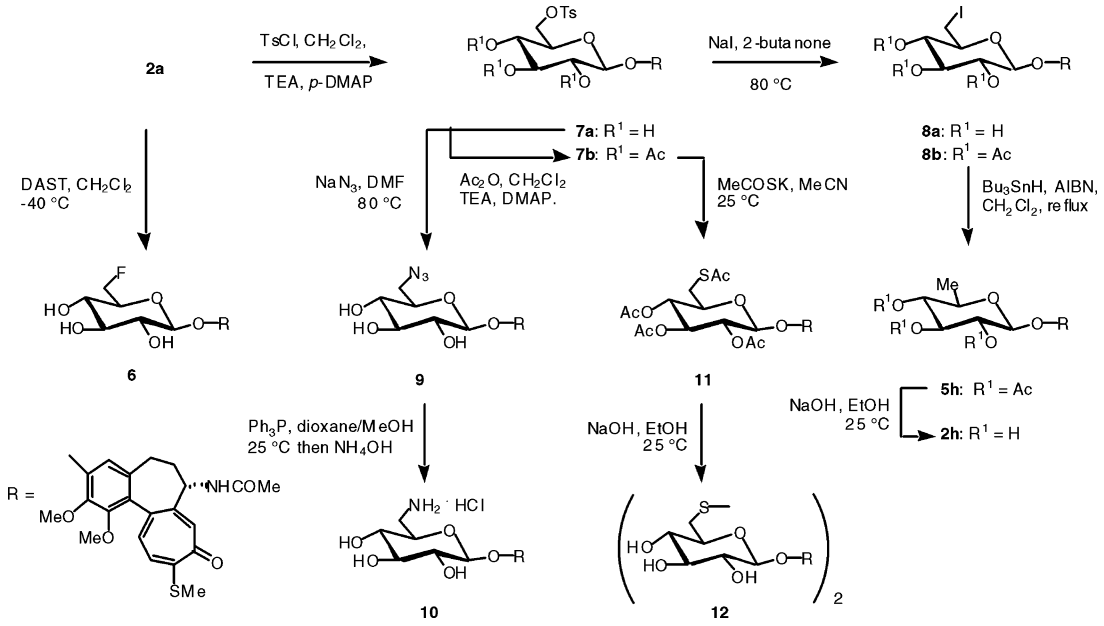
**Scheme 1.** Synthesis of 3-*O*-Glycosyl-3-demethylthiocolchicines **2**




**Scheme 2.** Synthesis of 3-*O*-Glycosyl-3-demethylthiocolchicines **6**−**12**, **5h**, **2h**




respectively. The oxygen atoms were directly deprotected, and **5b**−**e** were transformed into the corresponding pure $\beta$-glycosyl derivatives **2b** (70%), **2c** (70%), **2d** (70%), and **2e** (70%) and into the mixture of $\beta$,α-anomers **2f** (77%) and **2g** (77%). The stereochemical outcome of the reaction of **1b** and **4** is dependent on the relative stereochemistry of 1,2-substituents of the fluoro sugar. According to the mechanism proposed by Yamaguchi,[13] the 1,2-trans derivatives were formed as single isomers in the case of sugars **4a**,**c**,**e**, having an axial fluorine and an equatorial 2-acetoxy group. In the case of **4f** and **4g**, where the above groups are in the equatorial and axial positions, a small amount of the 1,2-cis epimer was also detected, via oxonium intermediate.[13] The 1,2-cis isomers were formed using sugars **4b** and **4d** in which the above groups are in the trans position. In this case, acetonitrile can form complexes with the oxonium cation, thus affecting the orientation of the incoming O-nucleophile.[15]

The second part of this research was devoted to the direct functionalization of the C-6′ position of **2a** with heteroatoms to give **6**−**12** and to prepare the 6′-desoxy derivative **2h** (Scheme 2). The reaction of **2a** with bis(2-methoxyethyl)-aminosulfur trifluoride (DAST) in $CH_2Cl_2$ at −40 °C gave the 6′-fluoro derivative **6** (40%). The reaction of **2a** with *p*-toluenesulfonyl chloride in $CH_2Cl_2$ and triethylamine (TEA) in the presence of a catalytic amount of *p*-dimethylaminopyridine (DMAP) afforded **7a** (75%). The iodo derivative **8a** (77%) was obtained from **7a** by using NaI in 2-butanone at 80 °C (16 h). The reaction of **7a** with $NaN_3$ in DMF (80 °C, 16 h) gave the azido derivative **9 (**75%). Its reduction with triphenylphosphine in dioxane/MeOH afforded the 6′-amino derivative **10** (22%) as the hydrochloride.

