Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 2207–2214

BIOORGANIC & MEDICINAL CHEMISTRY

1-Trichloromethyl-1,2,3,4-tetrahydro-β-carboline (TaClo) and Related Derivatives: Chemistry and Biochemical Effects on Catecholamine Biosynthesis†

Gerhard Bringmann,[a,*] Doris Feineis,[a] Ralf God,[a] Karl Peters,[b] Eva-Maria Peters,[b] Joachim Scholz,[c] Franz Riederer[c] and Andreas Moser[c,*]

[a]*Institut für Organische Chemie, Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany*
[b]*Max-Planck-Institut für Festkörperforschung, Heisenbergstraße 1, D-70506 Stuttgart, Germany*
[c]*Klinik für Neurologie, Universitätsklinikum Lübeck, Ratzeburger Allee 160, D-23538 Lübeck, Germany*

Received 11 October 2001; accepted 31 January 2002

Abstract—1-Trichloromethyl-1,2,3,4-tetrahydro-β-carboline (TaClo, **2**) is a mammalian alkaloid that readily originates in the human organism, by Pictet–Spengler condensation of endogenously present tryptamine (Ta) and the non-natural hypnotic agent trichloroacetaldehyde (chloral, Clo). Due to its structural analogy to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **1**), TaClo is discussed to possibly contribute to the pathogenesis of Parkinson's disease acting as an environmental toxin. Previous investigations on rats and neuronal cell cultures revealed **2** to be capable of inducing severe disturbances on the dopamine metabolism. In this paper, we report on the effects of **2** on the activity of tyrosine hydroxylase [L-tyrosine, tetrahydropteridine/oxygen oxidoreductase (3-hydroxylating), EC 1.14,16.2; TH] in vitro using rat brain homogenates prepared from the TH-rich nucleus accumbens. TaClo (**2**) dose-dependently inhibited basal TH activity ($IC_{50} = 3\,\mu M$), and after enzyme activation by pituitary adenylate cyclase-activating polypeptide (PACAP-27), it also reduced L-DOPA formation ($IC_{50} = 15\,\mu M$). Moreover, two presumable TaClo metabolites, 2-methyl-TaClo (*N*-Me-TaClo, **3**) and 1-dichloromethylene-1,2,3,4-tetrahydro-β-carboline (1-CCl_2-THβC, **4**), which were synthesized in good yields, also proved to be potent inhibitors of TH, with the strongest effect on basal activity (similar to TaClo) being observed for **3** ($IC_{50} = 3\,\mu M$). In contrast to TaClo, however, **3** and **4** showed biphasic effects after TH activation with PACAP-27, inducing a marked increase of enzyme activity in the nanomolar range ($< 0.1\,\mu M$), while TH activity was nearly completely blocked at high concentrations ($IC_{100} = 0.1$ mM). An X-ray diffraction investigation on the 3-dimensional structure of the 1-CCl_2-THβC-derived trifluoroacetamide **7** revealed the voluminous and quite rigid dichloromethylene substituent to be only moderately twisted out of the β-carboline ring 'plane', thus resulting in an inreased ring strain of the partially hydrogenated pyrido moiety accompanied by a strong steric hindrance of Cl(1), Cl(2), C(13), and N(2), which pushes the *N*-trifluoroacetyl group upwards to an even higher extent than for the TaClo-related trifluoroacetamide **8**.

Introduction

Since 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **1**) (Fig. 1), a contaminant of 'synthetic heroin', has been reported to be a potent neurotoxin that induces parkinsonism in humans,[1–3] environmental compounds structurally similar to MPTP have been suggested to contribute to cell death of dopaminergic neurons and to act as inhibitors of the enzymes related to catecholamine metabolism. Various xenobiotic agents, among them alkanes,[4,5] heavy metals,[6,7] pesticides (e.g., rotenone),[8,9] and, in particular, endogenously formed isoquinolines[10–12] and β-carbolines[13–15] have been discussed to initiate nigrostriatal degeneration in Parkinson's disease.

Highly chlorinated tetrahydro-β-carbolines (THβCs), which are characterized by a huge lipophilic and radical inducing CCl_3 moiety at C(1), constitute structurally most unusual representatives of mammalian alkaloids exhibiting a strong impairment of the dopaminergic system.[16,17] We mainly focus on 1-trichloromethyl-

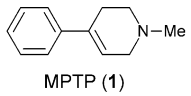
*Corresponding authors. G. Bringmann. Tel.: +49-931-888-5323; fax: +49-931-888-5323; A. Moser. Tel.: +49-451-500-2930; fax: +49-451-500-2489; e-mail: bringman@chemie.uni-wuerzburg.de; moser_a@neuro.mu-luebeck.de

†Part 39 in the series, Endogenous Alkaloids in Man; for part 38, see ref 24.

