IDENTIFICATION AND SYNTHESIS OF MAJOR OXIDATIVE DEGRADATION PRODUCTS OF TIAGABINE†

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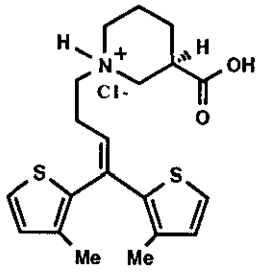
Abstract-Tablet formulations of tiagabine (an anti-epileptic drug currently in Phase III trials) revealed the presence of some degradation products. The isolation, spectroscopic characterization and synthesis of these products are described.

Epilepsy is a disorder characterized by recurrent spontaneous electrical discharges within the brain which are manifested by clinical seizures.[1] Four million patients in the USA are afflicted with this ailment. Current targets for therapeutic intervention include blocking of receptors of excitatory amino acids, modulating excitatory membrane ion channels and enhancing the neurotransmittory effect of γ-aminobutyric acid (GABA).[1,2]

Tiagabine (I),[3] currently in phase III clinical trials, is a single enantiomer and a highly potent, selective *in vitro* inhibitor of GABA uptake. Maintaining the extra cellular concentration of GABA leads to calming of excitatory synaptic currents thereby preventing seizures. The synthesis of tiagabine,[4] its 5-hydroxy human metabolite[5] and analogs such as desmethyltiagabine[6] has been reported.



I

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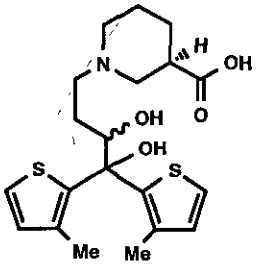
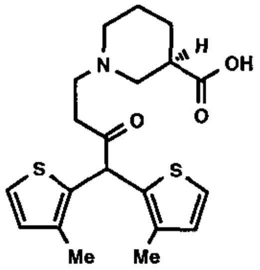
†This paper is dedicated to Dr. Arnord Brossi.

Initial liquid and tablet formulations of tiagabine had a short shelf life of 1-2 years and revealed the presence of two major and other minor oxidative degradation products. The degradation products could be suppressed by addition of anti oxidants to the formulation. This indicated the degradation process to be of oxidative origin. The products could also be seen in stressed solutions of bulk drug exposed to ultra violet light in presence of air.

We now report the isolation, structure elucidation and synthesis of the degradates. The major products were "dihydroxytiagabine" (II) and "ketotiagabine" (III).



II



III

Dihydroxytiagabine (II):

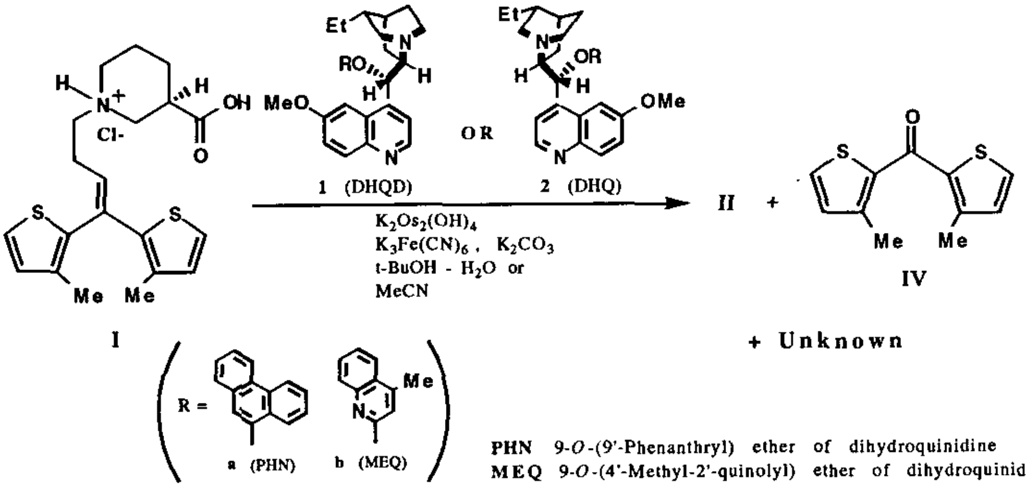
A solution of tiagabine was placed in a lightchamber and exposed to 6000 lux for 1 week. The degradation product was separated by hplc with ethanol/trifluoroacetic acid as the eluent. Neutralization of the eluates followed by desalting and freeze drying provided the pure product.

