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Alfred Burger Award Address

Bioactive Alkaloids. 4. Results of Recent Investigations with Colchicine and Physostigmine[†,‡]

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Prologue

After I received the Charles Mentzer Award from the French Society of Medicinal Chemistry in the Fall of 1988 and the Hanuš Medal from the Czechoslovak Chemical Society in April of 1989, I thought I could relax a little and take matters easy. The announcement by the President of the American Chemical Society made in the summer of 1989 that I was chosen to be the recipient of the 1990 Alfred Burger Award in Medicinal Chemistry, which came unexpected, made me realize that there would be no easy going ahead. Nevertheless, the announcement made me feel proud and happy. I felt for the first time that the 38 years of work with Roche (1952–1975) and the NIH (1976–1990) were not wasted. Thank you for giving me this precious feeling and for allowing me to stand here.

Looking back over 38 years tells me that I was very fortunate. With Jeger, Plattner, and Ruzicka I got an excellent education in chemistry. My 23 years with Roche taught me that guiding and implementing research in a pharmaceutical company cannot be done from a desk alone but needs constant interaction with science. Working at the NIH with Witkop supported my belief that good work needs good people, and as a matter of fact is directly proportional to the number of good scientists one works with. Last but by far not least is my family, a great spouse and fantastic children, who let me do my hobby and were happy when I was happy. I thought I had to mention these essential ingredients of my career.

Colchicine

Introduction

Colchicine, present as a major alkaloid in *Colchicum autumnale*, is an old drug used in medicine in acute gout attacks and in Famililal Mediterranean Fever (FMF). Colchicine and demecolcine, a naturally occurring congener, are also effective in chronic myelocytic leukemia, but the therapeutic effects are only observed at toxic or nearly toxic doses. Partially synthetic thiocolchicoside, a sugar derivative of 3-demethylthiocolchicine, is used in France as a long-acting muscle relaxant under the name Colcamyl (Figure 1).

Colchicine inhibits cellular mitosis, which disrupts the formation of microtubules, resulting in dissolution of the microtubule network.[1] This probably accounts for most of the therapeutic properties of colchicine and its analogues. The effect on tubulin can be assessed in vitro by measuring inhibition of tubulin polymerization and binding of radiolabeled colchicine to tubulin.[2] Although no clear relationship between antitubulin effect and antitumor activity exists, all colchicinoids found active in vivo did show good antitubulin activity.[3,4]

Only a few colchicinoids had been evaluated for antitubulin activity when we started our program in 1978. This lack of information, together with reports that colchicine was found to be clinically effective in patients with liver cirrhosis,[5] stimulted our interest in this old alkaloid and prompted us to have a new look at it. Ultimately, this

[†] This is the text of the Alfred Burger Award Lecture which was delivered by Dr. Arnold Brossi at the 199th National Meeting of the American Chemical Society in Boston, MA, on April 25, 1990.

[‡] Lecture 3 was given at the Fifth International Conference on Chemistry and Biotechnology of Biologically Active Natural Products in Varna, Bulgaria, Sept 18–23, 1989.

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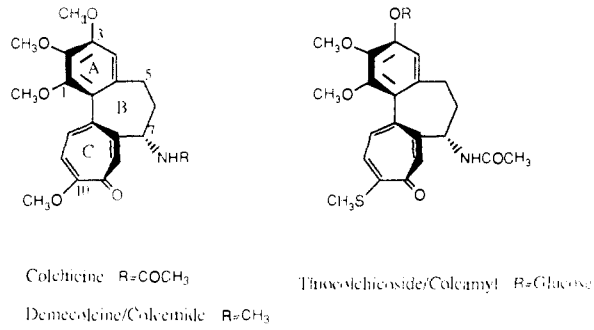

Figure 1.

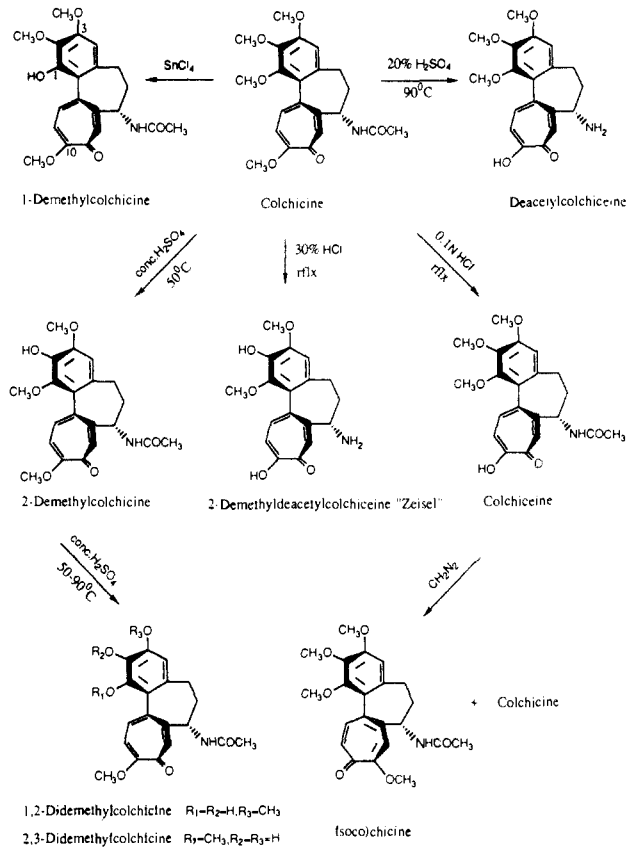

Figure 2.

research is directed to a better characterization of the colchicine binding site on tubulin and to a better understanding of the mechanism by which colchicine and other spindle toxins bind to tubulin.