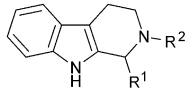
0968-0896/02/$ - see front matter
PII: S0968-0896(02)00060-3

1,2,3,4-tetrahydro-β-carboline (TaClo, **2**)[16,18–20] (Fig. 1), a progressively acting neurotoxin[21,22] that was found to be formed in humans in trace amounts, by Pictet–Spengler condensation of the biogenic amine tryptamine ('Ta') and therapeutically administered chloral hydrate ('Clo').[23,24] Animal studies on rats revealed TaClo to easily penetrate the blood–brain barrier[20,25] and to display distinct toxic effects towards dopaminergic[17,26] and serotonergic[27] neurons. As demonstrated by pulse voltammetric measurements,[16,17,26] a unilateral single intranigral injection of TaClo severely affected the striatal dopamine metabolism with marked biochemical disturbances to be observed one week after toxin application. The impairment of the dopaminergic system was even more pronounced three and six weeks after TaClo administration. Moreover, exposure of TaClo to dopaminergic neurons in primary cell cultures[28,29] resulted in a significant decrease in dopamine uptake at concentrations of 25 μM and higher, and led to a marked loss of tyrosine hydroxylase (TH) immunoreactive neurons. Besides TaClo itself, two related derivatives and presumable metabolites, 2-methyl-TaClo (*N*-Me-TaClo, **3**) and 1-dichloromethylene-THβC (1-CCl_2-THβC, **4**) (Fig. 1), also turned out to strongly damage TH positive neurons, as became obvious from a dose-dependent inhibition of dopamine uptake accompanied by a significant reduction of cell number and size.[29]

Since tyrosine hydroxylase [L-tyrosine, tetrayhydropteridine:oxygen oxidoreductase (3-hydroxylating), EC 1.14,16.2; TH] is known to be the rate-limiting enzyme of catecholamine biosynthesis,[30,31] TaClo (**2**) and its analogues **3** and **4** have been studied more closely for their interaction with TH in vitro using homogenized tissue of the rat nucleus accumbens, which was proven to be an excellent enzyme source[32] of high TH activities.



MPTP (**1**)



Compd	R^1	R^2	
2	CCl_3	H	TaClo
3	CCl_3	Me	*N*-Me-TaClo
4	$=CCl_2$	H	1-CCl_2-THβC

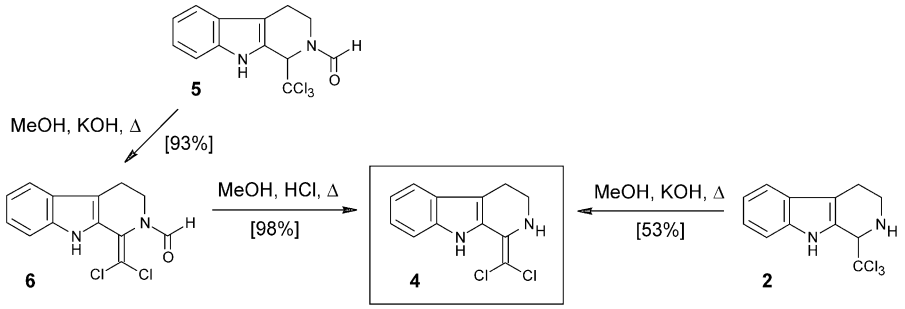
Figure 1. The dopaminergic neurotoxin MPTP (**1**), the structurally closely related highly chlorinated mammalian alkaloid TaClo (**2**), and its presumable metabolites, *N*-Me-TaClo (**3**) and 1-CCl_2-THβC (**4**).

This paper deals with the evaluation of the inhibitory capacities of **2**, **3**, and **4** towards TH, besides reporting on appropriate synthetic pathways leading to **3** and **4**. Furthermore, a comparative X-ray diffraction study on the three-dimensional structures of the trifluoroacetyl derivatives of TaClo (**2**) and 1-CCl_2-THβC (**4**) was performed to investigate the steric demand of their chlorine-containing substituents at C-1, in particular, looking at the influence of the quite flexible trichloromethyl group versus the more rigid dichloromethylene moiety on the conformation of the tetrahydropyrido ring system.