The ^{1}H nmr spectrum revealed a doublet of a doublet and two additional doublets (2H) in the aromatic region (7.10 and 6.70 ppm) compared to the four clearly separated doublets (1H) in the tiagabine spectrum.[4] The aliphatic portion of the spectrum was difficult to interpret due to numerous conformational possibilities. The resonance (triplet) in the vinylic hydrogen in tiagabine was replaced by a new resonance at 4.50 (m, $CDCl_3$). This was suggestive of the functionalization of the double bond.

The ^{13}C spectrum revealed resonances of eight carbons in the aromatic region and two additional carbons at 73 and 78 ppm indicative of carbons bound to -OH groups. Mass spectrometry (thermospray interface) revealed a M+1 quasi molecular ion at m/z=410. This was in agreement with two extra oxygen atoms having been introduced. The peak at m/z=392 (p-H_2O) suggested an aliphatic positioning of the hydroxy functionality. This was also seen in a lc/ms/ms experiment, where the M+1 ion at m/z=410 was collision activated to further fragmentation. An m/z=142 ion confirmed the nipecotic acid portion of the molecule to be intact. The structure was assigned as *R*(-)1-(4,4-bis(3-methylthien-2-yl)-3,4-dihydroxy-1-butyl)piperidine-3-carboxylic acid (II).

Preparation of dihydroxytiagabine (II) was accomplished by the method shown in Scheme A.

Scheme A



PHN 9-*O*-(9'-Phenanthryl) ether of dihydroquinidine
MEQ 9-*O*-(4'-Methyl-2'-quinolyl) ether of dihydroquinidinol

The synthesis proved difficult in that the central double bond is hindered and proved to be refractory to attack by reagents such as *m*-chloroperbenzoic acid, hydrogen peroxide etc. The putative epoxide was not detected under a variety of reaction conditions; a complex mixture of products was always obtained. Reaction with osmium tetroxide/pyridine/*N*-methylmorpholine-*N*-oxide was slow and yielded the requisite diol in low yield but extraction of the product from water proved to be a problem.

The Sharpless procedure[7] for effecting osmium catalyzed, ligand accelerated asymmetric dihydroxylation was successfully utilized; the reaction could also be scaled up. Bis (3-methylthien-2-yl) ketone (IV) was also a product in these reactions and was accompanied by an impurity whose structure has not been elucidated. Optimum yields of the dihydroxy material

(II) were obtained in dioxane-water/*t*-butanol-water mixtures. Use of osmium tetroxide instead of potassium osmate led to slower reaction and increased formation of undesired products.

The material derived from synthesis revealed complete identity with the tablet degradate; any diastereomers that formed were not resolved under our chromatographic conditions. Attempted functionalization of the vicinal dihydroxy groups (acetate, acetonide, triflate) was unsuccessful and led to complex mixtures of products.

Ketotiagabine (III):

The abundance of this product in the tablet degradates increased with the length of the storage. When dihydroxytiagabine (II) was stored under acidic conditions, it was slowly converted to (III). A 70 eV solid probe mass spectrum showed no parent ion peak. The peak at m/z=207 gave evidence for benzylic cleavage. A m/z=142 peak indicated the nipecotic acid portion of the molecule to be intact.