The more stable peracetyl tosylate **7b** (65%) was obtained by a "one-pot" procedure from **2a**, via tosylate **7a**, which was then protected with $Ac_2O$, TEA, and DMAP (catalytic amount). The 6′-thioacetyl derivative **11** (70%) was prepared from **7b** with potassium thioacetate in MeCN. The deprotection of both the oxygen and sulfur atoms with 1 N NaOH in EtOH at 25 °C (1 h) gave disulfide **12** (45%). The thiol derivative was not detected. Starting from the iodo derivative **8b**, obtained in 65% yield by reacting **7b** with NaI in 2-butanone, the 6′-deoxy derivative **5h** was prepared by reduction with tributyltin hydride and 2,2-azobisisobutyrylnitrile (AIBN) in $CH_2Cl_2$ at reflux. Its hydrolysis with 1 N NaOH in EtOH gave **2h** (65% overall yield).

**Effect of 3-*O*-Glycosyl-3-demethylthiocolchicines 2a**−**h, 6, 8a, 9, 10, and 12 on the Binding of [$^3$H]Strychnine and [$^3$H]-Muscimol.** The ability of the novel 3-*O*-glycosyl-3-demethylthiocolchicines to interact with strychnine-sensitive glycine and GABA receptors, localized in rat sc and cx, was evaluated in competition studies using synaptic membranes and the selective radioligands [$^3$H]strychnine and [$^3$H]muscimol. Dis-

**Table 1.** Relative Potency ($IC_{50}$ ($\mu$M)) of 3-*O*-Glycosyl-3-demethylthiocolchicines **2a**−**h**, **6**, **8a**, **9**, **10**, and **12**

| | [$^3$H]strychnine (sc) | [$^3$H]muscimol (sc) | [$^3$H]muscimol (cx) |
|---|---|---|---|
| strychnine | 0.014 ± 0.001 | | |
| glycine | 15.8 ± 0.5 | | |
| GABA | | 0.015 ± 0.002 | 0.024 ± 0.003 |
| $\beta$-**2a** | 1.5 ± 0.1 | 1.9 ± 0.5 | 3.4 ± 0.1 |
| $\beta$-**2b** | 2.3 ± 0.6 | 4.3 ± 0.8 | 3.8 ± 1.2 |
| $\beta$-**2c** | 2.6 ± 0.4 | 5.9 ± 0.9 | 8.3 ± 2.8 |
| $\beta$-**2d** | 3.0 ± 1.0 | 4.2 ± 1.1 | 2.2 ± 0.3 |
| $\beta$-**2e** | 2.6 ± 0.6 | 5.0 ± 0.3 | 3.99 ± 1.2 |
| **2f**[a] | 0.5 ± 0.1 | 2.5 ± 0.6 | 3.1 ± 1.3 |
| **2g**[a] | 0.6 ± 0.04 | 4.0 ± 1.2 | 5.6 ± 1.3 |
| $\beta$-**2h** | 1.9 ± 0.1 | 3.5 ± 1.1 | 6.2 ± 0.8 |
| $\beta$-**6** | 1.3 ± 0.1 | 2.0 ± 0.5 | 3.8 ± 0.6 |
| $\beta$-**8a** | >100 | 8.0 ± 1.0 | 17.3 ± 0.8 |
| $\beta$-**9** | 4.3 ± 0.9 | 8.2 ± 2.3 | 7.8 ± 2.0 |
| $\beta$-**10** | 4.7 ± 1.4 | 14.2 ± 0.5 | 10.7 ± 1.9 |
| $\beta$-**12** | 4.7 ± 0.3 | 7.8 ± 0.5 | >100 |

[a] Mixture of anomers.

placement curves were constructed for each product and compared with the potency of strychnine, glycine, GABA, and **2a**. Representative competition studies illustrating the ability of increasing concentrations of selected compounds to displace [$^3$H]strychnine and [$^3$H]muscimol from their specific binding sites in the two regions of the central nervous system (CNS) are in Figure FS1 of Supporting Information. The quantitative evaluation of the displacement curves obtained by the calculation of the specific $IC_{50}$ is reported in Table 1. The data demonstrate that all the compounds were able to compete with both receptors. On [$^3$H]strychnine binding, all compounds except **8a** showed a good potency, which like **2a** is in the low micromolar range. Exceptions to this pattern were **2f** and **2g**, which appear to be the most potent compounds ($IC_{50}$ is 3-fold lower than for **2a**). Therefore, the absence of a substituent on C-5 gave a notable improvement to the activity. In [$^3$H]muscimol binding, the compounds maintain the activity of **2a** with the exception of **8a**, **10**, and **12**, which are at least 1 order of magnitude less active than **2a**. Compound **6**, in which 6′-OH has been replaced for a fluorine, was equipotent to **2a** on all the receptors. Such a result is of great interest because the activity has been maintained while abolishing the susceptibility to direct oxidation to the glucuronyl derivative, making **6** a good candidate for further development.

