3-Demethoxy-3-glycosylaminothiocolchicines: Synthesis of a New Class of Putative Muscle Relaxant Compounds

Maria Luisa Gelmi,*[,§] Donato Pocar,[§] Guido Pontremoli,[§] Sara Pellegrino,[§] Ezio Bombardelli,[‡] Gabriele Fontana,[‡] Antonella Riva,[‡] Walter Balduini,[†] Silvia Carloni,[†] Mauro Cimino,[†] and Francis Johnson[||]

Istituto di Chimica Organica "A. Marchesini", Facoltà di Farmacia, Università di Milano, Via Venezian 21, I-20133 Milano, Italy, Indena S.p.a., Via Ortles 19, Milano, Italy, Istituto di Farmacologia e Farmacognosia, Facoltà di Farmacia, Università di Urbino "Carlo Bo", Via S. Chiara 27, 61029 Urbino, Italy, and Department of Chemistry, State University of New York at Stony Brook, Stony Brook, New York 11790-3400

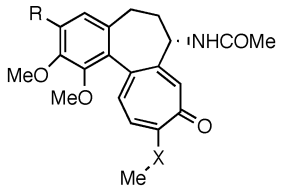
Received May 17, 2006

A novel class of 3-demethoxy-3-glycosylaminothiocolchicines (**7**) was prepared and tested for muscle relaxant activity. The syntheses were performed starting from the new 3-amino-3-demethoxythiocolchicine (**5**) prepared in good yield from 3-*O*-demethylthiocolchicine (**1c**) using the Buchwald−Hartwig reaction. The condensation of **5** with a series of pentose and hexose sugars (**6**) gave a series of 3-demethoxy-3-glycosylaminothiocolchicines (**7**). Their preparation was accomplished by adapting and improving a previous procedure for the preparation of *N*-arylglycosylamines. In particular, replacing traditional heating with microwave irradiation represents the key improvement of the process. The biological activity of the 3-demethoxy-3-glycosylaminothiocolchicines (**7**) was evaluated on GABA and strychnine-sensitive glycine receptors present in rat brain and spinal cord.

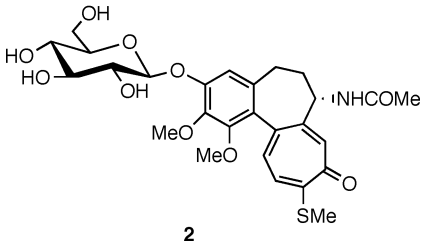
Introduction

Colchicine (**1a**) as well as the semisynthetic compound thiocolchicine (**1b**) (Figure 1) are alkaloids characterized by different pharmacological activities ranging from antiproliferative to antiinflammatory effects.[1−8] Thiocolchicoside (**2**, Figure 1), the glucopyranosyl derivative of the semisynthetic 3-*O*-demethylthiocolchicine (**1c**), is well-known as a muscle relaxing agent[9] and as an antiinflammatory drug substance. This compound is registered in different Countries under the tradenames of Coltramyl, Coltrax, Miorel, and Musco-Ril. Muscle spasm is one of the main factors responsible for chronic pain, and because this particular drug reduces muscle tone, it is used in therapy for the treatment of contractures and inflammatory conditions that affect the muscular system.[10] The action of thiocolchicoside has been attributed, at least in part, to the activation of GABA receptors. Indeed, it has been reported that thiocolchicoside can interact with GABA receptors, and, as a result, it is able to inhibit the tonic seizures induced by picrotoxin. Recently, it has been proposed that the activity of thiocolchicoside may also be due to its ability to interact with strychnine-sensitive glycine receptors as demonstrated by its ability to delay the appearance of strychnine-induced seizures and to displace[^{3}H]strychnine from its binding site.[11,12] Although the therapeutic properties of thiocolchicoside have been known since the 1970s, few efforts have been made to find new and more efficient analogues of thiocolchicoside. To our knowledge, only a few examples of glycosyl derivatives have been reported, namely 3-*O*-glucorunylthiocolchicine,[13,14] which is characterized by myorelaxant activity, and a series of acylated thiocolchicosides.[15] The biological activity of these latter compounds was not reported.

Recent studies revealed that thiocolchicoside is the effective drug but under physiologic conditions is hydrolyzed to 3-*O*-demethylthiocolchicine (**1c**).[13] Compound **1c** is inactive as a myorelaxant but, most importantly, is cytotoxic.[13] The formation in vivo of **1c** is therefore undesirable, and its serum concentration must be kept at the lowest possible level to avoid toxic effects. As a consequence, we initiated a new structure−activity relationships (QSAR) program based on thiocolchicoside, devoted to finding new analogues having muscle relaxing properties but also displaying increased metabolic stability of the glycosidic bond. We report here a part of this program in which we try to modulate the kinetics of the cleavage of the glycosyl derivatives to their common aglycone **1c**, by replacing the *O*-glycosyl moieties with *N*-glycosyl units. We thought that such a goal might be readily achieved by condensing 3-amino-3-demethoxythiocolchicine **5** with a series of hexoses or pentoses **6** (Figure 2).



1a: X = O; R = OMe
1b: X = S; R = OMe
1c: X = S; R = OH



2

Figure 1. Colchicine and thiocolchicine derivatives.

The new compound **5** was prepared from **1c** by a modification of the Buchwald−Hartwig reaction, previously described for

* Corresponding author. Phone: +390250314481. Fax: +390250314476. E-mail: marialuisa.gelmi@unimi.it.
[§] Università di Milano.
[‡] Indena SPA.
[†] Università di Urbino.
[||] State University of New York at Stony Brook.