A thermospray mass spectrum showed an M+1 ion at m/z=392, suggesting the product to be generated by loss of water from dihydroxytiagabine. Further, loss of water gave a dominant peak at m/z=374. A daughter ion spectrum was obtained by collision activation of the m/z=392 ion with argon at 10 eV. This spectrum had characteristic m/z=142 and 130 ion peaks of nipecotic acid moiety.

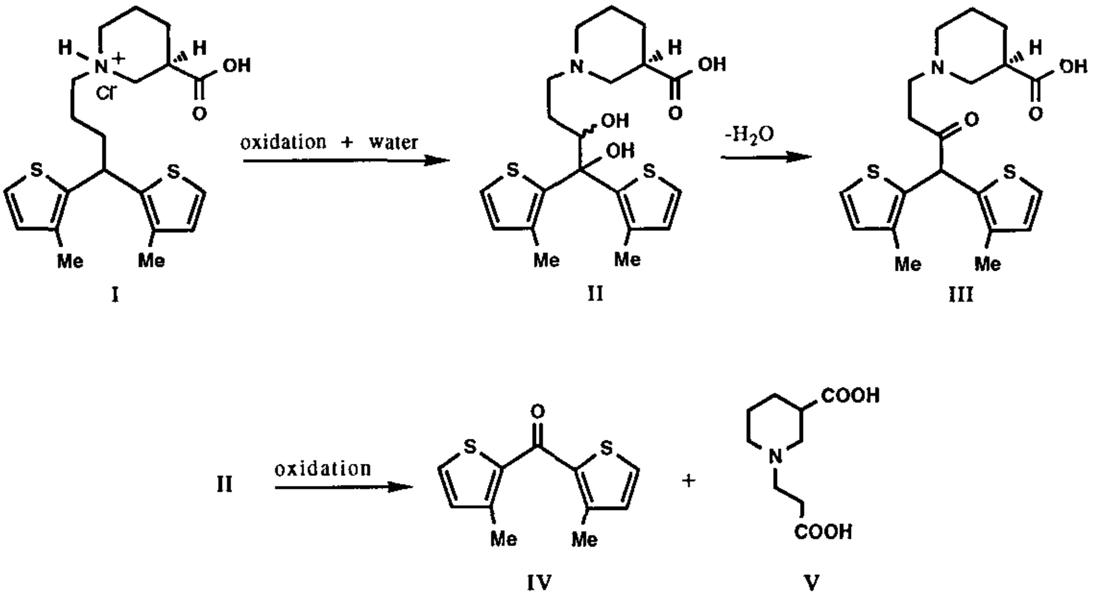
The ^{1}H nmr spectrum showed the presence of two closely spaced doublets at 6.80 ppm and a doublet at 7.15 ppm in the aromatic region arising from the four protons in the 4 and 5 positions in the two thiophene rings. The singlet (1H) at 5.60 ppm disappeared when the sample was mixed with deuterated water. No other peaks were present in the unsaturated part of the spectrum. In the aliphatic portion of the spectrum, the two methyl groups on the thiophenes were present as two singlets at 2.20 ppm. Further, a complex pattern from the other aliphatic hydrogens was observed from 1.30-3.70 ppm (13 H).

^{13}C Nmr showed the presence of an aliphatic carbonyl carbon at 202 ppm and a carboxy carbon at 173 ppm. In the aromatic region of the spectrum, four signals were present. These are due to the four aromatic carbons in the thiophene rings. Signals at 162 ppm and 110-135 ppm indicated the isolated substance to be a trifluoroacetic acid salt. In the aliphatic part of the spectrum, two methyl carbons were at 14 ppm and eight other aliphatic carbons were seen.

It was concluded that the carbonyl group was adjacent to the carbon benzylic to the two thiophenes, because the hydrogen on this carbon is a singlet. Enolization of the carbonyl will cause this hydrogen to be exchanged with deuterated water. Hence, the structure can be assigned as *R*-1-(4,4-bis(3-methylthien-2-yl)-3-oxo-1-butyl)piperidine-3-carboxylic acid (III). Increased conjugation with an aromatic system provided the driving force for the elimination of water. It was not possible to determine to what extent this dehydration occurs during sample preparation. If a base is used in the extraction medium, the ketotiagabine (III) is replaced with other impurities with longer retention times. The identity of these peaks is under investigation.

