Pergamon

Tetrahedron Vol. 50, No. 29, pp. 8699-8710, 1994

0040-4020/94 $7.00+0.00

0040-4020(94)00467-6

The Synthesis of Novel GABA Uptake Inhibitors. Part 2. Synthesis of 5-Hydroxytiagabine, a Human Metabolite of the GABA Reuptake Inhibitor Tiagabine

Knud E. Andersen [a], Mikael Begtrup [b], Mukund S. Chorghade [c], Elaine C. Lee [c], Jesper Lau [a], Behrend F. Lundt [a], Hans Petersen [a], Per O. Sørensen [a]*, and Henning Thøgersen [a]

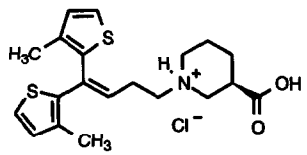
[a] Novo Nordisk A/S, Novo-Nordisk Park, DK-2760 Måløv Denmark.

[b] Department of Organic Chemistry, The Royal Danish School of Pharmacy, Universitetsparken 2 DK-2100 Copenhagen Denmark.

[c] Department 54P, Abbott Laboratories, Abbott Park, Illinois 60064.

Abstract: (*R*)-1-(4-(2,5-Dihydro-3-methyl-5-oxothien-2-ylidene)-4-(3-methyl-2-thienyl)butyl)-3-piperidinecarboxylic acid (5-hydroxytiagabine) **13**, has been prepared in 8 steps from 2-bromo-3-methylthiophene **3**. Key steps are Grignard reactions, displacement of heteroaromatic chlorine with methoxy, and simultaneously demethylation and opening of a hydroxymethylcyclopropane with bromotrimethylsilane. An alternative approach involving acylation of 2-lithio-3-methylthiophene **17a** was found less satisfying. A metalloporphyrin assisted hydroxylation of tiagabine **1** also yielded the target metabolite. The structure of 5-hydroxytiagabine was confirmed by NMR-data including COSY, ROESY, HMQC and HMBC experiments.

Tiagabine, (*R*)-1-(4,4-bis(3-methyl-2-thienyl)-3-butenyl)-3-piperidinecarboxylic acid hydrochloride **1** [1] (Fig. 1), acts centrally as an anticonvulsant by selectively inhibiting both glial or neuronal reuptake of γ-amino

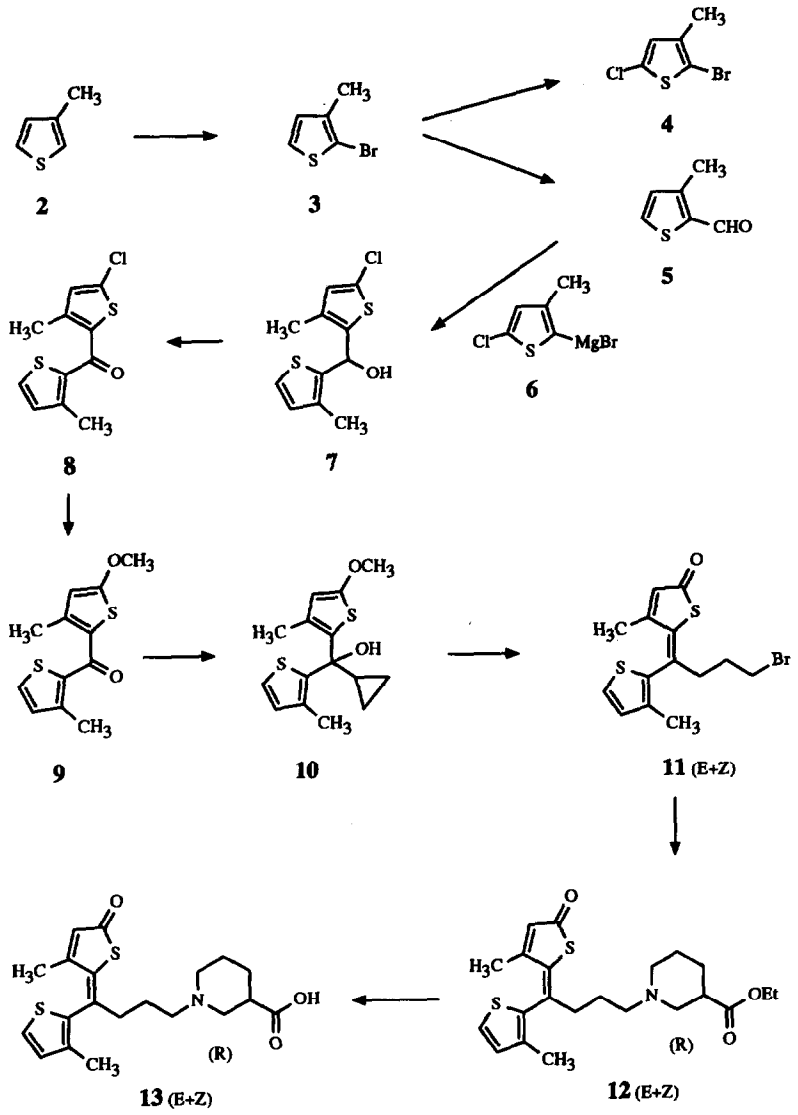


1

Fig. 1. Tiagabine

butyric acid (GABA), a mechanism of action which recently has gained proof of concept.[2] Tiagabine has progressed to phase III human clinical trials and is expected to be used in the treatment of epilepsy. After oral or intravenous administration of ^{14}C-tiagabine (30 mg/kg) to rats, the metabolic profile in urine was characterized by two major peaks which together accounted for about 90 % of the urinary radioactivity or 10-15 % of the ^{14}C-

dose. Upon isolation, each peak was shown to convert to the other and eventually equilibrate to an approximate 1:1 mixture. Identical protonated molecular ions (m/z=392) and daughter ion fragmentation patterns suggested thiophene ring oxidation (+16 amu). The ^{1}H NMR spectrum of an equilibrium mixture of the two metabolites revealed a pair of thiophene ring proton singlets (δ 6.42 and 6.44 ppm) and the absence of the olefinic proton present in the tiagabine spectrum (δ 5.98). Based on these data and literature precedents for the existence of hydroxythiophenes as keto tautomers, it is anticipated that oxidation in one of the thiophene rings of **1** formed 5-



Scheme 1

hydroxytiagabine **13**. This was believed to be present entirely as *E* and *Z* isomers of the tautomeric 5-oxo form (**13**, *E* and *Z*). The urinary metabolites were also identified as human metabolites following oral administration of ^{14}C-tiagabine to adult male subjects. To assure the position of oxidation in tiagabine a total synthesis starting from well characterized starting materials was performed.