Results and Discussion

Chemistry

By its still closer structural analogy to MPTP (**1**), the *N*-methylated compound **3** warranted a more detailed investigation on its mode of action and its presumable occurrence in vivo as a TaClo metabolite. It was easily prepared in good yields (>60%) by treatment of TaClo (**2**) with methyl iodide in the presence of sodium hydrogencarbonate. β-Elimination of HCl from **2** generated the THβC **4**, a probable in vivo TaClo degradation product, which was found to exhibit by far the most lipophilic character within these highly chlorinated THβCs **2**–**4** described in our study. 1-CCl_2-THβC (**4**) was synthesized following a rational two-step reaction sequence (see Scheme 1) via its formamide **6**, giving rise to analytically completely pure, crystalline material of **4**. Starting from *N*-formyl-TaClo (**5**), a most useful stable stock compound in TaClo synthesis,[16,18] the intermediate **6** was obtained by elimination of hydrochloric acid in refluxing methanol under mild basic conditions. Finally, smooth *N*-deformylation of **6** in methanolic hydrochloric acid yielded the desired target molecule 1-CCl_2-THβC (**4**) as its hydrochloride salt in an excellent overall yield of about 91%. The likewise attempted direct dehydrohalogenation of TaClo (**2**) (see Scheme 1), by contrast, gave less favorable yields (≤53%).



Scheme 1. Two alternative synthetic pathways to 1-CCl_2-THβC (**4**).

X-ray crystallography

With respect to the strong interaction of TaClo (**2**) and its presumable metabolites **3** and **4** with enzymes related to energy metabolism[17,29] and catecholamine biosynthesis[16,17,29] (see also next section), the knowledge of the steric effects exhibited by a voluminous trihalogenmethyl or a dichloromethylene group at C(1) on the conformation of a tetrahydro-β-carboline ring system seemed desirable. In contrast to the authentic bioactive compound **4**, which unfortunately did not give crystals suited for an X-ray investigation, the trifluoroacetamide **7** as obtained from **4** by trifluoracetylation did afford material of sufficient quality for this purpose. As expected, due to the additional sp^2-center at C-1 of the 1-CCl_2-THβC derivative **7** as compared to the TaClo derivative **8**, on which we reported previously,[17] the partially hydrogenated pyrido part of **7** is partially planarized adopting a half-chair conformation, with only C(3) and N(2) significantly located out of the β-carboline ring 'plane' (see Fig. 2). The dichloromethylene substituent at C(1) of **7**, in contrast to the CCl_3 group of **8**,[17] was found to be twisted out of the ring system only to a relatively small degree. This becomes manifest from the interplanar angle between C(5)–C(13)–C(1) and the plane C(13)–C(1)–C(14), which is only 24.4°, whereas the CCl_3 group of **8** occupies a nearly pseudo-axial position, with a significantly larger interplanar angle of 64.3° between the planes C(5)–C(13)–C(1) and C(13)–C(1)–C(14). While in **8** the three chlorine atoms at C(14) adopt a perfectly staggered orientation, thus resulting in a minimization of their steric interactions with C(13) and the *N*-trifluoroacetyl function, the more rigid dichloromethylene moiety of **7** leads to an enhanced ring strain of the six-membered heterocyclic part, accompanied by a high steric hindrance of the atoms Cl(1), Cl(2), C(13), and N(2), also influenced by the large van der Waals radius of chlorine (1.8 Å). As illustrated in Figure 2(b), the increased steric effects on the THβC framework exhibited by the $=CCl_2$ group as compared to those by the CCl_3 substituent are best visualized by a match plot of the crystal structures of **7** and **8**, demonstrating the trifluoracetyl substituent at N(2) to be pushed upwards by the demanding substituent at C-1 for **8** and, to an even higher degree, for **7**. The strain that, in turn, the *N*-trifluoroacetyl group exerts on the C-1 substitutent, even leads to a slight distortion of the—otherwise planar—C(1)=C(14) alkene system, as seen from the dihedral angles N(2)–C(1)–C(14)–Cl(1) (1.5°) and C(13)–C(1)–C(14)–Cl(2) (3.7°), and more significantly, from the combination of these effects, the interplanar angle between the planes C(13)–C(1)–N(2) and Cl(2)–C(14)–Cl(1), which amounts to 5.6° in a *P*-helical manner.