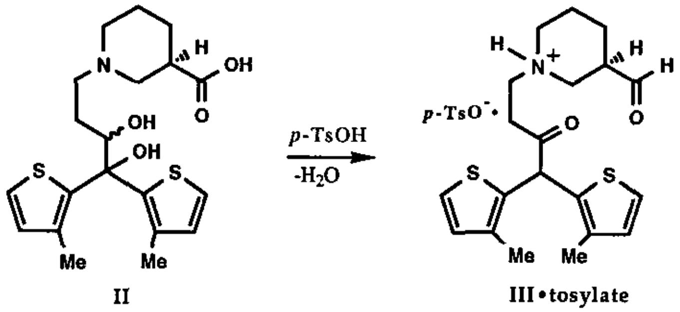
The degradates identified hitherto are summarized in Scheme B. In some cases, the chromatographic degradates do not account for the total degradation (hplc assay/peak areas); other degradation routes are possible. Alternately, binding of the transient epoxide to the hydroxy or carboxy functionalities in exipients may occur. These cases are under investigation in our laboratories.

Scheme B

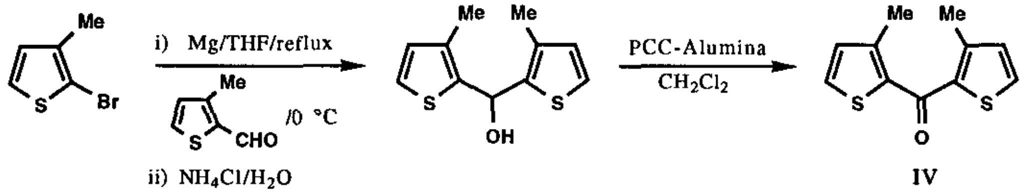


Synthesis of (III), (IV) and (V) was achieved as shown in Schemes C, D and E respectively.

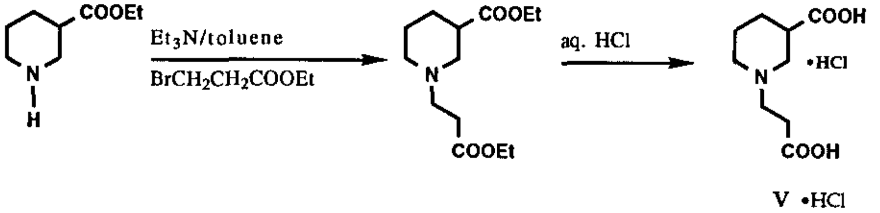
Scheme C



Scheme D



Scheme E



EXPERIMENTAL SECTION

General Procedures. Purification of degradates was done on a Varian Vista series 5000 preparative chromatograph. Semipreparative work was done on a Merck Hitachi instrument equipped with a nucleosil 5 µC18 (250x4, 200x4 or 200x25 mm) reverse phase columns; 20-80% gradient of MeCN/TFA 0.1% as eluent, 1.0 ml/min flow, and the detector operating at 250 nm. ^{1}H And ^{13}C nmr spectra were obtained on Bruker 200 MHz or 400 MHz spectrometers and are reported in parts per million (ppm) downfield from tetramethylsilane (TMS). 70 eV E.I. spectra were obtained on a Finnigan 5100 instrument using the direct insert inlet system . Thermospray mass spectra and CID spectra was obtained on a Finnigan TSQ instrument using a TSP2 interface and argon as collision gas. Uv spectra were recorded on a Shimadzu UV 260 instrument in quartz 1 cm cuvettes. Elemental analysis was done on a Perkin Elmer 240B C, H, N instrument. Karl Fisher titrations were conducted on a Methrom 652 KF coulometer. Synthetic reactions were monitored by high performance liquid chromatography using Spectra Physics P-4000, Autosampler AS-3000 and Chromjet 4400 equipped with 15 cm x 4.6 mm, C-18 Nucleosil column, with H_3PO_4 (pH~2.2)/MeCN as eluent, 1.0 ml/min flow with Spectra Physics UV-Visible detector (UV-2000) operating at 254 nm. All chemicals and solvents used for the studies were of analytical grade and *commercially available.*