The three approaches for the preparation of 5-hydroxytiagabine **13** shown in Scheme 1-3 have been studied. 3-Methylthiophene **2** served as the starting material in the sequences shown in Scheme 1 and 2. It was brominated with *N*-bromosuccinimide by standard procedures [3] to 2-bromo-3-methylthiophene **3** which then was chlorinated to give 2-bromo-5-chloro-3-methylthiophene **4**. Initial attempts to selectively chlorinate **3** at the 5 position failed. Chlorination of **3** with *N*-chlorosuccinimide [4] or sulfuryl chloride [5] has been reported to give **4** in 62-90 % yield. In our hands these procedures gave mixtures of **4**, 2,5-dichloro-3-methylthiophene and 2,5-dibromo-3-methylthiophene in the ratio 4.7:1:1 according to GC/MS analysis. The two sideproducts probably formed by halogen exchange could also be observed in high field ^{1}H NMR spectra. A similar mixture was obtained using chlorine in dichloromethane in the presence of mercuryoxide.[6]

The structure of **4** was assessed from its ^{13}C-NMR spectra (Table 1). The substituent pattern of the thiophene ring in **4** followed from the splittings of the ^{13}C-NMR signals caused by C-H couplings (see Table 1).

Compound	δ (J/Hz)								
	C-2	C-3	C-4	C-5	C-2'	C-3'	C-4'	C-5'	CH_3
4	105.6 (H-4: 7.0) (CH_3: 7.0)	136.9 (H-4: 2.3) (CH_3: 6.4)	128.1 (H-4: 172.2) (CH_3: 4.3)	128.1					15.2 (H-3: 128.9) H-4: 1.9)
8	[a]	[a]	131.0 (H-4: 171.8) (CH_3: 4.9	135.3	[a]	[a]	131.8 (H-4': 167.1) (H-5': 4.6) (CH_3': 4.6)	129.8 (H-4': 8.0) (H-5': 184.8)	16.0 16.3 CO: 180.6
9	122.7 (H-4: 9.0) (CH_3: 4.5)	146.2 (H-4: 3.5) (CH_3: 6.4)	110.2 (H-4: 167.6) (CH_3: 4.9)	171.2 (H-4: 2.3) (CH_3: 4.7)	135.7 (CH_3: 4.4)	142.6 (CH_3: 5.9)	131.1 (H-4': 166.3) (H-5') (CH_3')	128.1 (H-4': 7.1) (H-5': 183.3)	15.7 17.1 60.0 CO: 181.3

[a] The signals from C-2, C-3, C-2' and C-3' at 145.0 (m), 144.7 (d qua, J=4.0 and 6.5 Hz), 135.0 (sex, J=4.3 Hz) and 134.3 (sex, J=4.6 Hz) could not be assigned unambiguously.

Table 1. ^{13}C NMR spectroscopic data of thiophenes in $CDCl_3$ with the solvent peak (δ 76.90) as an internal standard

The magnitude of the one bond C-H coupling constants, which in thiophene are 167.0 and 185.3 Hz, respectively,[7] indicated that the unsubstituted carbon atom was situated at the β– and not at the α–position to the sulfur atom. One of the signals originating from a quaternary carbon atom was not split by long range C-H couplings between a ring carbon atom and the methyl group at C-3 and must therefore be assigned to C-5. The carbon atom which resonated at 105.6 ppm coupled with H-4 with a coupling constant of 7.0 Hz, a value close

to that observed in thiophene itself.[7] The shift displacement of C-2 of **4** as compared to C-2 of thiophene itself corresponds to the shift displacement effected by bromine in other aromatic compounds.[7]

2-Bromo-5-chloro-3-methylthiophene **4** was converted to 5-chloro-3-methylthien-2-ylmagnesium bromide **6** which was added to 2-formyl-3-methylthiophene **5** producing the bis(thien-2-yl)methanol **7**. 2-Formyl-3-methylthiophene **5** in its turn was prepared by formylation of 3-methylthien-2-ylmagnesium bromide **17b** with *N,N*-dimethylformamide using known methodology. Bis(thien-2-yl)methanol **7** was sensitive to acid but it could be oxidized to the corresponding ketone **8** under controlled conditions. Manganese dioxide, and pyridinium chlorochromate supported on basic aluminium oxide were tried, the latter providing the best yields.

The structure of the chloroketone **8** was verified from its coupled ^{13}C-NMR spectra as described below for the methoxyketone **9** and assigning the signal to C-5, which like the signal assigned to C-5 in compound **4**, was not split by long range couplings (see Table 1).

The chlorine in bis(thien-2-yl)ketone **8** was displaced with a methoxy group to give compound **9** by treatment with sodium methoxide in *N,N*-dimethylformamide-methanol (9:1). These conditions seemed particularly effective by substitution of weakly reactive chlorine with methoxide ions. In the present case, sodium methoxide in methanol left the starting material **8** unchanged whereas sodium methoxide in dimethylformamide in the absence of methanol gave slow conversion to **9** and formation of several unidentified by-products. While displacement of the chlorine in compound **8** could be achieved by this procedure corresponding displacement of bromine and iodine only afforded dehalogenation.