The tropolonic structure of colchicine, proposed by Dewar in 1945,[6] is in full agreement with results obtained by its chemical degradation and is supported by several total syntheses which were reviewed.[7] The reviews also discuss structural modifications of colchicine reported by Šantavý, Lettré, and Velluz and their collaborators, and the important contribution made by Corrodi and Hardegger in converting natural (−)-colchicine into its unnatural (+)-antipode via racemic colchicine.[8] In planning

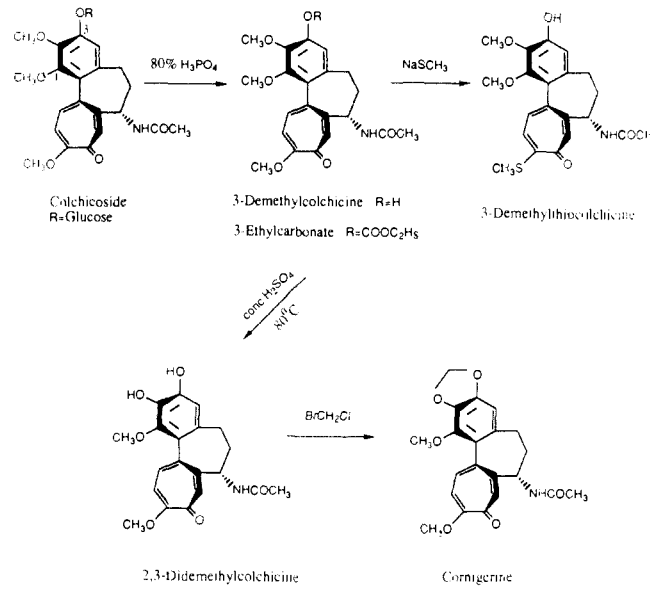

Figure 3.

our work, we decided first to have a closer look at the importance of the methoxy groups present in rings A and C of the alkaloid and the acetamido group in ring B, and how their modification would affect binding to tubulin. To make this report up-to-date I also include results reported by other investigators.

Modifications of Rings A, B, C

It is possible, as shown in Figure 2, to cleave, regioselectively, three of the four methoxy groups in colchicine [C(1), C(2), and C(10)], leaving the acetamido group at C(7) intact. Hydrolysis of the methoxy group at C(1) with stannic tetrachloride in acetic anhydride followed by hydrolysis of the acetate was accomplished by Bladé-Font. Hydrolysis of colchicine with strong aqueous acid also cleaves the acetamido group to afford deacetylcolchiceine (TMCA) and its 2-demethyl congener already obtained by Zeisel in 1888.[9] Hydrolysis of the methoxyl at C(10) is a disadvantage since O-methylation of colchiceine affords a mixture of natural and iso isomers which require separation. This complication does not exist in the thio series (not shown) where the OCH_3 group at C(10) is replaced by a SCH_3 group, thus stabilizing the tropolonic moiety toward acid hydrolysis. Treatment of colchicine with concentrated sulfuric acid at 50 °C affords 2-demethylcolchicine and results in a mixture of 1,2-didemethylcolchicine and its 2,3-didemethyl congener when the temperature is raised to 60–90 °C.[10]

The only monophenol which cannot be obtained by regioselective ether cleavage of colchicine is the naturally occurring 3-demethylcolchicine. This phenol is a microbial metabolite, but this bioconversion has not yet been perfected to a point where it could be considered to be practical.[11] The preparation of larger quantities of this phenol rests, at the moment, entirely on the availability of colchicoside, a sugar alkaloid present in *Colchicum autumnale* and in *Gloriosa superba*,[12] and given to us over the years by Dr. V. Šimánek.[13]

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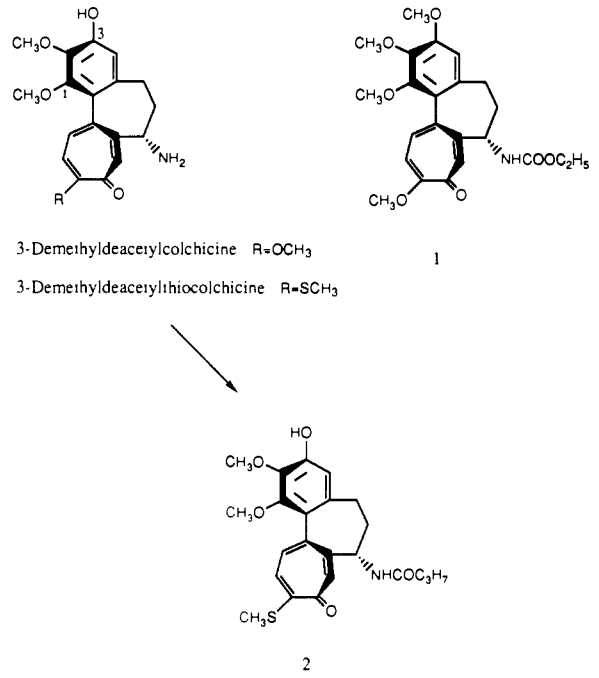

Figure 4.

Colchicoside, as shown in Figure 3, can conveniently be converted into 3-demethylcolchicine. The latter compound was used to prepare thioether analogues, catecholic congeners, and cornigerine. Of the three monophenols only 3-demethylcolchicine shows good antitubulin effects. These effects are considerably lower in the 2-demethyl congener and are practically lost in 1-demethylcolchicine.[4] Cornigerine resembles colchicine in its interactions with tubulin in vitro.[14]

Modification of the amide group in colchicine can be accomplished with deacetylcolchicine, obtained from TMCA after O-methylation with diazomethane and separation of the iso isomer.[15] In applying chemical methodology already discussed, we have succeeded in making several potent spindle toxins. Two of these are shown in Figure 4. Both compounds, the ethyl carbamate of deacetylcolchicine (1) and 3-demethyl-*N*-butyryldeacetylthiocolchicine (2), are potent antitubulin compounds.[2]

Further elaboration of the amide group in colchicine is shown in Figure 5 with a practical synthesis of demecolcine[16] and a synthesis of the alkaloid 2-demethylspeciosine also named speciocolchine.[17] The NH in the trifluoroacetamide of deacetylcolchiceine is sufficiently acidic to allow N-methylation and affords, after O-methylation with diazomethane, separation of isomers and mild hydrolysis of the natural isomer demecolcine (3). Selective ether cleavage of 3 with concentrated sulfuric acid at 60 °C affords 2-demethyldemecolcine[18] and 2-demethylspeciosine (4) on reaction of the former compound with 2-acetoxybenzyl bromide and mild hydrolysis of the acetate. This