Isolation of Dihydroxytiagabine (II): 200 ml of a solution of tiagabine (10 mg/ml) were placed in a lightchamber and exposed to 6000 lux for approximately 1 week. The degradate peak constituted 12% of the mixture. The solution was subjected to hplc on a 5 ml C-18 column with ethanol/trifluoroacetic acid 0.1% mixture (32:68). The fractions containing the degradate were neutralized with 10% aqueous ammonia and the solvents evaporated *in vacuo*. The product was further purified by semipreparative hplc with a mobile phase of acetonitrile/trifluoroacetic acid (0.1%) of 39:71. The combined pure fractions were neutralized and freeze dried. The product was desalted by applying an aqueous solution of the sample to a semipreparative hplc column, washing with water and eluting with acetonitrile/water (60:40). After evaporation and subsequent freeze-drying, a 110 mg of sample was obtained. Karl Fisher titration showed water content of 4.03%. A spike experiment was performed where the isolated dihydroxytiagabine sample was added to an aged tablet formulation. The experiment verified that the isolated sample had the same retention time as the degradation product. Elemental analysis corresponded to dihydroxytiagabine hydrate: Anal. Calcd for $C_{20}H_{29}NO_5S_2$: C, 56.29; H, 6.82, N, 3.28. Found: C, 56.73; H, 6.68; N, 3.20.

Isolation of Ketotiagabine (III): An aqueous solution of tiagabine (13 mg/ml) was left for 6 months at room temperature. After this period of storage, the peak at rt=0.72 corresponded to 10% of the total area of peaks in the chromatogram. Using *semipreparative chromatography,* 7 injections of 2 ml aliquots of the stored solution were chromatographed and the rt=0.72 peak was collected. After removal of acetonitrile by evaporation of the pooled fractions, the product was applied to a 4x200 mm C-18 column previously washed with acetonitrile and water. Elution with 80% acetonitrile and 20% water and freeze drying the desired fraction yielded 19 mg of a powder. The overall purification procedure was repeated by chromatography on the semipreparative column and after desalting, a white powder (10.2 mg) was isolated as a trifluoroacedic acid salt.

Analytical hplc showed chromatographic purity at 94.4% with dihydroxytiagabine (3.2%) and tiagabine (2.1%) being the main impurities. Elemental analysis Anal. Calcd for $C_{22}H_{26}NO_5F_3S_2$: C. 52.26; H, 5.13; N, 2.77. Found: C, 51.91; H, 5.30; N, 2.61.

Synthesis of Dihydroxytiagabine (II): To a well stirred mixture of DHQD PHN (216 mg, 0.40 mmol, 26 mol %), potassium ferricyanide (2.94 g, 9.0 mmol, 6 eq.) and potassium osmate dihydrate (12.0 mg, 0.030 mol, 1 mol%) in dioxane-water (1:1, 20 ml) was added tiagabine (I) (1.2 g, 3.0 mmol). The flask was stoppered and stirring continued overnight. The initial pale orange solution gradually turned green and then to black. Sodium sulfite (10.0 g) was added and the mixture was stirred for 1 h. The aqueous solution was saturated with sodium chloride and extracted with ethyl acetate (2x25 ml). Hplc indicated a 2:1 mixture of two products. Drying and evaporation of the organic layer *in vacuo* furnished a foam which was redissolved in a minimum amount of hot ethyl acetate. On cooling, a colorless glass precipitated (730 mg). It revealed complete spectroscopic identity with the degradate sample. The product was filtered and the filtrate separated into two "oxo" components by column chromatography (methanol:chloroform, 1:3). One of the compound was shown to be **(IV)** (200 mg, 15%); the other has not been conclusively identified as yet.