The structure of the methoxyketone **9** was verified from its coupled ^{13}C NMR spectra (Table 1). The signals from the disubstituted ring all showed two- and three bond C-H couplings to a methyl group and <u>two</u> other ring protons, while the signals originating from the trisubstituted ring showed long range coupling to a methyl group and <u>one</u> ring proton. The compound possessed one unsubstituted carbon atom α–situated and two β–situated with respect to a sulfur atom as indicated by the one bond coupling constants 183, 166 and 168 Hz, respectively. C-2 is distinguished from C-3 and C-5 through the larger three bond coupling to H-4, a feature also observed in thiophene.[7] C-5 was identified through its low field shift, characteristic of methoxy substituted aromatic carbon atoms.[7]

5-Methoxyketone **9** was treated with cyclopropylmagnesium bromide to give cyclopropylmethanol **10**. Treatment of this compound with bromotrimethylsilane resulted in demethylation, in opening of the cyclopropyl ring with elimination of trimethylsilanoxide, and in migration of the double bond thus formed to give the conjugated system **11**. The aliphatic bromine atom of compound **11** was displaced with ethyl (*R*)-3-piperidinecarboxylate and the resulting ester **12** was hydrolysed in a standard fashion with production of 5-hydroxytiagabine **13**. Acidic hydrolysis was found superior to basic providing the acid **13** in virtually quantitative yield.

The substituent pattern in the two thiophene rings of compound **13** was verified through its 2-D ^{1}H-^{1}H double quantum filtered COSY and ROESY NMR spectra and its 2-D ^{1}H-^{13}C HMQC and HMBC NMR spectra.* Upon dissolution in dimethylsulfoxide the ratio between the two isomers of **13** was 1:9. Upon stan-

* COSY correlated spectroscopy, ROESY rotating frame nuclear Overhauser enhanced sopectroscopy, HMQC heteronuclear multiple quantum coherence, HMBC heteronuclear multiple bond correlation.

ding this ratio approached 1:1. In both forms the presence of the 3-methylthien-2-yl fragment was verified from the ROESY spectrum (Fig. 2) which shows that one thiophene proton is spatially close to one methyl group and a second thiophene proton. The coupling between these two heteroaromatic protons was 5.1 Hz. For both isomers the cross peaks in the COSY and the ROESY spectra (Fig. 2) indicate that the third thiophene proton coup les with a spatially close methyl group situated at C-3. Therefore the third proton is situated at C-2 or C-4.

Compound	Chemical shift	Position ppm										
		2	3	4	5	3-Me	2'	3'	4'	5'	3'-Me	6
13, major isomer	$\delta 13_C$	137.9	159.7	132.9	190.9	16.6	133.1 [a]	136.8 [a]	130.3	127.1	14.3	138.2
	$\delta 1_H$			6.44		1.68			6.99	7.66	2.10	
13, minor isomer	$\delta 13_C$	139.2	158.6	133.2	192.7	19.4	137.4 [a]	136.2 [a]	130.2	126.4	14.9	139.7
	$\delta 1_H$			6.46		2.58			6.94	7.58	2.12	

[a] The chemical shifts of C-2' and C-3' of the individual isomers may have to be interchanged

Table 2. ^{13}C and ^{1}H NMR chemical shifts in ppm of the heteroaromatic part of compound **13** in dimethylsulfoxide-d_6 with the solvent peak (^{13}C: $\delta = 39.7$ ppm; ^{1}H: $\delta = 2.50$ ppm) as an internal standard