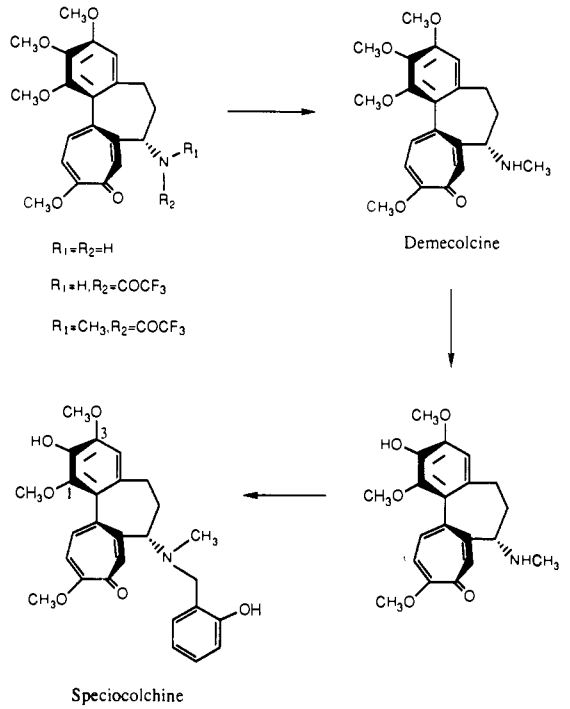

Figure 5.

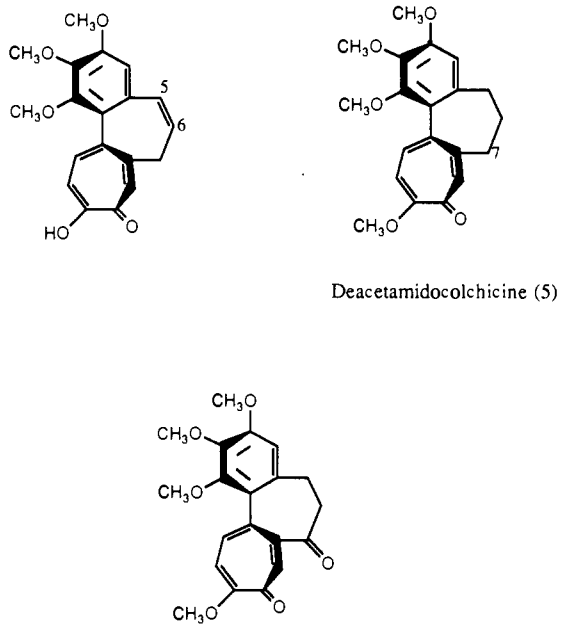

Figure 6.

compound was found identical with the natural alkaloid.

The importance of the acetamido group at C(7) in colchicine for binding to tubulin has been assessed by testing the compounds shown in Figure 6. Deacetamidocolchicine (5), prepared from deacetylcolchiceine by exhaustive methylation, followed by Hofmann degradation, catalytic reduction of olefins, O-methylation, and separation of isomers,[19,20] is fully active in antitubulin assays in vitro.[21]

(13) We would like to thank Prof. V. Šimánek from the Institute of Medicinal Chemistry, Medical Faculty, Palackŷ University, Olomouc 775 15, Czechoslovakia, for having supplied us over the years with substantial amounts of this precious alkaloid.

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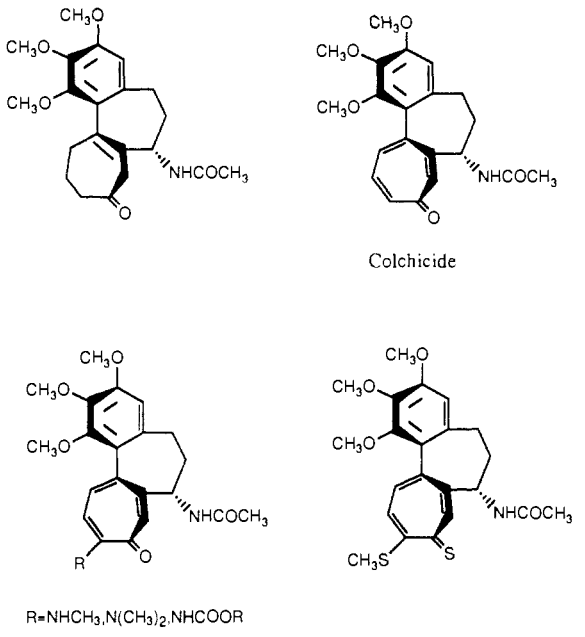

Figure 7.

Substantial activity is maintained in 7-oxodeacetamidocolchicine (**6**), found as a microbial metabolite,[11,22] and prepared by synthesis.[23] Ketone **6** represents an interesting compound for further investigations.

Modification of the tropolonic ring C in colchicine has resulted in several compounds which are shown in Figure 7. Catalytic reduction of colchicine affords a tetrahydro compound as a major product which is inactive in the antitubulin assays.[24] Elimination of the methoxy group at C(10) can be accomplished by Raney Ni catalyzed desulfurization of thiocolchicine (R = SCH_3) to give colchicide, which is not very active.[25] Biologically active compounds are obtained by replacing the OCH_3 group at C(10) with amines[26] and amino acid esters.[27] The activity of these compounds lies between that of the isosteres with OCH_3 and SCH_3 substituents, with the latter being the most potent.[2]

Thioketones, obtained from thiocolchicine with Lawesson's reagent, are highly potent cytotoxic agents.[2] Their relative instability and the fact that they are amorphous and highly mutarotating in solution make them less attractive for a pharmacological evaluation.

Absolute Configuration of Natural Colchicinoids and Derived Allo Compounds

Modification of substituents in colchicine and derived allo compounds with a benzenoid ring C showed the methoxyls at C(1), C(2), and C(10) in colchicine and at C(9) in allo compounds to be important for binding to tubulin since their conversion into phenols, or their replacement by hydrogen, afforded much less potent compounds.[28]

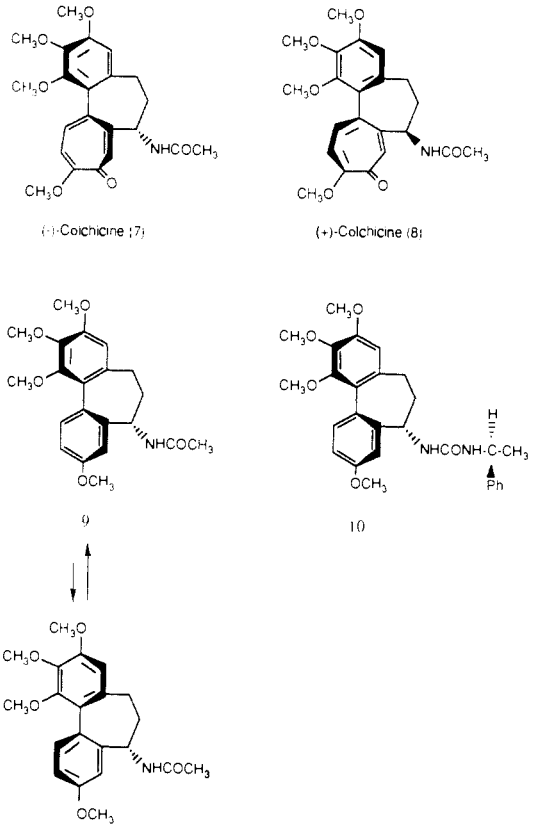

Figure 8.