## Conclusions

Two series of new 3-glycosyl-3-*O*-demethylthiocolchicines containing natural and unnnatural sugar moieties were prepared from **1b** and **2a**. The condensation of **1b** with 1-fluorosugars **4** afforded 3-*O*-glycosylthiocolchicines **2a**−**g** in good yield and with high diastereocontrol. By functionalization of the C-6′ position of **2a**, 6′-heterosubstituted compounds **6**−**12** and the 6′-desoxy derivative **2h** were prepared. Their biological activities were evaluated on GABA and strychnine-sensitive glycine receptors present in rat brain and spinal cord. Arabinosyl, lixosyl, and 6′-fluoro derivatives **2f**, **2g**, and **6** display a higher activity on the binding of [$^3$H]strychnine and a similar potency on the binding of [$^3$H]muscimol compared with **2a**, suggesting that the new compounds may be considered good candidates for further studies aimed at elucidating their involvement in myorelaxation.

## Experimental Section

**General Procedure for the Condensation of 3-*O*-Demethylthiocolchicine 1b with Fluorosugars 4a−g.** At 25 °C, under $N_2$ and stirring, **1b** (2.2 mmol) and sugar **4** (3.3 mmol) were suspended in anhydrous MeCN (100 mL). TMG (0.83 mL, 6.6 mmol) was added and then $BF_3{\cdot}Et_2O$ (2.2 mL, 17.6 mmol) (TLC, $CH_2Cl_2$/MeOH, 10:2). After 2.5 h, aqueous $NaHCO_3$ (30 mL) was added and the solution was extracted with AcOEt (2 × 20 mL). The organic layer was washed with aqueous $KHSO_4$ (50 mL) and brine (50 mL) and dried over $MgSO_4$. As an example, **5a** (830 mg, 97%) was isolated as yellow solid after chromatography ($SiO_2$, $CH_2Cl_2$/MeOH, 1:0 to 0:1).

**General Procedure for the Deacetylation Reaction. Synthesis of Compounds 2a−g.** Compound **5** (0.5 mmol) was dissolved in EtOH (4 mL), and 1 N NaOH (2 mL) was added. The solution was stirred at 25 °C (3 h). Compound **2** was obtained as yellow solid after chromatography ($SiO_2$, $CH_2Cl_2$/MeOH, 1:0 to 0:1) and crystallization (MeOH/$^i$Pr$_2$O). Yields are given on the starting compound **1b**.

***β*-2a:** yield 97%; mp 259 °C (mp 220 °C, lit.[16]); $[\alpha]^{25}_D$ −253° (*c* 1.0, MeOH).

***β*-2b:** yield 47%; mp 210−212 °C; $[\alpha]^{25}_D$ −182° (*c* 0.4, MeOH).

***β*-2c:** yield 44%; mp 214−216 °C; $[\alpha]^{25}_D$ −168° (*c* 0.4, MeOH).

**α-2d:** yield 49%; mp 165−168 °C; $[\alpha]^{25}_D$ −220° (*c* 0.4, MeOH).

***β*-2e:** yield 43%; mp 193 °C; $[\alpha]^{25}_D$ −201° (*c* 1.0, MeOH).

**2f:** mixture of anomers ($\beta/\alpha$, 1:6; 55%).

**2g:** mixture of anomers ($\beta/\alpha$, 1:3; 53%).

**6′-Fluorothiocolchicoside 6.** At −40 °C under $N_2$ and stirring, DAST (0.14 mL, 1.03 mmol) was added in 30 min to a suspension of **2a** (100 mg, 0.17 mmol) in dry $CH_2Cl_2$ (2.0 mL). The mixture was stirred (30 min, −40 °C) and then at 25 °C (3 h) (TLC, $CH_2Cl_2$/MeOH, 10:2). After the mixture was cooled at −20 °C, MeOH (0.5 mL) and $NaHCO_3$ (100 mg) were added. The solvent was evaporated, and the mixture was chromatographed ($SiO_2$, $CH_2Cl_2$/MeOH, 1:0 to 0:1) to afford pure **6** (39 mg, 40%) as a yellow solid: mp 178−180 °C (MeOH/$^i$Pr$_2$O); $[\alpha]^{25}_D$ −197° (*c* 0.5, MeOH).