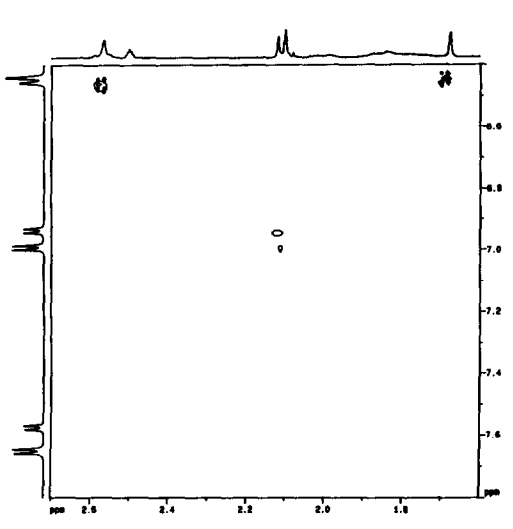


Fig. 2. ROESY Spectrum of 5-hydroxytiagabine **13**

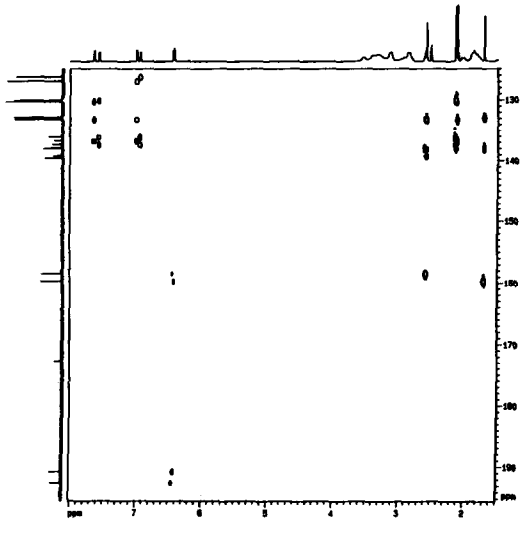
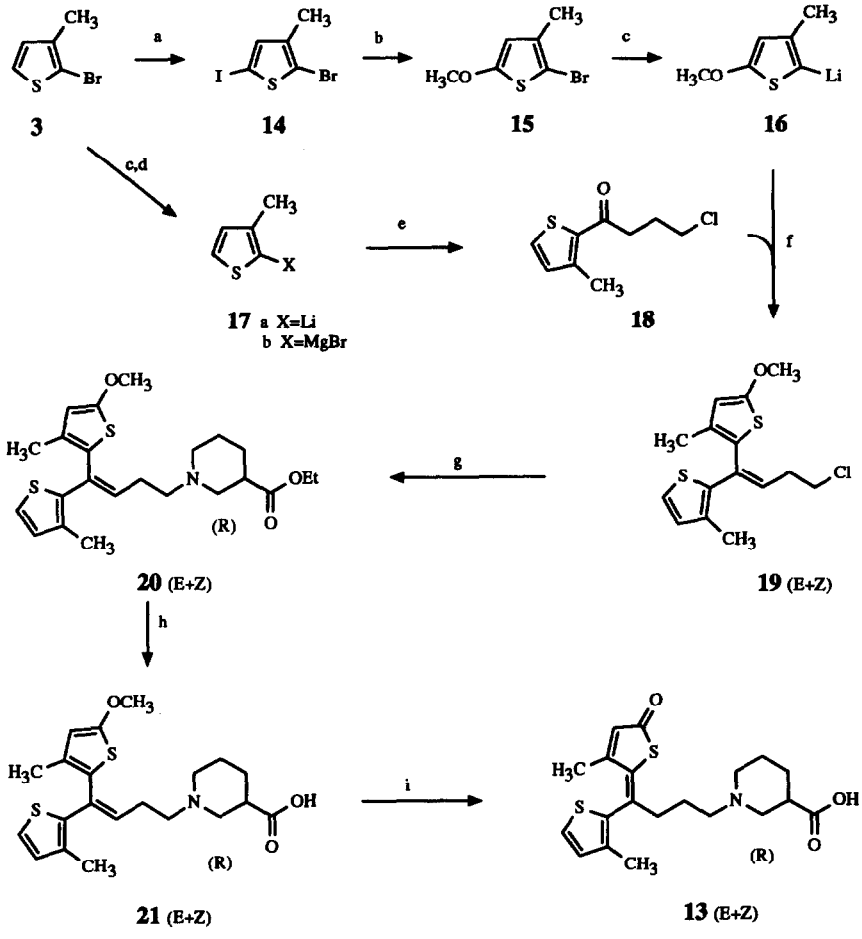


Fig. 3. HMBC Spectrum of 5-hydroxytiagabine **13**

According to ^{1}H-^{13}C HMBC spectra (Fig. 3) the third proton in both isomers is correlated to a carbonyl carbon atom which shows no connectivity to the methyl protons. This indicates that the third thiophene proton is situated at C-4 adjacent to a C-5 carbonyl group confirming that compound **13** adopted the 5-oxothien-2-ylidene

tautomeric form exsisting as a mixture of *Z*- (major) and *E*- isomers with respect to the C-2 C-6 double bond. The complete assignment of the thienyldihydro-oxothienylidenemethyl moiety of compound **13** is shown in Table 2.

An alternative route to the target compound **13** shown in Scheme 2 proved less satisfactory. All steps were feasible but the intermediate 2-bromo-5-methoxy-3-methylthiophene **15** was attainable in only 27 % yield.



Scheme 2

(a) NIS, MeCOOH, 40 °C, 18h + reflux 1 h, 63% (b) MeONa, CuO, MeOH, reflux 4 d, 27% (c) Bu^nLi, Et_2O, -20 °C, 1 h (d) Mg, THF, reflux 1 h (e) [1] $Cl(CH_2)_3CN$ [2] H_3O^+, 7% (f) [1] -70 °C, 3 h [2] NH_4Cl [3] flash chromatography [4] 4M HCl, Pr^nOH, 20 °C, 1 h [5] flash chromatography (g) ethyl (*R*)-3-piperidinecarboxylate, $(Bu^n)_2O$, K_2CO_3, 150 °C, 4 h, 47% (h) [1] NaOH (aq) [2] HCl (aq), 74% (as the hydrochloride) (i) Me_3SiBr, CH_2Cl_2, 20 °C, 3 h, 100%.

In addition **15** was unstable and very difficult to handle. Another serious draw back was the reaction between 3-methylthien-2-ylmagnesium bromide **17b** and 4-chlorobutyronitril which produced the desired 2-(4-chlorobutyroyl)-3-methylthiophene **18** in only 7 % yield. The corresponding lithium compound **17a** did not produce compound **18** at all. The remaining steps of the sequence from **18** to **13** all proceeded in acceptable yields. Although the shortcomings of the approach shown in Scheme 2 both were situated at an early stage of the sequence and this route later was abandoned, we succeeded in isolating a compound which according to ^{1}H-NMR spectroscopy, FAB-mass spectra and HPLC was identical to **13**.5-hydroxytiagabine is formed in vivo by a regiospecific cytochrome P-450 mono oxygenase mediated hydroxylation; extensive work has established the intermediacy of high valent iron porphyrin intermediates in similar transformations.[8] Sterically protected and electronically activated metalloporphyrins have been studied as synthetic models for cytochrome P-450 mediated epoxidations and hydroxylations. These catalysts are robust, not destroyed under strongly oxidizing conditions and effect catalytic oxidations with high turnover numbers.[8,9,10,11,12,13,14,15]

The central double bond in tiagabine is hindered and relatively inert to epoxidation under a wide variety of reaction conditions. Treatment with hydrogen peroxide, sodium hypochlorite or m-chloroperbenzoic acid did not lead to significant amounts of epoxide formation. Porphyrin assisted oxidation was therefore anticipated to be directed towards hydroxylation of the thiophene ring. This prediction was confirmed by experiment. We found octabromo tetrakis(2,6-dichlorophenyl)porphyrin Fe(III)Cl [Cl$_8$Br$_8$Fe(III)TPP] and octafluoro tetrakis(pentafluorophenyl)porphyrin Fe(III)Cl ["perfluoro Fe(III)TPP"] (see Fig. 4) to be very effective in achieving hydroxylation of the thiophene ring.