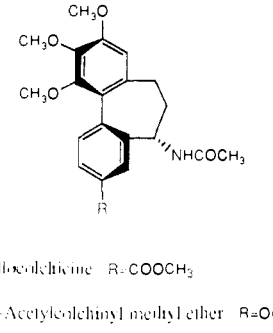

Figure 9.

A most important issue concerns atropisomerism of bridged and skewed phenyltropolones and biphenyls, represented by colchicine and allocolchicine, respectively. ORD studies by Šantavý pointed out that these molecules exhibit a strong negative Cotton effect at 260 nm, which implies that they have an axial *aS* configuration.[29] This is confirmed by an X-ray analysis of an analogue of the thio series.[30] The conclusion that (−)-colchicine is represented by structure **7** and (+)-colchicine by structure **8** (Figure 8) has now been substantiated by additional data. The optical rotation of **8** does not change in the presence of tubulin, but the negative rotation of **7** is markedly lowered. In addition, deacetamidocolchicine, which lacks chirality at C(7), has a positive rotation when measured in the presence of tubulin. It can be concluded that only

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(28) Muzaffar, A.; Brossi, A.; Hamel, E. *J. Nat. Prod.* 1990, in press. 1-Demethylcolchicine was inactive in antitubulin assays,[4] and 2-demethylcolchicinoids have constantly shown lower potency than the fully methylated compounds.
(29) Hrebek, J.; Jennings, J. P.; Klyne, W.; Šantavý, F. *Collect. Czech. Chem. Commun.* **1964**, *29*, 2822.
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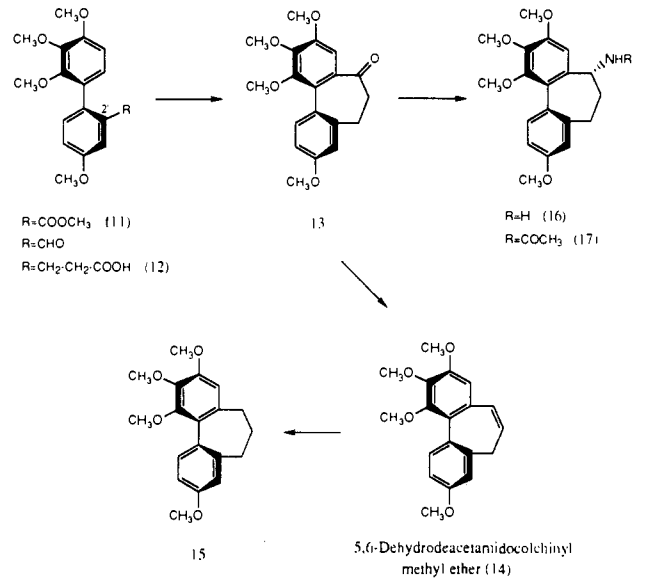

Figure 10.

colchicinoids with an *aS* configuration bind to tubulin.[31] This also is the case for the potent allo compound **9** (R = $COCH_3$). All these compounds show strong negative Cotton effects at 260 nm and that of (+)-colchicine (**8**) is strongly positive as expected.[32] *N*-Acetylcolchinyl methyl ether (**9**) isomerizes in chloroform solution to a mixture of **9** and its *aR*, 7*s* epimer **9A**. This is clearly visible in the CD spectrum with a drop of the strong negative rotational value at 260 nm to a lesser negative value and by chemical shifts of H-C(7) observed in the ^1H NMR. The X-ray analysis of urea **10**, prepared from deacetylcolchinyl methyl ether and (*R*)-(+)-phenylethyl isocyanate, has the a*S*,7*S*,15*R* configuration.[32]

Allo Compounds of the Colchicine Series

Treatment of colchicine and isocolchicine with sodium methoxide in methanol affords allocolchicine by ring contraction.[33] *N*-Acetylcolchinyl methyl ether is obtained by ring contraction with alkaline oxidizing agents.[34,35] Both compounds, shown in Figure 9, are potent inhibitors of tubulin polymerization.[36,37] Although not very active as antitumor agents in experimental animals, the inhibition data obtained in vitro with allo compounds attest that the seven-membered tropolonic ring C in colchicine can be converted into a six-membered ring without loss in antitubulin activity. We have, for this reason, further explored allo compounds which are synthetically more accessible than tropolonic analogues. A novel synthesis of allo structures shown in Figure 10, starts from the readily accessible biphenyl ester **11**.[37] Lengthening of the side chain at C(2′) affords propionic acid **12** and the important ketone **13** on reaction of **12** with trifluoroacetic anhydride in the presence of trifluoroacetic acid. Ketone **13** affords, after reduction and dehydration, dehydro compound **14** converted by catalytic reduction into desaminocolchinyl methyl ether **15** already prepared by Loudon and Cook.[38] Ketone **13** also served to prepare the optically active amine **16** of the 5-substituted series and its *N*-acetyl derivative **17**.[39] The *aS*-configurated acetamides equilibrate in chloroform, and to a much lesser extent in polar solvents, to afford diastereomers of the *aR* series of biphenyls. This is signaled in the CD spectrum of **17**, which shows a much less intense Cotton effect at 260 nm due to formation of the opposite configurated biphenyl. Equilibration of **17** and the 7-substituted isomer **9** discussed earlier also can be measured by ^1H NMR.[39] It is interesting to note that equilibration does not occur with the amine **16** or its salts and therefore rests on the presence of an acyl group on the nitrogen atom. The 5-substituted amine **16** and the acetamide **17** do not inhibit tubulin polymerization, suggesting that the area covered by the substituent at C(5) interferes with binding.