Inhibition of tyrosine hydroxylase (TH) activity by TaClo (2) and its derivatives 3 and 4

Tyrosine hydroxylase (TH; EC 1.14.16.2) catalyzes the first step in catecholamine biosynthesis by hydroxylating L-4-hydroxyphenylalanine (L-tyrosine) to L-3,4-dihydroxyphenylalanine (L-DOPA).[30,31,33] Inhibition of this physiologically important enzyme is suggested to be one of the common characters of dopaminergic neurotoxins.[33–36] Since some of the acute symptoms caused by the parkinsonism-inducing agent MPTP (**1**) are attributed to the ability of **1** to massively reduce both, striatal TH activity and TH protein synthesis,[37,38] studies on the regulatory mode of action of naturally occurring TH inhibitors have gained increasing relevance. Isoquinolines and β-carbolines resembling the structure of MPTP are interesting candidates for endogenously present compounds that might possibly affect tyrosine hydroxylation.[36] Indeed, several tetrahydroisoquinolines among them salsolinol[34,35] and *N*-methyl-norsalsolinol[39] are known to strongly act on TH activity in vitro and to induce reduction of TH expression in vivo (e.g., *N*-methyl-tetrahydroisoquinoline[40]).

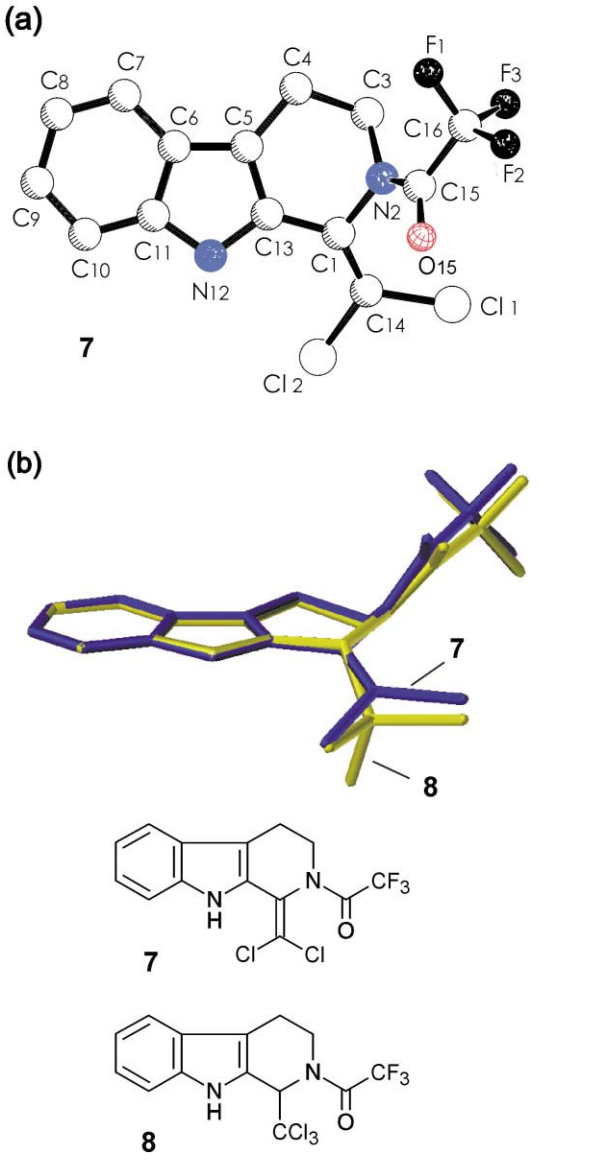


Figure 2. (a) SCHAKAL plot of the crystal structure of *N*-trifluoroacetyl-1-dichloromethylene-1,2,3,4-tetrahydro-β-carboline (**7**) (viewed peripendicular) with a guide of the atomic numbering system adopted in the X-ray investigation. (b) Joint plot of the structures of **7** (blue) and the TaClo-derived *N*-trifluoroacetyl derivative **8** (yellow) (cf, lit.[17]) in the crystal, matched with respect to the indole part of the molecules, viewed horizontal to the tetrahydropyrido ring planes (hydrogen atoms omitted for reasons of clarity).

Surprisingly, only few and in part contradictory results have been reported on the interaction of β-carbolines with TH.[36] For example, *N*-methyl-β-carbolinium ion was shown to cause TH inhibition,[41] while, by contrast, the benzodiazepine inverse agonist *N*-methyl-β-carboline-3-carboxamide was found to stimulate TH activity in the rat prefrontal cortex.[42]

Employing TH-rich homogenates of the rat nucleus accumbens as the enzyme source, the chloral-derived THβC TaClo (**2**) was found to exert a concentration-dependent reduction of basal enzyme activity (11 pmol L-DOPA/min/mg protein), with an IC_{50} value of approximately 3 μM (see Fig. 3 and Table 1). Addition of 0.1 mM of **2** to the incubation mixture resulted in a complete TH inhibition. Comparable to the parent compound **2**, its *N*-methylated derivative **3** also displayed dose-dependently a pronounced inhibitory effect on TH in the micromolar range ($IC_{50} = 3$ μM, see Table 1). Interestingly, the degree of basal TH inhibition induced by **2** and **3** was similar to that measured for the tetrahydroisoquinoline alkaloid *N*-methyl-norsalsolinol ($IC_{50} = 10$ μM) in our assay, as reported earlier.[39] And even for the artificial neurotoxin MPTP (**1**), a nearly identical trend has been described for its inhibitory capacity on TH in tissue slices of the rat nucleus accumbens, again with an IC_{50} value of approximately 10 μM.[37]