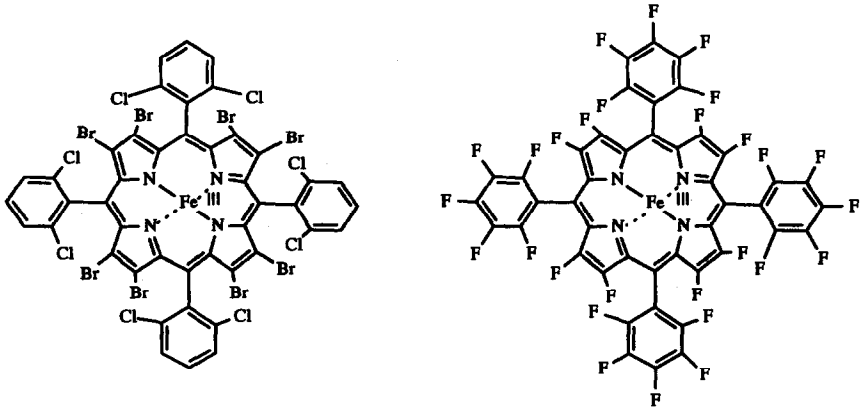
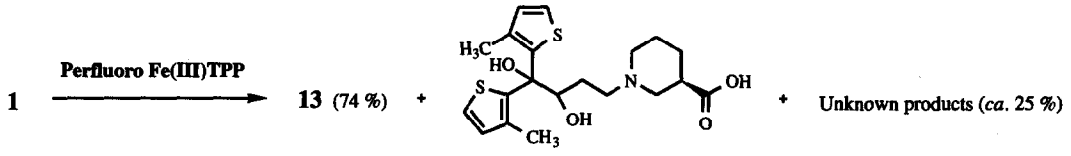


Fig. 4. Structure of the porphyrins Cl$_8$Br$_8$Fe(III)TPP and perfluoro Fe(III)TPP

The hemins were synthesized by the methods of Traylor, Dolphin and Tsuchiya.[15,16,17] Condensation of the appropriate aldehydes (2,6-dichlorobenzaldehyde and pentafluorobenzaldehyde respectively) with pyrrole in presence of anhydrous zinc chloride in refluxing lutidine yielded the Zn complexes of the porphyrins after

chromatographic purification. Halogenation of the pyrrole moiety with *N*-bromosuccinimide in refluxing CCl_4 or with anhydrous Ag(I)F in refluxing CH_2Cl_2 furnished the perhalogenated materials. Demetallation with trifluoroacetic acid followed by conversion to the hemin ($Fe(II)Cl_2$/DMF at reflux) by the method of Adler [18] and Kobayashi [19] provided the hemins after alumina chromatography.

Treatment of **1** with the synthesized hemins in dichloromethane/water biphasic system with NaOCl or 30% H_2O_2 as the exogenous oxygen donor gave 5-hydroxytiagabine, H_2O in 62-74%. yield. Phase transfer



22 (trace amounts)

Scheme 3

catalysts were not required. Conversion of **1** to the base followed reaction with the hemin and t-butyl hydroperoxide in dichloromethane. The yields were lower (40%) and more side products were discernible. The synthesized metabolite revealed complete spectroscopic and chromatographic identity with the authentic sample of the human metabolite. "Dihydroxytiagabine" **22** arising, presumably, via epoxidation of the central double bond followed by epoxide ring opening formed only in small amounts. This product was confirmed to be the dihydroxy material by independently subjecting **1** to the Sharpless asymmetric dihydroxylation.[20,21] Polar and apolar side products of the metalloporphyrin assisted hydroxylation have not been conclusively identified.

Porphyrin	Substrate	Co-oxidant	Solvent	Isolated yield	% Yields
Perfluoro Fe(III)TPP	1	NaOCl (5.5%)	H_2O/CH_2Cl_2	75 mg	74%
PerfluoroFe(III)TPP	1	H_2O_2 (30%)	H_2O/CH_2Cl_2	64 mg	62%
$Cl_8Br_8Fe(III)TPP$	1	NaOCl	H_2O/CH_2Cl_2	68 mg	66%
Perfluoro Fe(III)TPP	1 base	t-BuOOH	CH_2Cl_2	41 mg	40%

Table 3. Yields in metal porphyrin assisted hydroxylations of tiagabine **1**

This method is amenable to large scale synthesis of human metabolites. Experiments are under way to use the iron or manganese porphyrins in the synthesis of human metabolites of drugs currently under clinical investigation. Availability of larger amounts of metabolites would facilitate biochemical and toxicological studies.

EXPERIMENTAL

General. N,N-Dimethylformamide (DMF) and tetrahydrofurane (THF) were dried over 3 Å and 4 Å molecular sieves, respectively. Solutions were dried over magnesium sulfate when not otherwise stated. All starting materials were commercially available, except when noted.

2-Bromo-5-chloro-3-methylthiophene 4. The crude product prepared from 2-bromo-3-methylthiophene **3** (185.0 g, 1.04 mol) according to the literature procedure [4] was submitted to fractional distillation through a column (60 × 3 cm) containing Rashig rings and refractionated through a column (60 × 3 cm) containing Fenske rings to give 46.3 g, (20 %) of *2-Bromo-5-chloro-3-methylthiophene 4*, b.p. 78 °C/8 mmHg, reported [4] b.p. 79 °C/10 mmHg. ^{1}H-NMR ($CDCl_3$, 400 MHz) 2.13 (3H, s), 6.62 (1H, s); GC/MS: M^+ calcd. m/z: 210. Found 210 $(M^+/(M+2)^+/(M+4)^+$ 14:18:5, 97.44%), 166 $(M^+/(M+2)^+/(M+4)^+$ 36:25:2, 2.12%), 210 $(M^+/(M+2)^+/(M+4)^+$ 36:45:20, 0.44%).