Potential Markers of the Colchicine Binding Site on Tubulin

Data reported support the notion that it is not clearly established whether colchicine binds to the α- or β-subunit of tubulin.[40,41] There is an obvious need to prepare other labeled analogues of colchicine which would interact more specifically with the colchicine binding site either by formation of a drug–tubulin complex or by covalently interacting with prosthetic groups of the receptor molecule, allowing to establish the locus of interaction after sequential amino acid analysis. Colchicine and thiocolchicine ($R^3 = SCH_3$) with appropriate labels at R^1 and R^2, chosen to mark the binding site, are shown in Figure 11 with compounds **18–20**.

Biologically active amides labeled with deuterium or tritium in the NH-acyl group are represented by the deuterated butyryl amide **18**, prepared from deacetylcolchicine with deuterated butyric acid made from crotonic acid.[42] Isothiocyanates of biologically active molecules have successfully been used by Rice and his colleagues to map subunits of the opiate receptor.[43]

We have prepared isothiocyanate **19** with a ^{14}C label at the C(2)-OCH_3 from labeled thiocolchicine by hydrolysis of the amide group and reaction of the amine with thiophosgene.[43] Structure **19** is fully established by spectral data and by its reaction with ethylamine, which afforded a crystalline thiourea.

We also have substituted the amino group in deacetylcolchicine with a dihydrofluoresceyl group as shown in the DADF amide **20**. This amide, when chromatographed on TLC plates and then exposed to ammonia and iodine vapors, gives red dyes of erythrosine-based structure which are highly visible by the their UV maxima at 546 nm.[44] Unfortunately **20** does not inhibit tubulin polymerization in vitro.

We were unable to prepare isothiocyanates at C(10) of colchicine. Reaction of colchiceinamide with thiophosgene

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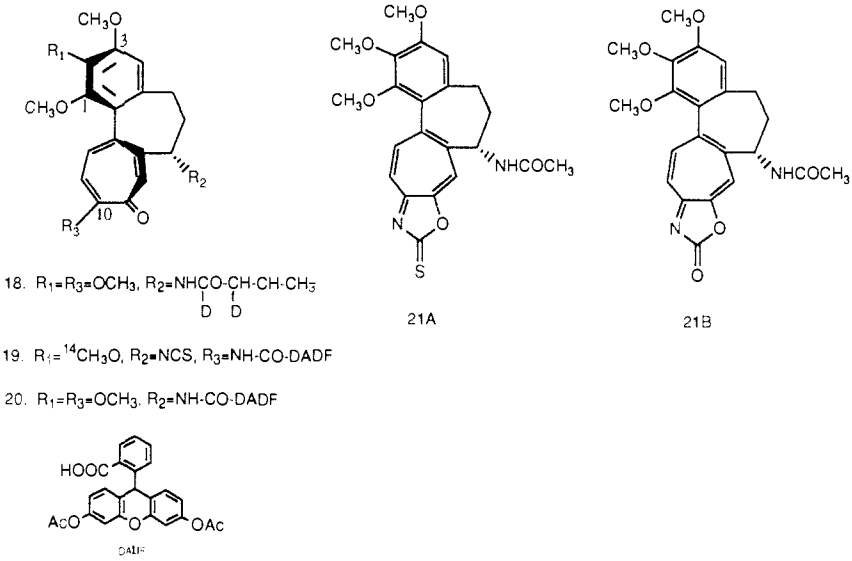


Figure 11.

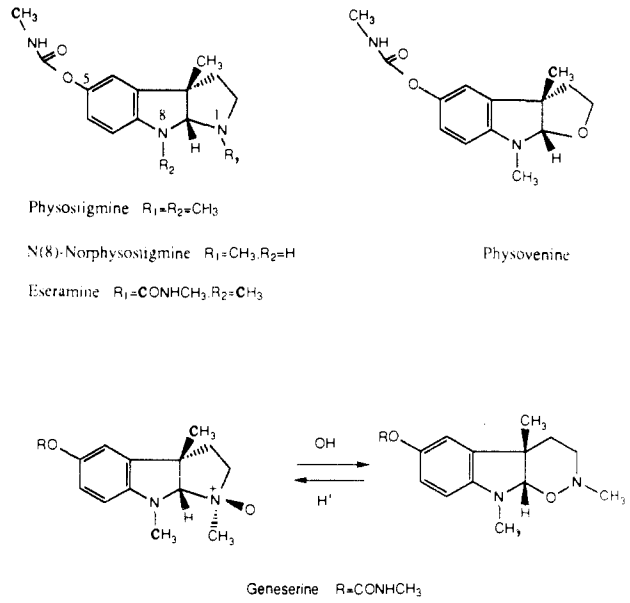
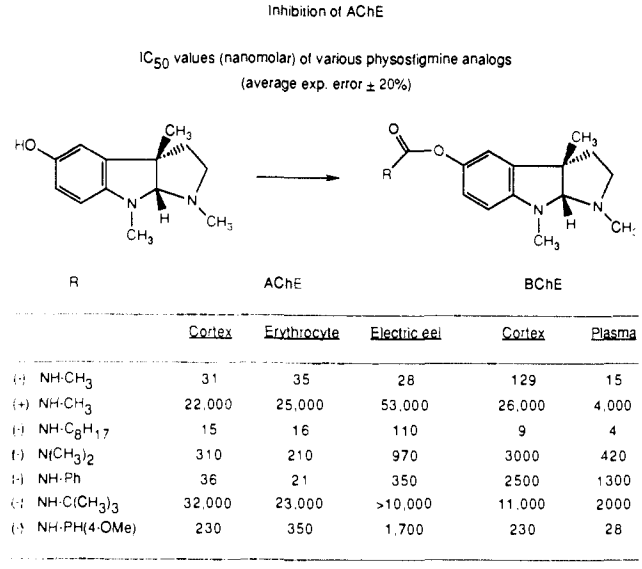


Figure 12.