Large Scale Reaction of the Synthesis of Dihydroxytiagabine (II)[8]: To a well stirred mixture of DHQD PHN (7.0 g, 14.0 mmol), potassium ferricyanide (176 g, 534 mmol, 3 eq.), potassium carbonate (150 g, 1.08 mol, 6 eq.) in dioxane-water mixture (1:1, 1l) was added tiagabine (I) (72 g, 174 mmol) and potassium osmate(VI)dihydrate (0.72 g, 0.73 mmol). The mixture was stirred at ambient temperature under nitrogen for 72 h. Sodium sulfite (150 g) was added and the resulting mixture was stirred at ambient temperature for 1 h. Ethyl acetate (1 l) was added and stirring was continued for 0.5 h. The organic phase was separated; the aqueous phase was back extracted with ethyl acetate (0.5 l) and acetonitrile (1x500 ml, 1x250 ml). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to leave a solid residue (86 g). Preparative hplc purification yielded dihydroxytiagabine (31 g, 41%).

Synthesis of Ketotiagabine Tosylate (III•Tosylate): Dihydroxytiagabine (0.41 g, 1.0 mmol) was dissolved in ethyl acetate (40 ml) and the mixture was stirred for 5 min under nitrogen. Then, *p*-toluenesulfonic acid monohydrate (0.19 g, 1.0 mmol) was added carefully. The reaction mixture was heated to 50 °C for 5 min, cooled to room temperature and stirred for 2 days at ambient temperature. The reaction mixture gave a weak red-violet gleam and produced a small greasy precipitate which was decanted off. The solvent was removed *in vacuo* to leave a tea-colored foam of the product (480 mg, 82 %). Product analysis showed hplc purity of 98%: ^{1}H Nmr ($CDCl_3$) δ; 2.19 (6H, 2s), 2.35 (3H, s), 5.64 (1H, s), 6.77 (2H, 2d, J=4.6 Hz), 7.08-7.20 (3H, 2d, J=4.6 Hz, J=9.2 Hz), 7.68 (2H, d, J=9.2 Hz). Anal. Calcd for $C_{27}H_{35}NO_7S_3$: C, 55.74; H, 6.06; N, 2.41. Found: C, 55.84; H, 6.22; N, 2.41.

Preparation of Bis(3-methylthien-2-yl)methanol: Magnesium (0.79 g, 0.03 mol) was overlaid with tetrahydrofuran (5 ml), 2-bromo-3-methylthiophene (5.63 g, 0.03 mol) in tetrahydrofuran (6 ml) was added and the reaction mixture was heated to reflux. After 1.5 h, the mixture was cooled to 5 °C and 3-methylthiophene-2-carboxaldehyde (3.99 g, 0.03 mol) in tetrahydrofuran (4 ml) was added. The temperature was maintained below 5 °C. The mixture was allowed to warm to ambient temperature and stirred overnight. The reaction was worked up by cooling to 0 °C, adding saturated ammonium chloride solution (30 ml), ether (30 ml), and filtering through dicalite. The layers were separated and the aqueous layer was back extracted with ether (2x35 ml). The combined organic solvents were washed with brine (30 ml), dried (anhydrous K_2CO_3) and concentrated under reduced pressure to yield an oil (5.22 g, 84%): R_f=0.40 (SiO_2, 70:30 n-heptane/THF); ir (film) ν_{max}: 3300, 2920, 1440, 1380, 1150, 1000, 710 cm^{-1}; ^{1}H nmr ($CDCl_3$)δ: 7.16 (d, 2H, J=5.2 Hz), 6.80 (d, 2H, J=5.2 Hz), 6.30 (d, 1H, J=3.7 Hz), 2.45 (d, 1H, J=3.7 Hz), 2.20 (s, 3H); ^{13}C nmr ($CDCl_3$) δ: 138.05, 134.61, 129.69, 124.18, 60.00, 13.77; *ms m/z (EI)*: 224(100), 207(55), 125(45), 99(40). Alcohol forms the ether dimer readily: Ir (film) ν_{max}: 2860, 1455, 1435, 1150, 1040, 1020 cm^{-1}; ^{1}H nmr δ ($CDCl_3$): 7.18 (d, 1H, J=5.1 Hz), 6.78 (d, 1H, J=4.8 Hz), 6.04 (s, 1H), 2.03 (s, 3H).