10.1021/jm060585t CCC: $33.50
Published on Web 08/04/2006

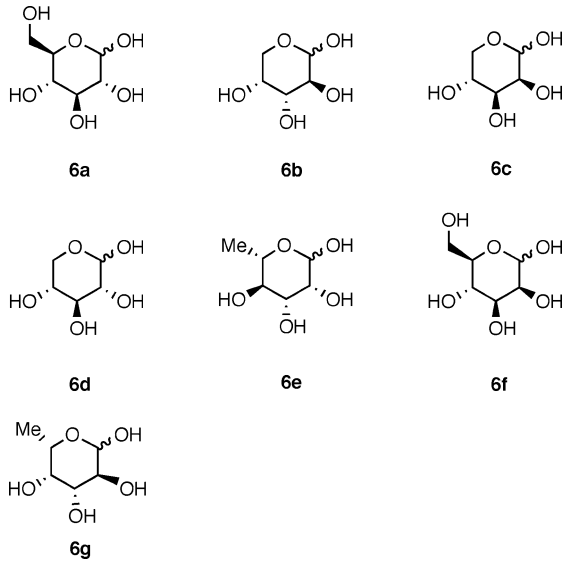


Figure 2. Pentoses and hexoses (**6**) used on this study.

3-*O*-demethylcolchicine in a patent application.[16] The glycosylation was then performed by adapting and improving a known procedure for the preparation of *N*-arylglycosylamines. In particular better yields and diastereoselectivities were obtained with microwave irradiation rather then the traditional heating method.

3-Demethoxy-3-glycosylaminothiocolchicines α-**7a**−**e** and *β*-**7a**−**d**,**f**,**g** were prepared and tested for their ability to interact with [^{3}H]strychnine and [^{3}H]muscimol binding sites in rat spinal cord and cerebral cortex.

Chemistry

3-Amino-3-demethoxythiocolchicine (**5**) is a new compound prepared by using as a key step the Buchwald−Hartwig (B−H) reaction[17−19] which it is well-known as a versatile method for generating an aromatic C−N bond under mild reaction conditions. The synthesis of the parent 3-amino-3-demethoxycolchicine was reported in a patent[16] in which 3-demethylcolchicine was transformed into the corresponding 3-demethoxy-3-benzylamino compound using a B−H reaction with the corresponding triflate. Catalytic reduction of the benzyl group gave the above compound, but no yield was reported. To avoid a synthetic step and considering the possibility that the presence of the sulfur atom on the thiocolchicine nucleus could inhibit the reduction reaction, we planned a different synthetic strategy starting from 3-*O*-demethylthiocolchicine **1c**, the aglycone of **2** which is available in large amount. The transformation of **1c** to **5** is outlined in Scheme 1. First, triflate **3** was prepared by allowing 3-*O*-demethylthicolchicine to react with triflic anhydride in dichloromethane in the presence of *p*-(dimethylamino)-pyridine (*p*-DMAP). Compound **3** was purified by column chromatography on neutral alumina (70% yield) since silica gel promotes decomposition.

Table 1. Reaction Conditions for the Coupling of **3** and Benzophenone Imine

entry	solvent[a]	catalyst[b]	ligand[c]	reaction time (h)	yield **5** (%)[d]
1	toluene	$Pd_2(dba)_3$	(*rac*)BINAP	16	28
2	toluene	$Pd_2(dba)_3$	DPPF	16	33
3	toluene	$Pd(OAc)_2$	DPPF	16	0
4	THF	$Pd(OAc)_2$	(*rac*)BINAP	16	25
5	toluene	$Pd(OAc)_2$	(*rac*)BINAP	16	70

[a] Reflux. [b] **3**/catalyst = 1:0.2. [c] **3**/ligand = 1:0.3. [d] Isolated compound.

Aryl triflates are good substrates in the cross-coupling reaction with nitrogen donors such as amines. We chose to introduce the amino group directly using the benzophenone imine as a surrogate for ammonia in these Pd-catalyzed aminations.[19] This reagent is commercially available and allows an easy deprotection of the amino function under mild conditions.

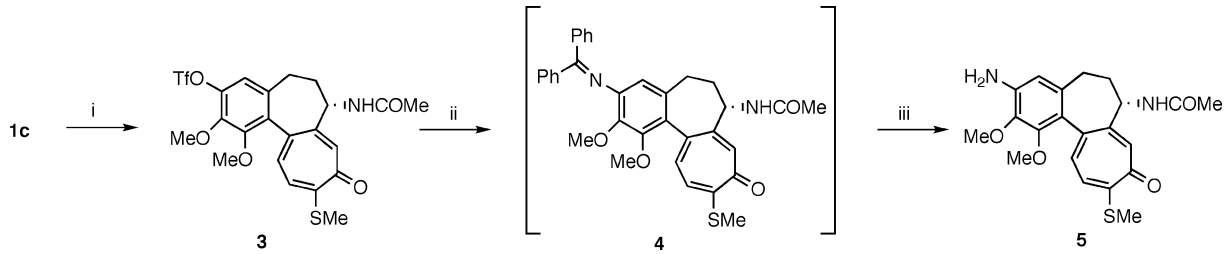
Triflate **3** and benzophenone imine were then subjected to the B−H reaction which gave intermediate **4**. This was not isolated but hydrolyzed immediately using MeOH in the presence of AcONa and $NH_2OH{\cdot}HCl$ which led to amine **5**. To achieve the best protocol for the coupling reaction, a systematic study was performed evaluating the choice of the catalyst, ligand, and solvent (Table 1). Cs_2CO_3 (1.4 equiv) was the base of choice since it is known that it avoids triflate hydrolysis.[19]

$Pd_2(dba)_3$ was first used as the catalyst either with BINAP (entry 1) or DPPF (entry 2) as ligands. In both cases low yields of **5** were found. The reaction failed when changing the catalyst and using DPPF (entry 3). Low yields were also observed when the reaction was performed uder the typical reaction conditions reported for coupling **5** with benzophenone imine (entry 4). Finally, simply by changing the solvent and using a simple combination of $Pd(OAc)_2$ and BINAP, amino compound **5** was obtained in good yield (entry 5).