**6′-Tosylthiocolchicoside 7a.** Under $N_2$ and stirring at 0 °C, **2a** (1 g, 1.72 mmol), TEA (0.5 mL, 3.61 mmol), DMAP (315 mg, 3.15 mmol), and TsCl (380 mg, 1.99 mmol) were added to dry pyridine (15 mL). After 20 h at 25 °C, the solvent was evaporated and the residue was dissolved in AcOEt/BuOH (3:1, 15 mL), washed with $H_2O$ (3 × 10 mL), aqueous $NaHCO_3$ (3 × 10 mL), and brine (3 × 10 mL), and then dried over $MgSO_4$. Yield 924 mg, 75%; mp 155 °C (MeOH/$^i$Pr$_2$O); $[\alpha]_D$ −84.3° (*c* 0.3, MeOH).

**6′-Tosylthiocolchicoside Triacetate 7b.** To a solution of **7a** (see above) were added TEA (2.2 mL, 15.78 mmol), DMAP (200 mg, 1.64 mmol), and $Ac_2O$ (2.5 mL, 24.49 mmol) under $N_2$ at 25 °C. After 1 h, $CH_2Cl_2$ (30 mL) was added and the organic layer washed with $H_2O$ (3 × 15 mL), aqueous $NaHCO_3$ (3 × 15 mL), and brine (20 mL) and then dried over $MgSO_4$. After chromatography ($SiO_2$, $CH_2Cl_2$/MeOH, 1:0 to 0:1), **7b** (890 mg, 65%) was obtained: mp 142−144 °C ($^i$Pr$_2$O); $[\alpha]^{25}_D$ −94.2° (*c* 0.58, MeOH).

**6′-Iodothiocolchicoside 8a.** NaI (32 mg, 0.214 mmol) and **7a** (110 mg, 0.142 mmol) in 2-butanone (1 mL) was heated at 80 °C (16 h). The solvent was evaporated and the mixture chromatographed ($SiO_2$, $CH_2Cl_2$/MeOH, 1:0 to 0:1), giving **8a** (70 mg, 77%) as a yellow solid: mp 110−112 °C (MeOH/$^i$Pr$_2$O); $[\alpha]_D$ −61.5° (*c* 0.52, MeOH).

**6′-Iodothiocolchicoside Triacetate 8b.** Pure **8b** (341 mg, 65%) was prepared as described for **8a** from **7b** (533 mg, 0.67 mmol) and NaI (120 mg, 0.80 mmol) in 2-butanone (3 mL) (6 h) after chromatography ($SiO_2$, $CH_2Cl_2$/MeOH, 5:1): mp 216−218 °C ($^i$Pr$_2$O); $[\alpha]_D$ −95.8° (*c* 0.5, $CHCl_3$).

**6′-Azidothiocolchicoside 9. 7a** (500 mg, 0.69 mmoli) and $NaN_3$ (90 mg, 1.39 mmol) in DMF (5 mL) were heated at 80 °C (16 h). After cooling, the mixture was taken up with AcOEt/BuOH (3:1, 10 mL), washed with $H_2O$ (3 × 10 mL), aqueous $NaHCO_3$ (3 × 10 mL), and brine (3 × 10 mL), and dried over $Na_2SO_4$. Compound **9** (300 mg, 75%) was obtained as a yellow solid after chromatography ($SiO_2$, $CH_2Cl_2$/MeOH, 1:0 to 0:1) and crystallization: mp 165−167 °C (MeOH/$^i$Pr$_2$O); $[\alpha]_D$ −141.3° (*c* 0.4, MeOH).

**6′-Aminothiocolchicoside Hydrochloride 10.** To a solution of $PPh_3$ (389 mg, 1.48 mmol) and **9** (300 mg, 0.49 mmol) in dioxane/MeOH (4.2 mL/0.8 mL) at 25 °C, after 1 h, $NH_4OH$ (1.1 mL, 35%)

was added. After 12 h, the solvent was evaporated and 1 N HCl (5 mL) was added. The aqueous solution was washed with toluene (3 × 5 mL) and evaporated, and the crude was taken up with benzene (3 × 5 mL) until dryness was obtained. Compound **10** (60 mg, 22%) was isolated as an orange solid: mp 148−150 °C (MeOH/$^{i}Pr_2O$).