5-Chloro-3-methylthien-2-yl (3-methylthien-2-yl)methanone 8. To magnesium turnings (3.54 g, 0.146 mol) under nitrogen was added 1/5 of a solution of **4** (31.73 g, 0.150 mol) in anhydrous THF (150 mL). The Grignard reaction was initiated by heating to reflux temperature and continued by dropwise addition of the remaining bromide solution. When addition was completed the reaction mixture was kept at reflux temperature for 45 min and then allowed to cool to room temperature. A solution of freshly distilled 2-formyl-3-methylthiophene [22] (19.87 g, 0.158 mol) in anhydrous THF (75 mL) was added dropwise. The mixture was then heated at reflux temperature for 4 h and stirred at room temperature for 14 h. Saturated aqueous ammonium chloride (100 mL) was carefully added keeping the temperature below 35 °C. Then water (50 mL) and brine (10 mL) were added and the mixture extracted with ether (100 mL). The phases were separated and the aqueous layer was extracted with ether (50 mL) .The combined organic phases were washed with 5% aqueous sodium hydrogencarbonate (50 mL) and dried for 15 min. After filtration the solvent was removed *in vacuo* and the residue was dissolved in dichloromethane (200 mL). This solution was added dropwise to a suspension of pyridinium chlorochromate (78.68 g, 0.365 mol) and alumina (basic, grade III, 300 g) in dichloromethane (600 mL). The mixture was stirred with a mechanical stirrer for 3 h, then filtered through Hyflo® and the solvent removed *in vacuo*. The residue was dissolved in ethyl acetate (200 mL) and washed twice with 5% aqueous citric acid (50 mL) and with 5% aqueous sodium hydrogencarbonate (30 mL) and dried. After filtration the solvent was removed *in vacuo* to give a solid which was dissolved in boiling ethanol (100 mL). Upon cooling a precipitate was formed which was collected and then suspended in 96% ethanol (100 mL), filtered and washed with cold 96% ethanol. The product was dried *in vacuo* to give *5-Chloro-3-methylthien-2-yl (3-methylthien-2-yl)methanone 8* (14.20 g, 38%). ^{1}H-NMR ($CDCl_3$, 400 MHz): 2.43 (3H, s) 2.50 (3H, s) 6.83 (1H, s) 6.98 (1H, d) 7.45 (1H, d). Mp.: 76.5-77.5 °C. Anal. $C_{11}H_9ClOS_2$ (256.78): calcd. C, 51.45; H, 3.53; Cl, 13.81. Found C, 51.53; H, 3.58; Cl, 13.80.

The mother liquor was concentrated to 75 mL and a second crop of the *title compound*, m.p. 72-74 °C was obtained bringing the total yield to (17.76 g, 47%).

5-Methoxy-3-methylthien-2-yl (3-methylthien-2-yl)methanone 9. To a solution of **8** (10.00 g, 0.039 mol) in dry DMF (400 mL) under a nitrogen atmosphere was added at 50 °C a 1 M solution of sodium methoxide in methanol (38.9 mL). The mixture was stirred at 50 °C for 20 h and then an additional 1 M solution of sodium methoxide in methanol (38.9 mL) was added and the stirring continued at 50 °C for 24 h. The reaction mixture was poured into water (1 L) and extracted three times with ether (400 mL). The combined organic extracts were washed twice with brine (200 mL) and dried. After filtration the solvent was removed *in vacuo* to give the crude product. The oily residue was stripped with xylene, 1-propanol and finally with tetrachloromethane to give *5-*

Methoxy-3-methylthien-2-yl (3-methylthien-2-yl)methanone ***9*** (4.85 g, 49%) as an oil. ^{1}H-NMR ($CDCl_3$, 200 MHz): 2.41 (3H, s) 2.45 (3H, s) 3.92 (3H, s) 6.17 (1H, s) 6.92 (1H, d) 7.87 (1H, d). Anal. $C_{12}H_{12}O_2S_2$ (252.36): calcd. C, 57.11; H, 4.79. Found C, 57.90; H, 4.77.

Cyclopropyl (5-methoxy-3-methylthien-2-yl) (3-methylthien-2-yl)methanol ***10***. To magnesium turnings (0.73 g, 0.030 mol) under nitrogen was added 1/3 of a solution of bromocyclopropane (3.64 g, 0.030 mol) in anhydrous THF (30 mL). The Grignard reaction was initiated by heating to reflux temperature and continued by dropwise addition of the remaining bromide solution. When addition was completed the reaction mixture was kept at reflux temperature for 30 min. and then cooled to 5 °C. A solution of **9** (3.80 g, 0.015 g) in anhydrous THF (30 mL) was added dropwise. The mixture was stirred at ambient temperature for 17 h. Saturated ammonium chloride (50 mL) was carefully added and then diethylether (50 mL) was added. The aqueous phase was neutralized with 2 M sulfuric acid and the phases were separated. The aqueous phase was extracted with diethylether (50 mL). The combined organic phases were washed with 5% aqueous sodium hydrogencarbonate (25 mL) and dried. After filtration the solvent was removed *in vacuo* to give a crude product (4.49 g) which was dissolved in dichloromethane (100 mL), silica gel (25 g, 15-40 μm) was added and the solvent removed *in vacuo*. The silica gel supported product was purified by column chromatography on silica gel (200 g, 40-63 μm, n-heptane/ethyl acetate 4/1). Fractions containing product were collected and the solvent was removed *in vacuo* to give *cyclopropyl (5-methoxy-3-methylthien-2-yl) (3-methylthien-2-yl)methanol* ***10*** (3.07 g, 69%) as an oil. ^{1}H-NMR ($CDCl_3$, 200 MHz): 0.5-0.7 (4H, m) 1.6-1.8 (4H, m) 2.10 (1H, s) 3.85 (3H, s) 5.90 (1H, s) 6.78 (1H, d) 7.10 (1H, d). Anal. $C_{15}H_{18}O_2S_2$ (294.44): calcd. C, 61.19; H, 6.16. Found C, 61.49; H, 6.34.