Different from **2** and **3**, however, 1-CCl_2-THβC (**4**) markedly enhanced TH activity in low concentrations varying between 1 nM and 10 μM, with a maximal increase of 300% over basal activity at a concentration of 0.1 μM. Interestingly, at concentrations higher than 10 μM, **4** was shown to significantly inhibit the enzyme. TH activity was reduced by 50% at approximately 20 μM (IC_{50}, see Table 1), and the enzyme was nearly completely blocked at a concentration of 0.1 mM.

As reported earlier,[43] we have demonstrated that pituitary adenylate cyclase-activating polypeptide (PACAP-27) is capable of enhancing TH activity in homogenate preparations of the rat nucleus accumbens through receptor-mediated cAMP formation assisted by proteinkinase A (PKA). For example, TH activation produced by PACAP-27 (1 μM) in the presence of the adenosine nucleotide App(NH)p (0.1 mM) led to a maximal increase of 520–550% over basal TH activity. Moreover, with respect to the fact that PACAP-27 takes part in the short-term TH regulation in dopaminergic neurons,[43] we examined the effects exhibited by the THβCs **2**–**4** on PACAP-activated TH in this study, too.

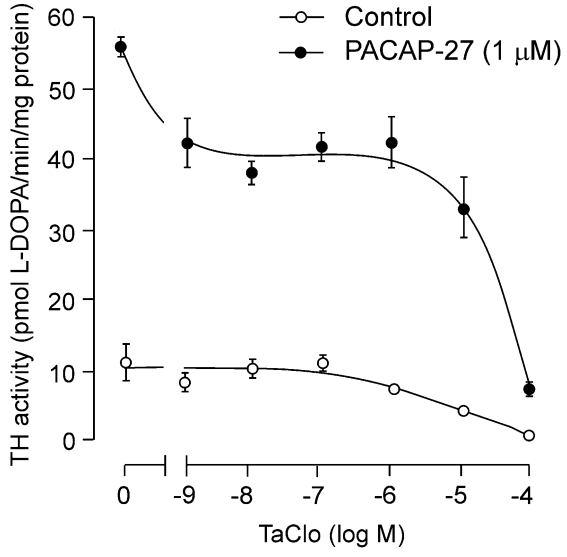


Figure 3. The effect of TaClo (**2**) on TH activity in the absence (○) and presence (●) of PACAP-27 (1 μM). $N = 6$ for each experimental condition.

As outlined in Table 1, besides being an effective inhibitor of basal tyrosine hydroxylation, TaClo (**2**) also distinctly reduced TH activity in homogenate preparations of the nucleus accumbens preincubated with 1 μM of PACAP-27. In this assay, the IC_{50} value for **2** was determined to be 15 μM, while a total inhibition of PACAP-activated TH was achieved at 0.1 mM (cf., Fig. 3). By contrast, when *N*-Me-TaClo (**3**) was added to the PACAP-activated incubation medium in nanomolar quantities (1 nM up to 1 μM), a strong enhancement of enzymatic L-DOPA formation was observed, with a maximal increase of 700% over basal TH activity at 0.3 μM. An even more pronounced stimulating effect on enzyme activity was found to be displayed by 1-CCl_2-THβC (**4**): Similar to **3**, **4** also strongly increased PACAP-induced L-DOPA production at concentrations in the nanomolar range (1 nM up to 100 nM), with a maximum of 790% increase over basal TH activity at 10 nM (see Table 1). At high concentrations of **3** and **4**, however, both agents proved to be potent inhibitors of PACAP-activated TH, discernible from IC_{50} values of 9 μM for **3**, and 5 μM for **4**. For both of these THβCs, total TH inhibition was achieved at a concentration of 0.1 mM. Thus, taken together, **3** and **4** produced biphasic effects on TH activated by PACAP-27. Both compounds induced a substantial enhancement of enzyme activity at low and caused a strong inhibition at high THβC concentrations.