Synthesis of Bis(3-methylthien-2-yl)methanone (IV): Bis(3-methylthien-2-yl)methanol (5.22 g, 0.023 mol) was dissolved in dichloromethane (90 ml) and pyridinium chlorochromate on alumina (38.88 g, 0.039 mol)[9] was added. The mixture was stirred vigorously for 45 min and filtered through a pad of silica gel. The filter cake was washed with dichloromethane (500 ml). The combined organic solutions were concentrated in vacuo to yield an oil which was further purified by flash chromatography to yield **(IV)** (4.27 g, 81%). ^{1}H Nmr δ (CDCl$_3$): 2.45 (s, 6H), 6.90 (d, 2H, J=5.0 Hz), 7.40 (d, 2H, J=4.5 Hz); ms m/z (EI): 222.

On large scale (90 g), an alternate literature procedure[10] was used. To obtain an analytically pure crystalline sample of the ketone, the distilled product was purified by flash chromatography using n-heptane as eluent. ^{1}H Nmr δ (CDCl$_3$): 2.45 (6H, s), 6.95 (d, 2H, J=5.0 Hz), 7.4 (d, 2H, J=4.5 Hz). Anal. Calcd for $C_{11}H_{10}S_2O$: C, 59.43; H, 4.53; S, 28.84. Found: C, 59.56; H, 4.73; S, 28.39. mp 36 °C.

Synthesis of 1-(2-Carboxyethyl)piperidine-3-carboxylic Acid Hydrochloride (V•HCl): To a mixture of ethyl piperidine-3-carboxylate (10.0 g, 0.064 mol) and triethylamine (10.0 g, 0.10 mol) in toluene (20 ml), ethyl 3-bromopropionate (11.6 g, 0.064 mol) was added over 30 min at ambient temperature. After stirring overnight, ether (100 ml) was added and triethylamine hydrobromide was filtered off. The filtrate was washed with water (2x30 ml) and saturated NH_4Cl solution (20 ml) and water (30 ml). The organic phase was dried with $MgSO_4$. Evaporation of the solvents yielded an oil (16.4 g, 100%) identified as ethyl 1-(2-ethoxycarbonyl)ethylpiperidine-3-carboxylate: ^{1}H Nmr (CDCl$_3$, 200 MHz) δ: 4.13 (q, 2H, J=6.9 Hz), 4.12 (q, 2H), 1.3-3.1 (m, 13H), 1.25 (t, 6H).

The above was suspended in 4N HCl (150 ml) and refluxed for 2 h. Evaporation of the solvents yielded (V) as a white powder (15.2 g, 100%): ^{1}H Nmr (DMSO-d$_6$, 200 MHz) δ: 9.00-13.50 ((br), 3H), 2.70-3.70 (m, 9H), 1.30-2.20 (m, 4H); ^{13}C nmr (DMSO-d$_6$, 200 MHz) δ: 21.5, 24.8, 28.4, 38.8-40.7; (DMSO-d$_6$ + 1C): 51.1, 51.6, 51.9, 171.5, 172.7. mp 203 °C. Anal. Calcd for $C_9H_{16}NO_4Cl$: C, 45.48; H, 6.78; N, 5.89; Cl, 14.92. Found: C, 45.29; H, 7.10; N, 5.90; Cl, 15.30.

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