**6′-Thioacetylthiocolchicoside Triacetate 11.** To a solution of **7b** (650 mg, 0.82 mmol) in dry MeCN (10 mL) under $N_2$ at 0 °C, MeCOSK (298 mg, 2.63 mmol) was added. The suspension was refluxed for 2 h. After cooling, $CH_2Cl_2$ (30 mL) was added and the solution washed with brine (3 × 20 mL) and $H_2O$ (3 × 20 mL) and dried over $MgSO_4$. Chromatography on silica gel ($CH_2Cl_2$/MeOH, 1:0 to 0:1) gave pure **11** (477 mg, 70%) as a red-orange solid: mp 140−142 °C ($^{i}Pr_2O$); $[\alpha]_D$ −117.5° (*c* 0.4, $CHCl_3$).

**6′-Disulfurthiocolchicoside 12.** Compound **11** (300 mg, 0.36 mmol) and 1 N NaOH (1.5 mL) in EtOH (2 mL) was stirred at 25 °C (1 h). The mixture was chromatographed ($SiO_2$, $CH_2Cl_2$/MeOH, 1:0 to 0:1) to give **12** (178 mg, 45%): mp 260 °C (MeOH/$^{i}Pr_2O$); $[\alpha]_D$ −262.5° (*c* 0.16, MeOH).

**6′-Desoxythiocolchicoside 2h.** To a solution of **8b** (100 mg, 0.12 mol) and AIBN (62 mg, 0.37 mol) in $CH_2Cl_2$ (4 mL) at reflux, $Bu_3SnH$ (4 × 0.15 mL) was added (4 times in 2 h), and heating was continued (15 h). After solvent evaporation **5h** was obtained and then stirred in EtOH (1 mL) and 1 N NaOH (1 mL) for 1 h. Compound **2h** (55 mg, 65%) was obtained as a yellow solid after chromatography ($SiO_2$, $CH_2Cl_2$/MeOH, increasing polarity): mp 185−187 °C (MeOH/$^{i}Pr_2O$); $[\alpha]_D$ −223° (*c* 0.36, MeOH).

**[$^3$H ]Strychnine Binding.** The synaptosomal membrane fraction from rat sc[17] was stored at −80 °C and used within 2 weeks. The binding assay was performed in a final volume of 1.2 mL of 50 mM sodium−potassium phosphate buffer (pH 7.1) containing 2 nM [$^3$H]strychnine (New England Nuclear, specific activity of 25.7 Ci/mmol), increasing concentrations of cold strychnine, glycine, or colchicoside derivatives, and membranes at a final protein concentration of 0.2−0.4 mg/1.2 mL. The assay was carried out at 4 °C for 10 min and rapidly filtered through Watman GF-B glass fiber filters. The filters were rapidly rinsed with NaCl (5 mL, 0.15 M). Nonspecific binding was determined in the presence of 0.1 mM unlabeled strychnine.

**[$^3$H]Muscimol Binding.** The interaction of **2a** with $GABA_A$ receptors was tested in a [$^3$H]muscimol binding assay.[18] Membranes were obtained by sc brainstem and cx of adult Sprague-Dawley rats[19] and incubated with 5 nM [$^3$H]muscimol and increasing concentrations of GABA, **2a**, or other colchicosides, in 50 mM Tris-citrate buffer, pH 7.1, in a final volume of 1 mL. After 30 min of incubation at 4 °C, the samples were rapidly filtered through Watman GF-B glass fiber filters and washed (three times) with ice-cold buffer (5 mL). Nonspecific binding was determined in the presence of 200 $\mu$M unlabeled GABA. Radioactivity was determined with a Wallach 1409 liquid scintillator counter with 50% efficiency.

**Protein Determination.** Protein content was determined by using the Bradford dye-binding procedure from Bio-Rad Laboratories.

**Data Analysis.** Each experimental point was run in triplicate, and each displacement curve used for the determination of $IC_{50}$ represents the average of three curves obtained from three independent experiments. Results were analyzed by nonlinear fitting using the computer program Prism (GrapPad Software). The *F* test was used to assess if the fitting using a two-site model equation was better ($P < 0.05$) than that obtained with a one-site model.

**Supporting Information Available:** Figure FS1 of binding assay results, spectroscopic data for **2** and **5**−**12**, and Table TS1 of elemental analysis results for **2** and **6**−**12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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