E/Z-*4-Bromo-1-(2,5-dihydro-3-methyl-5-oxothien-2-ylidene)-1-(3-methyl-2-thienyl)butane* ***11***. To a solution of **10** (2.88 g, 0.01 mol) in anhydrous dichloromethane (40 mL) under a nitrogen atmosphere was added dropwise a solution of bromotrimethylsilane (3.00 g, 0.02 mol) in anhydrous dichloromethane (10 mL) keeping the temperature below 30 °C. When addition was complete the mixture was stirred at ambient temperature for an additional hour and water (50 mL) was carefully added. After addition of brine (10 mL) the phases were separated and the aqueous phase was extracted with dichloromethane (50 mL). The combined organic phases were washed with 5% aqueous sodium hydrogencarbonate (20 mL) and dried. After filtration the solvent was removed *in vacuo* to give 2.77 g (82%) of crude **11** which was used in the next step without further purification. ^{1}H-NMR ($CDCl_3$, 200 MHz): 1.73 (3H, s) 1.95 - 2.10 (2H, m) 2.13 (3H, s) 2.72 (2H, dd) 3.40 (2H, t) 6.16 (1H, s) 6.88 (1H, d) 7.32 (1H, d); GC/MS:M^+ calcd. m/z: 342. Found 342 (M^+/$(M+2)^+$ 9:10, 78.59 %).

Ethyl E/Z-(R)-*1-(4-(2,5-dihydro-3-methyl-5-oxothien-2-ylidene)-4-(3-methyl-2-thienyl)butyl)-3-piperidinecarboxylate* ***12***. A suspension of **11** (0.77 g, 2.2 mmol), ethyl (*R*)-3-piperidinecarboxylate L-(+)-tartrate [23,24] (1.03 g, 3.4 mmol), potassium carbonate (0.93 g, 6.7 mmol) and potassium iodide (40 mg, 0.2 mmol) in acetone (20 mL) were stirred at room temperature for 48 h. The reaction mixture was filtered and the solvent was evaporated from the filtrate *in vacuo*. The residue was purified by column chromatography on silica gel (50 g, 40-63 μm, n-heptane/ethyl acetate gradient 4/1 to 2/1) to give the *title compound* (0.30 g, 32%) as an oil. ^{1}H-NMR ($CDCl_3$, 400 MHz), major isomer: 1.26 (3H, t) 1.71 (3H, s) 2.11 (3H, s) 2.36 (2H, dd) 2.60 (2H, dd) 4.13 (2H, q) 6.15 (1H, s) 6.85 (1H, d) 7.30 (1H, d).

E/Z-(R)-*1-(4-(2,5-Dihydro-3-methyl-5-oxothien-2-ylidene)-4-(3-methyl-2-thienyl)butyl)-3-piperidinecarboxylic acid hydrochloride* ***13, HCl.*** To a solution of **12** (40 mg, 0.1 mmol) in acetone (1 ml) was added 2 M hydrochloric acid (2 ml). The resulting mixture was stirred at 50 °C for 16 h. The acetone was removed *in vacuo* and the aqueous residue was washed with n-heptane (2 ml). The aqueous phase was concentrated *in vacuo* to give an oily residue which was stripped *in vacuo* with dichloromethane (3 ml) to give the *title compound* (28 mg, 69%). ^{1}H-NMR (DMSO-d_6, 400 MHz), major isomer: 1.70 (3H, s) 2.13 (3H, s) 6.45 (1H, s) 6.98 (1H, d) 7.65 (1H, d) 11.0 (1H, br s). An analytical sample was dissolved in a 1:1 mixture of water and acetonitrile and immobilised on a preparative HPLC column (RP 18, 20 mm × 250 mm). The column was washed with water and the immobilised material was eluted off the column with acetonitrile/water 9/1. The eluate was acidified to pH 2 with 2 M hydrochloric acid and the solvents were removed *in vacuo* to give an oil which was dissolved in acetone. Removal of the solvent gave the hydrochloride of **13** as an amorphous solid. Anal. $C_{20}H_{25}NO_3S_2$,.HCl, H_2O (446.03): calcd. C, 53.86; H, 6.33; N, 3.14; Cl, 7.95. Found C, 53.42; H, 6.28; N, 3.49; Cl, 7.66.

Metalloporphyrin assisted hydroxylation of tiagabine. -General Procedures and Starting Materials. Samples of Cl_8Br_8Fe(III)TPP and perfluoro Fe(III)TPP were prepared by minor modifications of literature procedures followed by chromatographic purification over alumina. Elution of the column with hot chloroform yielded greater and faster recovery of the products. The samples were characterized by UV absorption values (Soret bands) in accordance to those reported in the literature. The reactions were monitored high performance liquid chromatography using Spectra Physics P-4000, Autosampler AS-3000 and Chromjet 4400 equipped with 15 cm × 4.6 mm, C-18 Nucleosil column, with H_3PO_4 (pH ≈ 2.2)/CH_3CN as eluent, 1.0 mL/min flow with Spectra Physics UV-Visible detector (UV-2000) operating at 254 nm.