Few synthetic methods have been reported for the preparation of *N*-arylglycosylamines starting from aniline derivatives.[20−26] Usually, the reaction could be accomplished by heating in protic solvents for different periods of time. The *N*-arylglycosylamines were isolated in poor to good yield depending on the reactivity of the aniline derivative. The stereochemistry at the anomeric carbon depends on the sugar and on the steric features of both the amine and the sugar. In some cases a mixture of epimers was obtained. Optimization of the *N*-glycosylation of **5** in terms of yield, reaction time, and diastereoselection represented a significant challenge.

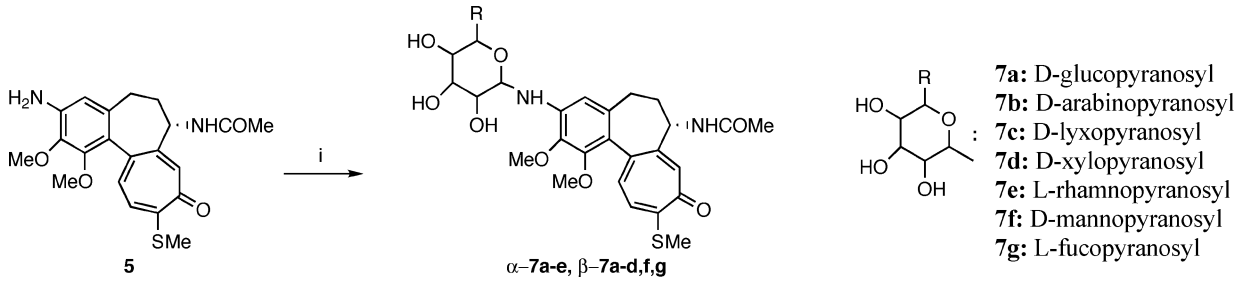
In a preliminary experiment we tested the classical reaction conditions indicated in the literature, consisting of the condensation of an aniline and an unprotected sugar in MeOH at reflux. The reaction of **5** with glucose **6a** (1 equiv) gave the espected

Scheme 1. Synthesis of 3-Amino-3-demethoxy-thiocolchicine **5**[a]



[a] Reagents and conditions: (i) $(TfO)_2O$, *p*-DMAP, CH_2Cl_2, 0 °C; (ii) Cs_2CO_3, benzophenone imine, $Pd(OAc)_2$, (±)BINAP, toluene, 25 °C (45 min.) then 120 °C (16 h); (iii) AcONa, $NH_2OH{\cdot}HCl$, MeOH, 25 °C.

Scheme 2. Synthesis of 3-Demethoxy-3-glycosylaminothiocolchicines **7**



[a] Reagents and conditions: (i) sugar **6**, MeOH, microwave.

N-glycosyl derivative **7a** (Scheme 2), but the reaction time required was very long (6 days) and the desired compound was obtained in low yield (35%). Only 10% of the starting material could be recovered. Prolonging the reaction time or using an excess of glucose (2 equiv) did not improve yield. In particular the latter conditions required an extensive purification procedure to obtain the product. ^{1}H NMR spectroscopy was used to monitor the glucose reaction and this showed the formation of a mixture of two anomers, α-**7a** and *β*-**7a** in a 20:80 ratio, respectively. These reaction conditions are general for the preparation of different *N*-glycosyl derivatives: epimers **7b** (α/*β*: 45:55; 38%) and the pure compound α-**7e** (35%) (Scheme 2) were prepared from **5** using D-arabinose (**6b**) and L-rhamnose (**6e**), respectively (Figure 2).

Although these reaction conditions permit the desired products to be obtained, they are not particularly efficient and other synthetic methodologies were therefore examined. Having in our hands triflate **3** and considering our previous experience with the B−H reaction, we attempted a palladium-catalyzed coupling reaction between 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosylamine and **3**. Two different reaction conditions were tested using either $Pd(OAc)_2$/BINAP/Cs_2CO_3 or $Pd_2(dba)_3$/DPPF/*t*BuOK and operating in toluene as the solvent, but in both cases the reaction was unsuccessful and the starting amine was recovered.

Alternatively, we tried to have compound **5** react with 2,3,4,6-tetra-*O*-tetraacetyl-*β*-D-glucopyranosyl fluoride, which is a good substrate for *O*-glucosylation,[27] but again the reaction failed.

A great improvement in the yields was obtained by the use of microwave irradiation which is known to be very effective in thermal reactions. By allowing **5** to react with **6a** (1 equiv) in MeOH, a mixture of α,*β*-anomers of **7a** was obtained operating at 110 °C and at 150 W. The reaction was examined at intervals of time. We found that the reaction time could be dramatically reduced. After 1 h, 50% of the starting material could be recovered, and this was reduced to 10% after 1.30 h, whereas after 4 h it completely disappeared. Furthermore, the yield of product was increased to 58%. This is a satisfactory result because it is well-known that electron-rich amines give very poor yields (13−30%) of *N*-glycosyl compounds when conventional heating methods are used.[21,24]

To our knowledge, this is the first application of the use of microwave heating in the condensation of sugars with anilines. This satisfactory synthetic result prompted us to verify the generality of the method. Both D-arabinose (**6b**) and L-rhamnose (**6e**) were subjected to the reaction with **5** and again an improvement in yield with respect to the classical thermal method was found. The *N*-glycosyl compounds **7b** (α/*β*: 45:55; 60%) and **7e** (α: 100; 60%) were obtained, respectively. Accordingly, this synthetic protocol was extended to the preparation of *N*-glycosyl compounds **7c** (α/*β*: 40:60; 60%), **7d** (α/*β*: 30:70; 55%), **7f** (*β*: 100; 60%), and **7g** (*β*: 100; 58%) (Scheme 2), using **5** and D-lyxose **6c**, D-xylose **6d**, D-mannose **6f**, and L-fucose **6g**, respectively (Figure 1). In all cases a substantial improvement in yield was observed together with a decrease in reaction time compared with conventional methods. Comparison of the the classical thermal method with the microwave method, when sugars **6a**,**b**,**e** were used, did not show any change in the diastereomeric ratio. Generally, however, a mixture of epimers is obtained, except in the rhamnose case where the *β*-epimer is the preferred form.