Outlook

TH is known to underly diverse short- and long-term regulatory mechanisms, involving feedback inhibition and enzyme phosphorylation at the short-term level, and transcriptional and translational mechanisms at the

Table 1. The inhibitory effect of TaClo (**2**), 2-Me-TaClo (**3**), and 1-CCl_2-THβC (**4**) on TH activity (IC_{50}) in the absence (basal) and presence of PACAP-27 (1 μM)

Compd	TH activity (basal) in the absence of PACAP-27 IC_{50} (μM)	TH activity in the presence of PACAP-27		
		IC_{50} (μM)	CA_{max}[a] (μM)	A_{max}[b] (%)
2	3	15	—	—
3	3	9	0.3	700
4	20	5	0.01	790

$N = 6$ for each experimental condition.
[a]CA_{max}, concentration (μM) of the tetrahydro-β-carbolines **2**, **3**, and **4** at maximal enzyme activation.
[b]A_{max}, maximal enzyme activation (%) over basal TH activity (100%).

long-term level (for a review see lit.[44]). Since PACAP-27 was found to activate TH through PKA-dependent phosphorylation of the regulatory N-terminus of the enzyme,[45] the results described above led us to suggest that **2**, **3**, and **4** interact with the PKA-TH complex. Although some trends are evident, nonetheless, detailed investigations on the mode of action considering both, the stimulating and the inhibitory effects on tyrosine hydroxylation displayed by these chloral-derived THβCs **2**–**4**, are sill missing. Further work is in progress.

Experimental

General methods

Melting points (uncorrected) were determined on a Reichert-Jung Thermovar hot-stage apparatus. Infrared spectra (IR) were obtained on a Perkin–Elmer Model 1420 spectrophotometer. KBr refers to a potassium bromide disk for infrared spectra. Proton and carbon-13 spectra were recorded on a Bruker AC 250 spectrometer at 250 MHz (for ^{1}H NMR), and at 63 MHz (for ^{13}C NMR). Chemical shifts (δ) are reported in parts per million (ppm), and are referenced to internal chloroform (^{1}H, δ = 7.26 ppm), acetone (^{1}H, δ = 2.01 ppm), methanol (^{1}H, δ = 3.33 ppm; ^{13}C, δ = 49.02 ppm) or dimethylsulfoxide (^{13}C, δ = 39.43 ppm) in the deuterated solvents. Coupling constants (*J*) are given in Hertz (Hz). For mass spectrometry (electron ionization, 70 eV), a Finnigan MAT 8200 instrument was used. The peaks listed are those arising from the molecular ion $[M]^{+\bullet}$, those attributable to loss of certain fragments (M^+ minus a fragment), and some other prominent peaks. Elemental analyses were performed by the Microanalysis Laboratory of the Institute of Inorganic Chemistry (University of Würzburg) on a Carlo Erba Elemental Analyzer M 1106 apparatus.

Materials

All reagents used were of commercial quality. NSD 1015 and pituitary adenylate cyclase-activating polypeptide (PACAP-27) were purchased from Research Biochemicals International (Cologne, Germany), 6,7-dimethyl-5,6,7,8-tetrahydropterin ($DMPH_4$) from Sigma-Aldrich GmbH (Deisenhofen, Germany). Organic solvents were dried and distilled prior to use. The petroleum ether used had a boiling range of 30–70 °C. 1-Trichloromethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole hydrochloride (**2**·HCl) was prepared from tryptamine and trichloroacetaldehyde (chloral) via 2-formyl-1-trichloromethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (**5**)[18] as described previously.[16,18] Reactions were monitored by thin-layer chromatography (TLC) on aluminum plates coated with silica gel 60 F_{254} (Merck, Darmstadt, Germany). Column chromatography was performed on Merck silica gel (0.063–0.200 mm).