General procedure for the hydroxylation of tiagabine. (1) (100 mg, 2.5×10^{-4} moles) was dissolved by gentle warming in water (10 mL), the solution was added to dichloromethane (10 mL). To the rapidly stirred mixture was added the metalloporphyrin (25 mg) followed by dropwise addition of 30% H_2O_2 (2.0 mL) or aqueous NaOCl 5.5%, 5.0 mL). The mixture was vigorously stirred at ambient temperature for 6-8 hours. HPLC revealed only small amount of **1** (< 2%) in the aqueous layer; the reaction mixture was filtered to remove a discolored residue. The aqueous layer was separated and saturated with sodium chloride and extracted with ethyl acetate (2 × 30 mL). The aqueous layer was lyophilized and the residue was triturated with ethyl acetate (30 mL). The organic layers were combined and concentrated *in vacuo*. HPLC analysis of the residue revealed the major product to be **13**. Chromatography over silica gel (50 g, 230-400 mesh) and elution with chloroform yielded a fraction running with the solvent front. This contained some apolar products that have not been characterized. The 5-hydroxytiagabine was eluted with chloroform/methanol (1:9); further elution with chloroform/methanol (1:3) furnished a small amount of the "dihydroxytiagabine" **22**. This was identified by comparison with an authentic sample prepared by an alternate method. Elution with chloroform/methanol (1:1) yielded a mixture of unidentified products. A blank reaction of **1** with H_2O_2 or NaOCl led to a mixture of products, none of which corresponded to 5-hydroxytiagabine.

Acknowledgement. We wish to acknowledge the contribution of Paw Bloch, Karina Madsen, Freddy Pedersen and Jan Sørensen for synthesis of target compounds and thank Dr. Barbara Bopp for an authentic sample of 5-hydroxytiagabine; Professor Dr. Dolphin for detailed procedures relating to the synthesis of the metalloporphyrins and for reference samples of octachloro and octachlorooctabromo Fe(III)TPP. We are grateful to Vibeke Bødstrup, Gitte Knudsen, Jytte Rasmussen, Dr. Abu Rustum and Anne Ryager for their assistance with HPLC and other analytical measurements.

REFERENCES

1. Andersen, K. E.; Braestrup, C.; Grønwald, F.; Jørgensen, A. S.; Nielsen, E. B.; Sonnewald, U.; Sørensen, P. O.; Suzdak, P. D.; Knutsen, L. J. S., *J. Med. Chem.* **1993**, *36*, 1716.
2. Richens, A.; Chadwick, D.; Duncan, J.; Dam, M.; Morrow, J.; Gram, L.; Mengel, H.; Shu, V.; Pierce, M., *Seizure* **1992**, *1, Suppl. A*, S37.
3. Kellog, R. M.; Schaap, A. P.; Harper, E. T.; Wynberg, H., *J. Org. Chem.* **1968**, *33*, 2902.
4. Yom Tov, B.; Gronowitz, S.; Ross, S. B.; Stjernström, N. E., *Acta Pharm. Suecia* **1974**, *11*, 149.
5. Binder, D.; Noe, C., *Eur. Pat.* **1992**, 32768.
6. Steinkopf, W.; Jacob, H., *Liebigs Ann. Chem.* **1935**, *515*, 273.
7. Kalinowsky, H. O.; Berger, S.; Braun, S. *Carbon-13 NMR Spectroscopy* Wiley: New York, 1988.
8. White, R. E.; Coon, M. J., *Annu. Rev. Biochem.* **1980**, *49*, 315.
9. Groves, J. T.; Nemo, T. E.; Myers, R. S., *J. Am. Chem. Soc.* **1979**, *101*, 1032.
10. Groves, J. T.; Nemo, T. E., *J. Am. Chem. Soc.* **1983**, *105*, 5786.
11. Groves, J. T.; Kniper, W. J.; Haushalter, R. C., *J. Am. Chem. Soc.* **1980**, *102*, 6375.
12. Groves, J. T.; Haushalter, R. C.; Nakamura, M.; Nemo, T. E.; Evans, B., *J. Am. Chem. Soc.* **1981**, *103*, 2884.
13. Renaud, J. P.; Battioni, P.; Bartoli, J. F.; Mansuy, D. J., *J. Am. Chem. Soc.* **1985**, *107*, 888.
14. Collman, J. P.; Tanaka, H.; Hembre, R. T.; Brauman, J. I., *J. Am. Chem. Soc.* **1990**, *112*, 3689.
15. Traylor, P. S.; Dolphin, D.; Traylor, T. G., *J. Am. Chem. Soc.* **1984**, *106*, 279.
16. Traylor, T. G.; Tsuchiya, S., *Inorg. Chem.* **1987**, *26*, 1338.
17. Tsuchiya, S.; Seno, M., *Chem. Lett.* **1989**, 263.
18. Adler, A. D.; Longo, F. R.; Kampos, F.; Kim, J., *Inorg. Nucl. Chem.* **1970**, *32*, 2443.
19. Kobayashi, H.; Higuchi, T.; Kaiza, Y.; Osada, H.; Aoki, M., *Bull. Chem. Soc. Jpn.* **1975**, *48*, 3137.
20. Sharpless, B.; Amberg, W.; Beller, M.; Chen, H.; Hartung, J.; Kawanami, Y.; Lübben, D.; Manoury, E.; Ogino, Y.; Shibata, T.; Ukita, T., *J. Org. Chem.* **1991**, *56*, 4585.
21. Chorghade, M. S.; Lee, E. C., *Heterocycles* **1993**, In Press.
22. Gronowitz, S.; Moses, P.; Hörnfeldt, A. B.; Håkansson, R., *Arkiv Kemi* **1961**, *17*, 165.
23. Akkerman, A. M.; DeJongh, D. K.; Veldstra, H., *Recl. Trav. Chim. Pays-Bas* **1951**, *70*, 899.
24. Bettoni, G.; Duranti, E.; Tortorella, V., *Gazz. Chim. Ital.* **1972**, *102*, 189.

(*Received in UK* 11 *March* 1994; *revised* 20 *May* 1994; *accepted* 27 *May* 1994)