The stereochemistry of the anomeric center was assigned on the basis of spectroscopic data (^{1}H NMR, ^{13}C NMR, HTCOR). We observed that the H′ proton is at lower field with respect to the other sugar protons. The α or *β* configuration was assigned on the basis that $J_{1,2trans}$ is larger than $J_{1,2cis}$. The chemical shifts and the *J* values for *β*-anomers **7a**−**d**,**f**,**g** and α-anomers **7a**-**e** are reported in the Table TS1 (Supporing information), together with signals associated with the H-4 and N*H*-3 of thiocolchicine nucleus.

Effect of *N*-Glycosylthiocolchicines 7 on the Binding of [^{3}H]Strychnine and [^{3}H]Muscimol. The ability of 3-demethoxy-3-glycosylaminothiocolchicines (**7**) and of amino compound **5** to interact with strychnine-sensitive glycine and GABA receptors localized in rat spinal cord and cerebral cortex was evaluated in competition studies carried out using synaptic membranes and the selective radioligands [^{3}H]strychnine and [^{3}H]muscimol. In particular, displacement curves were constructed and compared with the potency of reference compounds such as strychnine, glycine, GABA, and thiocolchicoside **2**. Representative competition studies, illustrating the ability of increasing concentrations of compounds **7** to displace [^{3}H]strychnine and [^{3}H]muscimol from their specific binding sites in spinal cord and cerebral cortex, are reported in Figure 3. Under our experimental conditions, [^{3}H]strychnine (2 nM) was incubated with synaptic membranes from spinal cord (Figure 3A,B) and [^{3}H]muscimol (5 nM) was incubated with either spinal cord (Figure 3C,D) or cerebral cortex (Figure 3E,F) membranes of adult rats, in the absence (100% specific binding) or presence of increasing concentrations of the compounds under examination. The data obtained demonstrate that all the compounds investigated were able to compete with both receptor populations. However, the displacing potency of most compounds was lower than that reported for thiocolchicoside. Compounds **7e** and **7f** were less active on the binding of [^{3}H]muscimol in the cerebral cortex. The quantitative evaluation of the displacement curves obtained by the calculation of the specific IC_{50} values (the concentration of compound that displaces 50% of specific binding) for the 3-demethoxy-3-glycosylaminothiocolchicines is reported in Table 2. On [^{3}H]strychnine binding, compounds **7c**,**d**, **7e**, and **7g** were the most potent 3-demethoxy-3-glycosylaminothiocolchicines examined, followed by **7b**, **7a**, and **7f**. When the same experimental procedure and data analysis were