2-Methyl-1-trichloromethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole hydrochloride (3·HCl). To a suspension of **2**·HCl[16,18] (1.00 g, 3.07 mmol) and sodium hydrogencarbonate (1.00 g, 11.9 mmol) in dry methanol (40 mL), methyl iodide (2.00 mL, 4.54 g, 32.0 mmol) was added. The methylation reaction was allowed to proceed in the dark at room temperature with intensive stirring for 3 days. The solution was then concentrated in vacuo, and the residue was purified by column chromatography on silica gel (eluent: petroleum ether/*tert*-butyl methyl ether, 2:1). Fractions containing the desired pure product were combined, and treated with methanol saturated with HCl. The solvent was removed under reduced pressure affording **3**·HCl (670 mg, 1.97 mmol, 64% yield) as a pale yellow amorphous solid: mp 96 °C (dec). IR (KBr, cm^{-1}) 3330 (indole NH), 2900 (CH), 2650, 1450, 1430, 1410 (CH), 815, 750 (CCl); ^{1}H NMR (CD_3OD): δ 3.18 (s, 3H, NCH_3), 3.26–3.33 (m, 2H, 4-H, overlay by solvent), 3.70 (dt, J_{gem} = 13.1 Hz, *J* = 4.5 Hz, 1H, 3-H), 4.31 (dt, J_{gem} = 13.1 Hz, *J* = 8.8 Hz, 1H, 3-H), 6.05 (s, 1H, 1-H), 7.11–7.17 (m, 1H, 6-H or 7-H), 7.24–7.31 (m, 1H, 6-H or 7-H), 7.48–7.52 (m, 1H, 5-H or 8-H), 7.59–7.63 (m, 1H, 5-H or 8-H); ^{13}C NMR (CD_3OD): δ 16.1 (C-4), 43.8 (C-3), 46.6 (C-1), 72.3 (CH_3), 111.3 (CCl_3), 111.8, 118.3, 118.7, 122.2, 125.3, 136.6; MS (EI, 70 eV) *m*/*z* (rel int) 308/306/304/302 (0.1/0.3/1.3/1.4) $[M]^{+\bullet}$, 270/268/266 (0.5/1.9/3.0) [M−HCl], 234/232 (2.3/7.8) [M−2 Cl], 185 (100) [M−CCl_3]. Anal. Calcd for $C_{13}H_{13}Cl_3N_2$·HCl: C, 45.91; H, 4.15; N, 8.24. Found: C, 45.80; H, 4.32; N, 7.96.

1-Dichloromethylene-2-formyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (6). To a stirred suspension of **5**[18] (1.00 g, 3.15 mmol) in methanol (50 mL), a solution of potassium hydroxide (1.00 g, 17.8 mmol) in methanol (30 mL) was cautiously added over a period of 30 min. The reaction mixture was heated at reflux for 15 min with intensive stirring, then cooled to room temperature, and evaporated to dryness. Purification of the crude product by filtration through a short silica gel column (eluent: petroleum ether/*tert*-butyl methyl ether, 2:1), and crystallization from methanol yielded **6** (0.82 g, 2.92 mmol, 93% yield) as beige-colored crystals: mp 187 °C (dec). IR (KBr, cm^{-1}) 3280 (indole NH), 3040, 2940, 2900, 2830 (CH), 1660 (C=O), 1445, 1395 (CH_2), 750 (CCl); ^{1}H NMR (C_3D_6O): occurrence of two distinct rotational isomers A and B, ratio 3:1. Isomer A: δ 2.92 (m_c, 2H, 4-H), 4.06 (m_c, 2H, 3-H), 7.04–7.12 (m, 1H, 6-H, 7-H, overlay by isomer B), 7.18–7.27 (m, 1H, 6-H or 7-H, overlay by isomer B and solvent), 7.48–7.57 (m, 2H, 5-H, 8-H, overlay by isomer B), 8.44 (s, 1H, NC*H*O). Isomer B: δ 3.01–3.07 (m, 2H, 4-H), 3.65–3.78 (m, 1H, 3-H), 4.34–4.42 (m, 1H, 3-H), 7.04–7.12 (m, 1H, 6-H or 7-H, overlay by isomer A), 7.18–7.27 (m, 1H, 6-H or 7-H, overlay by isomer A), 7.48–7.57 (m, 2H, 5-H, 8-H, overlay by isomer A), 8.29 (s, 1H, NC*H*O); ^{13}C NMR [$(CD_3)_2SO$]: Isomer A: δ 20.9 (C-4), 44.9 (C-3), 110.1 (C-1), 112.5, 115.1 (CCl_2), 118.9, 119.7, 124.0, 125.1, 137.2, 163.3 (C=O). Isomer B: δ 22.9 (C-4), 44.2 (C-3), 109.3, 112.4, 114.0, 118.8, 119.6, 123.8, 125.0, 137.1, 160.7 (C=O); MS (EI, 70 eV) *m*/*z* (rel int) 284/282/280 (2.3/13/19) $[M]^{+\bullet}$, 247/245 (38/100) [M−Cl], 219/217 (4.7/13) [247/245−CO], 181 (44) [219/217−HCl]. Anal. Calcd for $C_{13}H_{10}Cl_2N_2O$: C, 55.54; H, 3.59; N, 9.96. Found: C, 55.58; H, 3.77; N, 9.71.