	Cortex	Erythrocyte	Electric eel	Cortex	Plasma
(-) NH-CH_3	31	35	28	129	15
(+) NH-CH_3	22,000	25,000	53,000	26,000	4,000
(-) NH-C_8H_{17}	15	16	110	9	4
(-) $N(CH_3)_2$	310	210	970	3000	420
(-) NH-Ph	36	21	350	2500	1300
(-) NH-$C(CH_3)_3$	32,000	23,000	>10,000	11,000	2000
(-) NH-PH(4-OMe)	230	350	1,700	230	28

Figure 13.

afforded oxazolethione **21A**, and with phosgene, oxazolone **21B** was similarly obtained. We have so far been unable to design useful markers to elucidate the colchicine binding site on tubulin.

Physostigmine

Introduction

Seeds of the African vine *Physostigma venenosum* (Calabar beans) contain a number of alkaloids which are shown in Figure 12. The most important of the alkaloids is (-)-physostigmine (Phy), which is used medically for treating glaucoma, and in combination with atropine as an antidote in organophosphate poisoning. It was recently reported that Phy, when given in larger doses over an extended period of time, may be beneficial in Alzheimer's disease.[45] Research on Phy done in France after World War I by Polonovski, and important contributions made later by Robinson in Manchester, assured the absolute configuration of the alkaloids and that of Phy and is secured by an X-ray analysis.[46] The chemistry of Phy and that of its congeners has been reviewed repeatedly.[47a-e] Introduction of the synthetic drugs neostigmine and pyridostigmine into medicine greatly diminished the medical use of the relatively toxic Phy for the treatment of cholinergic disorders.

In realizing that relatively little chemistry was reported on structural modification of Phy, we decided in 1984 to change this situation. Modifying the carbamate group in Phy and improving the Julian total synthesis of Phy[48] were our immediate objectives.[49] Some of these efforts collided with similar intentions pursued by Italian investigators. This led to some controversy,[50] a natural phenomenon in an exciting field of international research activity. Reports that (-)-eseroline (ES), the phenolic metabolite of Phy, has

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(47) (a) Marion, L. *Alkaloids* **1952**, *2*, 438. (b) Coxworth, E. *Ibid.* **1965**, *8*, 27. (c) Robinson, B. *Ibid.* **1967**, *10*, 383. (d) Robinson, B. *Ibid.* **1971**, *13*, 213. (e) Takano, S.; Ogasawara, K. *Ibid.* **1989**, *36*, 225.
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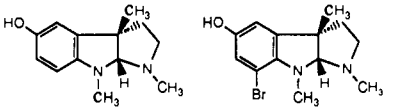
(50) Pomponi, M. *FEBS Lett.* **1989**, *252*, 158 and response by Brossi, A. *Ibid.* **1989**, *252*, 159.

Analgesic Activity of Eserolines



	Morphine	(-)-Eseroline	(-)-7-Bromo-eseroline
Hot plate assay[a]	1.3	1.6	N.T.
Tail-flick assay[a]	5.8	2.3	0.54
Writhing test[a]	0.23	0.7	0.18
Inhibition of binding to opiate receptors[b]			
IC_{50} μ / δ / χ	1:45:150		1:5:20

[a] sc, mg/kg; [b] K_i, nM.

Figure 14.

potent morphine-like analgesic effects[51,52] added additional momentum to our plan.

Modification of the Carbamate Side Chain

Preparation of ES from Phy by acid or alkaline hydrolysis in the presence of water is accompanied by formation of the red pigment rubreserine which is fully characterized by an X-ray analysis.[52] Alcoholysis of Phy in refluxing butanol in the presence of sodium butoxide, with isolation of ES as a fumarate salt, represents a vastly improved method for making this phenol.[53] Reaction of ES with isocyanates afforded carbamate analogues of Phy which were evaluated for inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) from various tissues in vitro.[54] The most interesting carbamates are shown in Figure 13. Although highly potent in vitro, and often more potent against BChE than Phy, it has to be demonstrated by additional pharmacological screening whether these carbamates are superior to Phy and whether they give measurable blood levels of drug or metabolites at therapeutic doses. Naturally the toxicity of these carbamates also has to be evaluated for an assessment.

Analgesic Eserolines

Before leaving the compounds which can be obtained form natural physostigmine, it is worth summarizing the analgesic properties of ES reported by Galli and Bartolini and co-workers in 1979[51a-c] and later verified in our Laboratory.[52] These data are shown in Figure 14. This evaluation includes the 7-bromo analogues of ES, developed at Hoechst-Roussel in Sommerville and named HP-736.[55]

HP-736 is a potent morphine-like analgesic. Its agonist actions are μ-receptor mediated, but it may have antagonist actions at the χ- and δ-receptors as well. The duration of action of HP-736 in mice is about half of that of morphine.

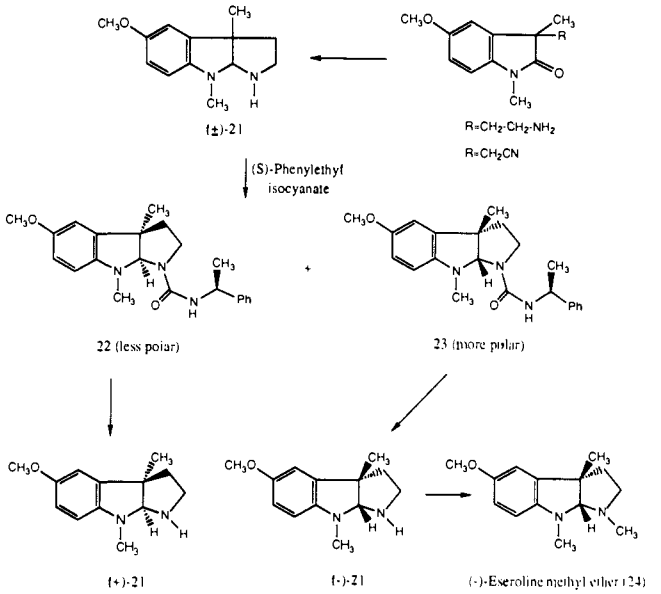


Figure 15.