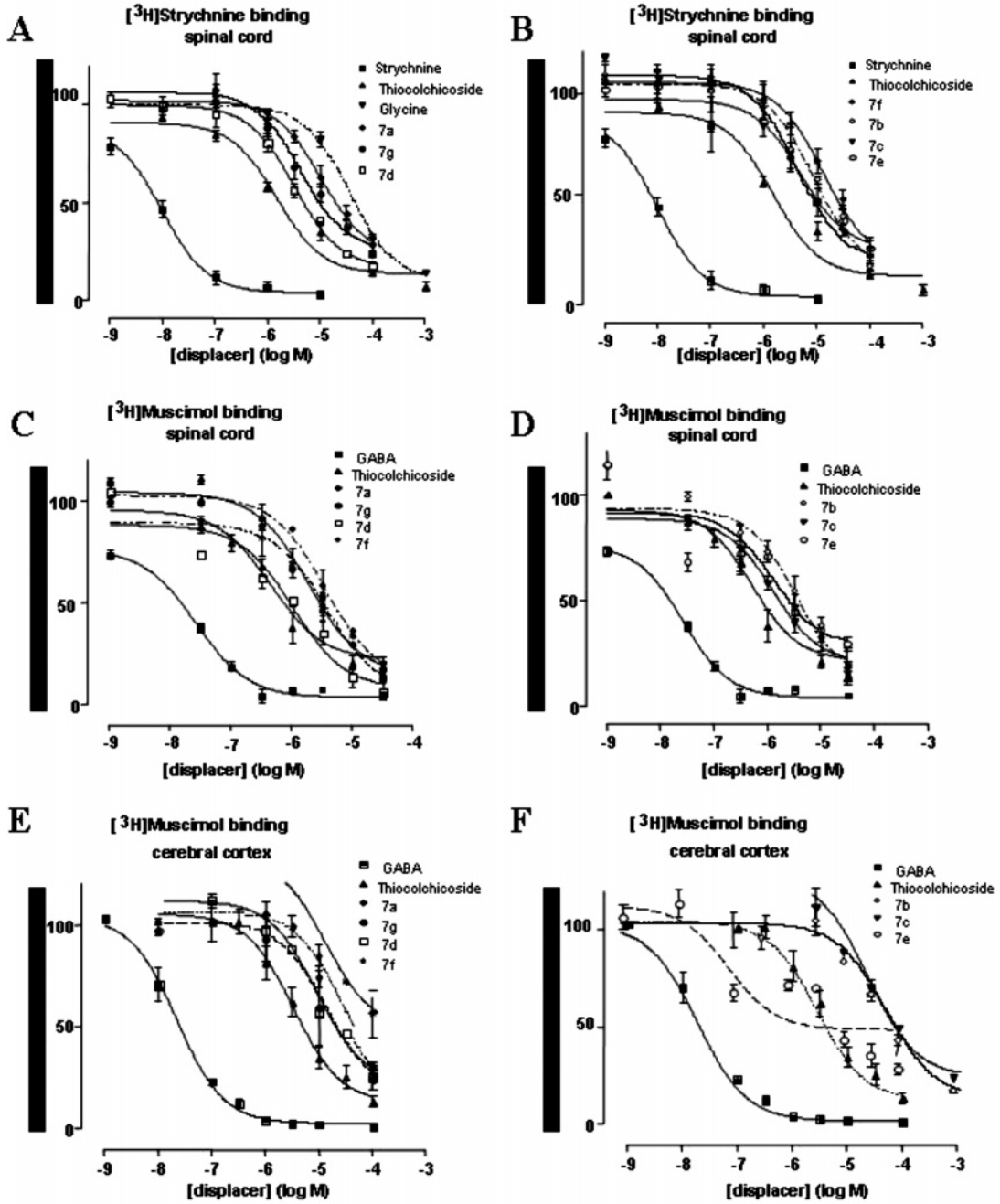


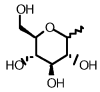
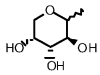
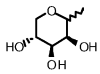
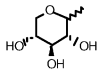
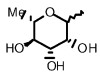
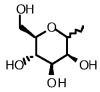
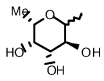
Figure 3. Representative displacement of [^{3}H]strychnine and [^{3}H]muscimol from spinal cord (A−D) and cerebral cortex (E, F) membranes by different concentrations of 3-demethoxy-3-glycosylaminothiocolchicines compounds, strychnine, glycine, GABA, and thicolchicoside. Results are the mean of three different experiments performed in triplicate.

applied to [^{3}H]muscimol binding, differences in the potency of the compounds between spinal cord and cerebral cortex were observed.

Indeed, in spinal cord, we found the same profile of activity described for the strychnine-sensitive glycine receptors, with compounds **7c**,**d**, **7e**, and **7g** being more potent than **7a**,**b** and **7f**. In contrast, when the membrane preparation of the cerebral cortex was used, **7d** exhibited the best displacement activity. Because compounds **7c**, **7d**, and **7g** display receptor activities comparable to those observed for thiocolchicoside, the data reported in the present study suggest that these newly synthesized molecules may be considered good candidates for further studies on myorelaxation.

From the present results it appears quite evident that, at least in vitro and except for amino compound **5**, the thiocolchicoside and the above *N*-glycosylthiocolchicines are more active with respect to glycine in the [^{3}H]strychnine binding assay and less active with respect to GABA for [^{3}H]muscimol binding. Comparing the activity of thiocolchicoside (**2**) with that of **7a**, which are functionalized with glucose and aminoglucose, respectively, a lower activity was found in the case of amino compound for all receptors tested. Nevertheless in the *N*-glycosyl-3-demethoxythiocolchicine series, we find interesting results concerning the sugar functionalization. These data suggest that the functionalization of C-6 position of the sugar moiety changes the activity of the above compounds. In fact, the presence of a polar group (i.e. CH_2OH group,) decreases the activity, so that the 6-desoxyhexoses **7e**, **7g** and the pentose derivatives **7b**, **7c**, **7d** are more active than the glucose and mannose derivatives **7a** and **7f**, respectively.

Table 2. Relative Potency of 3-Demethoxy-3-glycosylaminothiocolchicines **7a**−**g** on [^{3}H]Strychnine and [^{3}H]Muscimol Binding Sites in Rat Spinal Cord (sc) and Cerebral Cortex (cx)

Compounds	Sugar Moiety	IC_{50} (μM)		
		[^{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
Thiocolchicoside **2**		1.5 ± 0.1	1.9 ± 0.5	3.4 ± 0.1
3-Amino-3-demethoxythiocolchicine **5**		39.9 ± 2.4	1.9 ± 0.5	5.4 ± 0.8
7a[a]		10.6 ± 0.4	11.3 ± 4.8	18.0 ± 1.6
7b[a]		8.6 ± 1.3	10.5 ± 0.5	17.7 ± 5.2
7c[a]		4.1 ± 1.0	4.0 ± 0.2	9.7 ± 1.3
7d[a]		3.3 ± 0.6	3.3 ± 1.0	7.0 ± 0.7
α-**7e**		4.6 ± 1.0	3.8 ± 0.2	> 100
β-**7f**		13.1 ± 0.8	11.3 ± 1.2	> 100
β-**7g**		4.2 ± 0.4	5.9 ± 0.4	10.5 ± 2.2

[a] Mixture of anomers.

Concerning the cerebral cortex [^{3}H]muscimol binding, a lower activity was found for all compounds tested with respect to the binding with the other two receptors. The xylosyl derivative **7d** is the most active of the whole series. Among the compounds examined in this study, amino derivative **5** displayed the lowest activity on receptor sites labeled by [^{3}H]Strychnine, but its potency on both cortical and spinal cord [^{3}H]muscimol binding is comparable to that determined for thiocolchicoside, indicating a higher selectivity of **5** for GABA receptors.

Conclusions

In conclusion, the new 3-aminothiocolchicine (**5**) was prepared in good yield using B−H Pd-coupling chemistry. This compound is the starting material for the preparation of a series of new *N*-glycosylthiocolchicines **7**. The latter targets were achieved using a microware energy source which allowed a substantial increase in yield and a dramatic reduction in reaction time. These new aminoglycosides were tested on strychnine-sensitive glycine and GABA receptors localized in rat spinal cord and cerebral cortex. The substitution pattern on the sugar moiety gave information about the structure−activity relationship. Putative miorelaxant activity for these compounds can be hypothesized based on their demonstrated comparable affinity, relative to the thiocolchicoside, toward the receptors considered above.

Experimental Section

3-*O*-Trifluoromethanesulfonyl-3-*O*-demethylthiocolchicine (3). Operating under a nitrogen atmosphere and stirring at 0 °C, triflic anhydride (1.24 mL, 7.40 mmol) was added to a solution of 3-*O*-demethylthicolchicine **1c** (2 g, 4.98 mmol), *p*-DMAP (1.77 g, 15.78 mmol), and anhydrous CH_2Cl_2 (50 mL). Stirring was continued at 0 °C for 20 h and then at room temperature for 3 h. Reaction progress was monitored by TLC analysis (CH_2Cl_2/MeOH, 10:1; R_f: **1c** = 0.27, **3** = 0.38). After removal of the solvent, the residue was purified by column chromatography (neutral alumina) using mixtures of CH_2Cl_2/MeOH (increasing polarity). Compound **3** (1.84 g, 70%) was obtained as a yellow solid after crystallization from EtOH. Mp 140−142 °C; $[\alpha]^{25}_D$ −60° (*c* 0.9, $CHCl_3$); IR (Nujol) ν_{max} 1667, 1620 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.94 (d, J = 7 Hz, 1H), 7.44 (s, 1H), 7.28, 7.09 (AB system, J = 10.4 Hz, 1H), 6.84 (s, 1H), 4.65−4.55 (m, 1H), 4.05 (s, 3H), 3.68 (s, 3H), 2.63−2.54 (m, 1H), 2.45 (s, 3H), 2.39−2.25 (m, 2H), 1.98 (s, 3H), 1.90−1.80 (m, 2H); ^{13}C NMR (200 MHz, $CDCl_3$) 181.8, 170.4, 160.3, 151.9, 151.0, 145.6, 142.4, 136.9, 135.3, 134.8, 134.7, 128.7, 126.6, 121.2, 116.9, 62.2, 62.0, 36.4, 29.7, 23.3, 15.6.

3-Amino-3-demethoxythiocolchicine (5). An oven-dried Schlenk flask was purged with nitrogen and charged with Cs_2CO_3 (685 mg, 2.09 mmol), benzophenone imine (0.25 mL, 1.49 mmol), palladium acetate (68 mg, 0.29 mmol), (±)BINAP (290 mg, 0.44 mmol), triflate **3** (800 mg, 1.48 mmol), and dry outgassed toluene (3 mL). The flask was capped with a rubber septum and then purged with nitrogen. The reaction mixture was stirred for 45 min at room

temperature and then heated at 120 °C for 16 h. It was monitored by TLC analysis (CH_2Cl_2/MeOH, 20:1). The mixture was cooled to room temperature, diluted with EtOAc, filtered, and concentrated under reduce pressure. The crude material containing the imine adduct **4** was dissolved without purification in MeOH (15 mL) at room temperature, and NaOAc (509 mg, 6.21 mmol) and $NH_2OH\cdot$HCl (323 mg, 4.65 mmol) were added. After 30 min, the mixture was diluted with CH_2Cl_2 and acidified to pH 2 with HCl (2 N). The organic layer was separated, and the aqueous layer was basified with NaOH (25%) and extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to afford compound **5** which was then crystallized from EtOH (373 mg, 70%). TLC: R_f 0.43 (CH_2Cl_2/MeOH, 10:1.5). Mp 280−282 °C; $[\alpha]^{25}_D$ −292° (*c* 0.5, $CHCl_3$); IR (Nujol) ν_{max} 3340, 1667, 1620 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.38 (s, 1H), 7.33, 7.09 (AB system, J = 10.2 Hz, 1H), 6.37 (s, 1H), 3.95 (s, 3H), 3.65 (s, 3H), 2.45 (s, 3H), 2.40−2.24 (m, 2H), 2.00 (s, 3H), 1.89−1.81 (m, 2H); ^{13}C NMR (200 MHz, $CDCl_3$) δ 182.7, 170.2, 157.9, 152.4, 150.9, 141.0, 139.7, 139.5, 135.2, 134.8, 128.7, 127.1, 123.6, 110.8, 61.5, 61.0, 52.6, 36.7, 29.8, 23.1, 15.3.

General Procedure for the Synthesis of *N*-Glycosides 7. Method A. To a solution of **5** (404 mg, 1 mmol) in MeOH (8.5 mL), compound **6a**,**b**,**e** (1 mmol) was added and the mixture was heated at 80 °C for 6 days. The reaction was monitored by TLC analysis (CH_2Cl_2/MeOH, 10:1.5). The solvent was removed under reduced pressure, and the crude material was purified by column chromatography (silica gel; CH_2Cl_2/MeOH, 100:1 to 100:5) and gave two main fractions, corresponding to the unreacted starting material (8−10%) and compound **7** which was crystallized from MeOH/iPr$_2$O. A mixture of anomers α-**7a**/β-**7a** (20: 80, 34%) and α-**7b**/β-**7b** (45:55, 38%), which are not separable, and pure β-**7e** (35%) were isolated. **Method B.** To a solution of **5** (404 mg, 1 mmol) in MeOH (8.5 mL), compound **6** (1 mmol) was added and the mixture was heated in microwave oven for 4 h at 110 °C and 150 W. The reaction was monitored by TLC analysis (CH_2Cl_2/MeOH, 10:1.5): at the end of the period only trace amounts of the starting material were detected. The solvent was removed under reduced pressure, and the crude material was purified by column chromatography (silica gel; CH_2Cl_2/MeOH, 100:1 to 100:5). All attempts to separate anomeric mixtures failed. Compounds **7** (α-**7a**/β-**7a**: 20:80, 58%; α-**7b**/β-**7b**: 45:55, 60%; α-**7c**/β-**7c**: 40:60, 60%; α-**7d**/β-**7d**: 30:70, 55%; α-**7e**: 60%; β-**7f**: 60%; β-**7g**: 58%) were isolated after crystallization from MeOH/iPr$_2$O. Analytical and spectroscopic data are given in Supporting Information.