(+)-Physostigmine and Carbamate Analogs

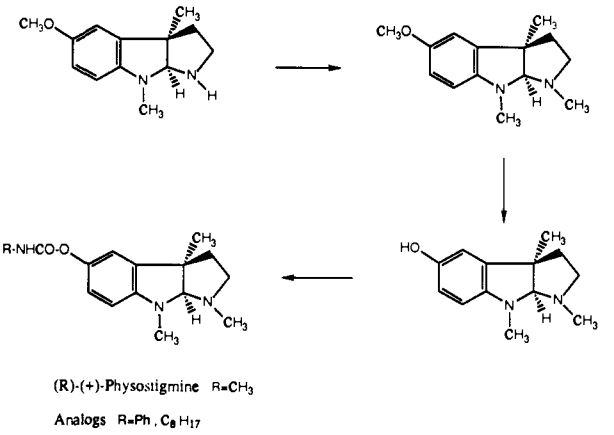


Figure 16.

The finding of potent analgesics among hexahydropyrroloindoles will undoubtedly stimulate the development of analgesics and analgesic antagonists in the indole alkaloid series, up to now almost exclusively a domain of isoquinoline alkaloids.

Efficient Synthesis of Optically Active Alkaloids

Chemical modification of the methyl group at N(1) of Phy and improving the optical resolution[57] required a reexamination of the Julian synthesis. The successful outcome is shown in Figure 15. Racemic *N*(1)-noreseroline methyl ether (NEM), obtained by reduction of the Julian amine with sodium in ethanol[48] or by direct reduction of the Julian nitrile with lithium aluminum hydride in tetrahydrofuran,[53] was resolved by the phenylethyl urea method.[58]

This is accomplished by reacting (±)-NEM with optically active 1-phenylethyl isocyanate, chromatographic

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(56) The data on ES and HP-736 presented in Figure 14 were obtained from Dr. H. P. Wolf, E. Merck, Darmstadt, West Germany, and from Dr. A. E. Jacobson, Chairman, Drug Testing Committee on Problems of Drug Dependence, NIH.

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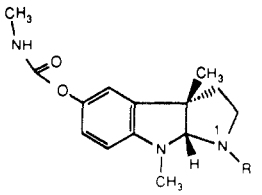
Inhibition of AChE

IC_{50} values (nanomolar) of various N(1)-substituted analogs of (-)-Physostigmine



R	AChE			BChE	
	Cortex	Erythrocyte	Electric eel	Cortex	Plasma
CH_3	31	35	28	129	15
H	23	21	57	35	3
CH_2-CH=CH_2	32	45	69	16	3
CH_2-CH_2-Ph	150	220	1,000	7	2
CH_2-Ph	190	330	1,000	55	10

Figure 17.

separation of urea diastereomers, and alcoholysis of the ureas in refluxing butanol in the presence of sodium butoxide[59] (Figure 15). To explore the unnatural (+) series and to prepare larger quantities of (+)-Phy already made by Robinson[60] required the less polar urea of mp 124–125 °C. This was converted into (+)-Phy as shown in Figure 16. Our experiences with the urea method which has been applied successfully in other series of alkaloids as well shows that pure ureas give pure amines, and they give both optical isomers at the same time.

Although (+)-physostigmine has little effect on AChE in vitro,[54,60] it is a weak, centrally acting cholinergic agonist. Unlike natural Phy, fully classic manifestations of muscarinic and nicotinic toxicity were not evident following iv doses of (+)-Phy as high as 12 mg/kg,[61] but (+)-Phy prevented organophosphate-induced damage at the neuromuscular synapse by a mechanism not related to cholinesterase carbamoylation.[62] It may depend on a direct blockade at the nicotinic acetylcholine receptor and its ion channel.[63] Whether (+)-Phy in combination with atropine could be useful in treating organophosphate poisoning remains to be seen.

Benzylation of natural NEM of the natural series affords *N*(1)-benzylnorphysostigmine by chemistry already discussed and *N*(1)-norphysostigmine on catalytic reduction over PdOH/C catalyst.[64] Alkylation of th nor compound with phenethyl bromide and allyl bromide afforded the corresponding analogues. Their activities as inhibitors of AChE and CHhE in vitro are shown in Figure 17. The nor compounds, including the potential metabolite *N*-(1)-norphysostigmine, represent interesting compounds with high potency. *N*(1)-Norphysostigmine also is useful for preparing analgesic antagonists of the ES series by

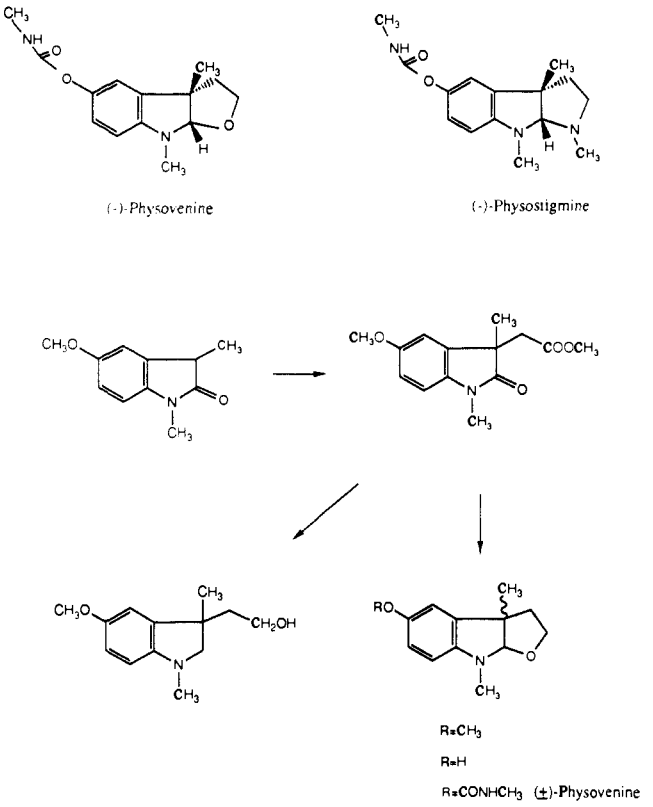


Figure 18.

avoiding acidic reaction conditions.

Minor Alkaloids

The improvements made in the synthesis of Phy suggested a simpler approach to physovenine, another alkaloid found in Calabar beans.[47c,e] This was accomplished from the oxindole of the Julian synthesis as shown in Figure 18 by C-alkylation with ethyl bromoacetate and reduction of the ester with the methyl ether group.[65] Natural physovenine could not be obtained by this route and was prepared from Phy as reported by Robinson.[60] The comparison of (−)-physovenine with the racemic mixture in assays measuring inhibition of AChE in vitro supports the data reported by Robinson and shows that (−)-physovenine is a potent inhibitor of AChE.