[^{3}H]Strychnine Binding. The synaptosomal membrane fraction from rat spinal cord was prepared by the reported method, [28] 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 25.7 Ci/mmol), increasing concentrations of either cold strychnine, glycine, or colchicoside derivatives, and membranes at a final protein concentration of 0.2−0.4 mg/1.2 mL. The reaction was carried out at 4 °C for 10 min and was terminated by rapid filtration trough Whatman GF-B glass fiber filters. The filters were rapidly rinsed with 5 mL of NaCl (0.15 M). Nonspecific binding was determined in the presence of 0.1 mM unlabeled strychnine.

[^{3}H]Muscimol Binding. The interaction of thiocolchicoside with $GABA_A$ receptors was tested in a [^{3}H] muscimol binding assay, which was performed according to the procedure of Beaumont.[29] Membranes were obtained by spinal cord−brainstem and cerebral cortex of adult Sprague−Dawley rats as described by Balduini et al. (1999).[30] The binding assay was carried out by incubating membrane aliquots with 5 nM [^{3}H] muscimol and increasing concentrations of either GABA, thiocolchicoside, or the other colchicoside derivatives under investigation, in 50 mM Tris-citrate buffer, pH 7.1, in a final volume of 1 mL. After 30 min incubation at 4 °C, the samples were rapidly filtered through Whatman GF-B glass fiber filters which were then washed three times with 5 mL of ice-cold buffer. Nonspecific binding was determined in the presence of 200 μM 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} values represents the average of three curves obtained from three independent experiments. Results were analyzed by nonlinear fitting using the computer program Prism (GraphPad Software). The *F*-test was used to assess whether the fitting using a two-site model equation was significantly better ($P < 0.05$) than that obtained with a one-site model.

Supporting Information Available: Significant chemical shifts and J values for anomers α-**7** and β-**7** are reported in Table TS1. Spectroscopic data for compounds **7**. Analytical data for compounds **3**, **5**, **7** are reported in Table TS2. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Capraro, H. G.; Brossi, A. *Tropolonic Colchicum Alkaloids. The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1984; vol. 23, p 1.

(2) Boyé, O.; Brossi, A. *Tropolonic Colchicum Alkaloids. The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1992; vol. 41, p 125.

(3) Sun, L.; Hamel, E.; Lin, C. M.; Hastie, S. B.; Pyluck, A.; Lee, K.-H. Antitumor agents. 141. Synthesis and biological evaluation of novel thiocolchicine analogues: *N*-acyl, *N*-aroyl-, and *N*-(substituted benzyl)deacetylthiocolchicines as potent cytotoxic and antimitotic compounds. *J. Med. Chem.* **1993**, *36*, 1474−1479.

(4) Shi, Q.; Verdier-Pinard P.; Brossi, A.; Hamel, E.; McPhail, A. T.; Lee, K.-H. Antitumor Agents. 172. Synthesis and Biological Evaluation of Novel Deacetamidothiocolchicin-7-ols and Ester Analogues as Antitubulin Agents. *J. Med. Chem.* **1997**, *40*, 961−966.

(5) Guan, J.; Zhu, X.-K.; Tachibana, Y.; Bastow, K. F.; Brossi, A.; Hamel, E.; Lee, K.-W. Antitumor Agents. 185. Synthesis and Biological Evaluation of Tridemethylthiocolchicine Analogues as Novel Topoisomerase II Inhibitors. *J. Med. Chem.* **1998**, *41*, 1956−1961.

(6) Gelmi, M. L.; Mottadelli, S.; Pocar, D.; Riva, A.; Bombardelli, E.; De Vincenzo, R.; Scambia, G. *N*-Deacetyl-*N*-aminoacylthiocolchicine Derivatives: Synthesis and Biological Evaluation on MDR−Positive and MDR-Negative Human Cancer Cell Lines. *J. Med. Chem.* **1999**, *42*, 2, 5272−5276.

(7) Danieli, B.; Giardini, A.; Lesma, G.; Passarella, D.; Silvani, A.; Appendino, G.; Noncovich, A.; Fontana, G.; Bombardelli, E.; Sterner, O. Synthesis and biological evaluation of paclitaxel-thiocolchicine hybrids. *Chem., Biodivers.* **2004**, *1*, 327−345.

(8) Kim, M.-H.; Chun, K.; Choi, J.-W.; Joe, B.-Y.; Park, S.-W.; Kim, K. H.; Oh, B.-K.; Choi, J.-H. Preparation of tricyclic *N*-deacetylcolchicine derivatives for use in pharmaceutical compositions as anticancer agents. PCT Int. Appl. PIXXD2 WO 2004113281 A1 20041229. *Chem. Abstr.* **2004**, *142*, 94015.

(9) Tuzun, F.; Unalan, H.; Oner, N.; Ozguzel, H.; Kirazli, Y.; Icagasioglu, A.; Kuran, B.; Tuzun, S.; Basar, G. Multicenter, randomized, double-blinded, placebo-controlled trial of thiocolchicoside in acute low back pain. *Joint, Bone, Spine* (revue du rhumatisme) **2003**, *70*, 356−361.

(10) Janbroers, J. M. Review of the toxicology, pharmacodynamics and pharmacokinetics of thiocolchicoside, a GABA-agonist muscle relaxant with antinflammatory and analgesic action. *Acta Ther.* **1987**, *13*, 221−225.

(11) Balduini, W.; De Angelis, V.; Mazzoni, E.; Depoortere, H.; Cattabeni, F.; Cimino, M. Autoradiographic localization of [3H]thiocolchicoside binding sites in the rat brain and spinal cord. *Neuropharmacology* **2001**, *40* 1044−1049.

(12) Cimino, M.; Marini, P.; Cattabeni, F. Interaction of thiocolchicoside with [3H]strychnine binding sites in rat spinal cord and brainstem. *Eur. J. Pharmacol.* **1996**, *318*, 201−204.

(13) Thenot, J. P.; Espie, P.; Allen, J.; Depoortere, H. Preparation of thiocolchicoside glucuroniate derivative as muscle relaxants. PCT Int. Appl. PIXXD2 WO 9961457 A1 19991202. *Chem. Abstr.* **1999**, *131*, 351618.

(14) Trellu, M.; Filali-Ansary, A.; Françon, D.; Adam, R.; Lluel, P.; Dubruc, C.; Thénot, J. P. New metabolic and pharmacokinetic characteristics of thiocolchicoside and its active metabolite in healthy humans. *Fund. and Clin. Pharm.* **2004**, *18*, 493−501.

(15) Danieli, B.; De Bellis, P.; Corea G.; Riva, S. Regioselective enzyme-mediated acylation of colchicoside and thiocolchicoside. *Gazz. Chim. Ital.*, **1991**, *121*, 123−125.

(16) Clark, D.; Frankmoelle, W.; Houze, J.; Jaen, C.; Medina, J. Preparation of tubulin-binding agents. PCT Int. Appl PIXXD2 WO 2000035865. *Chem. Abstr* **2000**, *133*, 43382.
(17) Yang, B. H.; Buchwald, S. L. Palladium-catalyzed amination of aryl halides and sulfonates. *J. Organomet. Chem.* **1999**, *576*, 125−146.
(18) Louie, J.; Driver, M. S.; Hamann, B. C.; Hartwig, J. F.Palladium-Catalyzed Amination of Aryl Triflates and Importance of Triflate Addition Rate. *J. Org. Chem.* **1997**, *62*, 1268−1273.
(19) Wolfe, J. P.; Ahman, J.; Sadighi, J. P.; Singer, R. A.; Buchwald, S. L.Ammonia Equivalent for the Palladium-Catalyzed Amination of Aryl Halides and Triflates. *Tetrahedron Lett.* **1997** *38*, 6367−6370.
(20) Bognar, R.; Nanasi, P. *N*-Substituted glycosylamines derived from sulfanilamide and *p*-aminosalicylic acid. *J. Chem. Soc.* **1953**, 1703−1708.
(21) Wang, L.; Maniglia, C. A.; Mella, S. L.; Sartorelli, A. C. *N*-(Substituted-phenyl)-D-glycopyranosylamines and their *O*-acetyl derivatives as potential modifiers of the formation of glycosaminoglycans. *J. Med. Chem.* **1983**, *26*, 1323−1326.
(22) Qian, X.; Li, Z.; Liu, Z.; Song, G.; Li, Z. Syntheses and activities as trehalase inhibitors of *N*-arylglycosylamines derived from fluorinated anilines. *Carbohydr. Res.* **2001**, *336*, 79−82.
(23) Das, Y. M.; Rao, C. P.; Kolehmainen, E. Synthesis and characterization of *N*-glycosylamines from the reaction between 4, 6-*O*-benzylidene-D-glucopyranose and substituted aromatic amines and also between 2-(o-aminophenyl)benzimidazole and pentoses or hexoses. *Carbohydr. Res.* **2001**, *334*, 261−269.
(24) Kublashvili, R. *N*-Glucosides of Aminobenzoic Acids and Aminophenols. *Chem. Nat. Compd.* **2003**, *39*, 586−588.
(25) Du, W.; Hu, Y. Synthesis of Fully Protected *N*-Arylglycosylamines and Factors Affecting the Configuration of C1−Substituents of *N*-Arylglycosylamines. *Synth. Commun.* **2004**, *34*, 2987−2992.
(26) Winum, J. Y.; Casini, A.; Mincione, F.; Starnotti, M.; Montero, J. L.; Scozzafava, A.; Supuran, C. Y. Carbonic anhydrase inhibitors: *N*-(p-sulfamoylphenyl)-α-D-glycopyranosylamines as topically acting antiglaucoma agents in hypertensive rabbits. *Biorg. Med. Chem. Lett.* **2004**, *14*, 225−229.
(27) Yamaguchi, M.; Horiguchi, A.; Fukuda, A.; Minami, T. Novel synthesis of aryl 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosides. *J. Chem. Soc., Perkin Trans. 1* **1990**, 1079−1082.
(28) Young, A. B.; Snyder, S. H. Strychnine binding in rat spinal cord membranes associated with the glycine synaptic receptor: cooperativity of glycine interaction. *Mol. Pharmacol.* **1974**, *10*, 790−796.
(29) Beaumont, K.; Chilton, S. W.; Yamamura, H. I.; Enna, S. J. Muscimol binding in rat brain: association with synaptic GABA receptors. *Brain Res.* **1978**, *148*, 153−162.
(30) Balduini, W.; Cimino, M.; Depoortere, H.; Cattabeni, F. Characterization of [3H]thiocolchicoside binding sites in rat spinal cord and cerebral cortex. *Eur. J. Pharmacol.* **1999**, *376*, 149−157.

JM060585T