Racemic physovenol prepared from the carbamate by alcoholysis did not show analgesic properties, but this has to be repeated with the phenol of natural configuration.

A third Calabar alkaloid investigated is geneserine, originally thought by Polonovski to be the *N*-oxide of Phy,[66] but the structure was later changed to that of an oxazine.[67] In repeating the oxidation of Phy with peracid we found that salts of geneseroline and geneserine had *N*-oxide structures, but that the free bases were the expected oxazines (Figure 11).[68] Geneserine itself has no inhibitory effect on AChE.[69]

Efforts to prepare the fourth Calabar alkaloid, *N*(8)-norphysostigmine, following a route developed for Phy by Speckamp,[70] has not yet afforded the desired alkaloid.

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This investigation is continuing despite an elegant total synthesis of (−)-*N*(8)-norphysostigmine just published by Japanese scientists.[71]

Conclusion

Analogues of colchicine (ethyl carbamate) and thiocolchicine (3-demethylthiocolchicine) which show interesting biological properties have to await further pharmacological and toxicological evaluation to establish their potential clinical usefulness.

The finding, that natural colchicinoids and derived allo congeners bind to tubulin as *aS*-configurated biaryls, will greatly help in further study of elucidating the mechanism by which they bind to the colchicine binding site on the protein. Systematic efforts to structurally modify compounds of the allo series paid off, since it clearly showed that the methoxy groups at C(1), C(2), and C(9) are required for the binding to tubulin and shifting the acetamido group from C(7) to C(5) afforded an inactive compound.

With efficient synthesis of Calabar alkaloids leading to both optical isomers on hand, it now is up to medicinal chemists to make further molecular changes, which hopefully may lead to clinically useful analgesics and cholinergic agents. Further pharmacological evaluation of (+)-physostigmine and of (−)-*N*(1)-norphysostigmine, which emerged as interesting compounds, is indicated. In both series of alkaloids discussed, the colchicines and the physostigmines, optical resolution of racemic mixtures and testing optically active isomers instead of racemic mixtures, was pivotal for obtaining useful information.

Acknowledgment. The research presented here could not have been accomplished without the input made by many devoted and hard working scientists. This, in the case of colchicine, included the following Ph.D's: H. G. Capraro, M. A. Iorio, M. Rösner, P. N. Sharma, P. Kerekes, R. Dumont, M. Chrzanowska, A. Muzaffar, Y. Itoh, and O. Boyé from our Laboratory, and C. F. Chignell, E. Hamel, H. I. C. Yeh, J. V. Silverton, G. Clifford, and F. Quinn from intramural collaborations. In the case of physostigmine they were Q. S. Yu, L. A. Chrisey, V. Pabuccuoglu, and M. Brzostowska from our Laboratory, and J. R. Atack, W. X. Albuquerque, and J. L. Flippen-Anderson from extramural collaboration. The interest of B. Witkop and of N. J. Leonard, who spent a year at the NIH as a Fogarty Scholar, in both topics and their many helpful discussions is gratefully acknowledged. I also would like to thank J. Peterson-Mark for editing and typing the manuscript.

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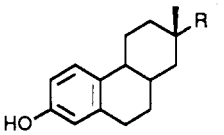
Communications to the Editor

Trifluoromethylacetylenic Alcohols as Affinity Labels: Inactivation of Estradiol Dehydrogenase by a Trifluoromethylacetylenic Secoestradiol

Estradiol dehydrogenase (EC 1.1.1.62) is a pyridine-nucleotide-dependent enzyme that interconverts estradiol and estrone.[1] Because estradiol is more potent than estrone, this enzyme may serve to modulate estrogenic potency in vivo. Also, the role of the enzyme in reproductive endocrinology and in estrogen-dependent neoplasms is of widespread interest.[2-4] Consequently, we have been involved in the development of inhibitors of estradiol dehydrogenase.

We have shown previously that acetylenic secoestradiol **1** is a mechanism-based inactivator of estradiol dehydrogenase.[5] Enzymatic oxidation of **1** (K_m = 79 μM) leads to ketone **2**, a Michael acceptor, which covalently modifies and inactivates the enzyme ($K_i$app = 2.8 μM, limiting $t_{1/2}$ = 12 min).[5-7] Our interest in developing other irreversible inhibitors led us to prepare and evaluate trifluoromethylacetylenic alcohol **3** as an inactivator of estradiol dehydrogenase. We report here that, in contrast to acetylenic alcohol **1** which is a substrate for estradiol dehydrogenase, trifluoromethylacetylenic alcohol **3** is an affinity label. This is the first report in which the (trifluoromethyl)acetylene group has been utlized as an enzyme affinity labeling group.



1: R = (17*S*)-CH(OH)C≡CH
2: R = C(=O)C≡CH
3: R = CH(OH)C≡CCF$_3$
4: R = CHO

Trifluoromethylacetylenic alcohol **3** was synthesized from optically pure secoaldehyde **4**[8] by reaction with lithium (trifluoromethyl)acetylide in 75% yield as a diastereomeric mixture (^{19}F NMR: δ = −50.74, s (broad)).[9] For enzymology, a small sample of the mixture was separated by HPLC, and diastereomers **3a** and **3b** were obtained as noncrystalline solids.[10] The absolute stereochemistry at C-17 in **3a** and **3b** has not been determined.

The diastereomers were evaluated separately as time-dependent inactivators of estradiol dehydrogenase.[11] The

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(8) Auchus, R. J.; Palmer, J. O.; Carrell, H. L.; Covey, D. F. *Steroids* **1989**, *53*, 77.
(9) Satisfactory electron-impact high-resolution mass spectroscopic data as well as NMR (^{1}H, ^{19}F) and IR spectroscopic data have been obtained. ^{19}F NMR values (δ) are reported relative to $CFCl_3$ (δ = 0) as internal standard.
(10) Diastereomers were separated as foams by high-performance liquid chromatography (HPLC) using three tandem Alltech (#60085) 5-μm silica cartridge columns (250 mm × 4.6 mm). Ethyl acetate (15%) in hexanes was used as eluent at a flow rate of 2 mL/min. Retention times for **3a** and **3b** were 25.5 and 26.5 min, respectively.

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