1-Dichloromethylene-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole hydrochloride (4·HCl). Method A. To a well-stirred solution of **2**·HCl[16,18] (2.00 g, 6.14 mmol) in methanol (100 mL), a suspension of potassium hydroxide (2.00 g, 35.7 mmol) in methanol (60 mL) was slowly added. The reaction mixture was heated at reflux for 15 min, then cooled to room temperature, and filtered through Celite to remove a precipitate of potassium chloride. The solvent was removed under reduced pressure, and the brown residue was purified by column chromatography on silica gel (eluent: petroleum ether/*tert*-butyl methyl ether, 2:1). Saturated methanolic HCl was added to the combined fractions. After evaporation to dryness, the crude product was recrystallized from methanol/diethyl ether affording **4**·HCl (0.94 g, 3.25 mmol, 53% yield) as an orange-colored crystalline powder, mp 175 °C (dec.). IR (KBr, cm^{-1}) 3400 (indole NH), 2850 (NH), 1620 (C=C), 1530 (aryl C=C), 780, 760 (CCl); ^1H NMR ($CDCl_3$): δ 2.89 (m_c, 2H, 4-H), 3.91 (m_c, 2H, 3-H), 7.02–7.15 (m, 1H, 6-H), 7.18–7.33 (m, 1H, 7-H), 7.43–7.64 (m, 2H, 5-H, 8-H); ^{13}C NMR (CD_3OD): δ 20.1 (C-4), 44.6 (C-3), 107.8, (C-1), 114.6 (CCl_2), 122.4, 123.3, 123.7, 124.0, 131.3, 132.3, 176.2; MS (EI, 70 eV) *m/z* (rel int) 256/254/252 (4.6/28/47) $[M]^{+\bullet}$, 219/217 (32/100) [M−Cl], 181 (28) [M− HCl−Cl]. Anal. Calcd for $C_{12}H_{11}Cl_2N_2$·HCl: C, 49.77; H, 3.83; N, 9.67. Found: C, 49.55; H, 3.84; N, 9.46.

Method B. Concentrated HCl (1.8 mL) was added to a solution of **6** (800 mg, 2.85 mmol) in methanol (20 mL). The mixture was refluxed for 15 min. The residue obtained after removal of the solvent was recrystallized from methanol/diethyl ether to give **4**·HCl (808 mg, 2.79 mmol, 98% yield) as orange-colored crystals, mp 174 °C (dec). All spectroscopic data are in agreement with those reported above.

1-Dichloromethylene-2-trifluoroacetyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (7). To a suspension of 4·HCl (100 mg, 0.35 mmol) in dry dichloromethane (15 mL), triethylamine (0.20 mL, 132 mg, 1.10 mmol) was added. The mixture was treated dropwise with trifluoroacetic anhydride (0.12 mL, 181 mg, 0.86 mmol) at 0 °C, then stirred at room temperature for 2 h, and extracted with water (3×). The organic phases were dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. Purification of the residue by crystallization from methanol/petroleum ether provided the title compound (99 mg, 0.28 mmol, 82% yield) as pale yellow crystals, suitable for X-ray structure analysis: mp 232 °C (dec). IR (KBr, cm^{-1}) 3330 (indole NH), 3040, 2900, 2840 (CH), 1685 (C=O), 1195, 1180, 1140 (CN, CF), 1040, 745; ^1H NMR ($CDCl_3$): δ 2.95–3.03 (m, 1H, 4-H), 3.09–3.23 (m, 1H, 4-H), 3.70–3.82 (m, 1H, 3-H), 4.47–4.55 (m, 1H, 3-H), 7.12–7.19 (m, 1H, 6-H or 7-H), 7.26–7.33 (m, 1H, 7-H or 6-H, overlay by solvent), 7.38–7.41 (m, 1H, 5-H or 8-H), 7.49–7.52 (m, 1H, 8-H or 5-H), 8.90 (br. s, 1H, indole NH); ^{13}C NMR ($CDCl_3$): δ 23.6 (C-4), 46.7 (C-3), 111.6 (C-1), 114.2 (CCl_2), 119.1 (CF_3), 120.7, 120.8, 124.8, 125.5, 125.6, 131.3, 136.1; MS (EI, 70 eV) *m/z* (rel int) 352/350/348 (2.5/13/20) $[M]^{+\bullet}$, 315/313 (32/100) [M−HCl], 249 (26), 154 (60). Anal. Calcd for $C_{14}H_9Cl_2F_3N_2O$: C, 48.16; H, 2.60; N, 8.02. Found: C, 48.17; H, 2.63; N, 7.89.

Single-crystal X-ray diffraction analysis of 7. The crystal chosen for X-ray investigations was a clear pale yellow lath with the approximate dimensions 0.20×0.65×0.05 mm. Data were collected on a Siemens R3m/V four-circle diffractometer using graphite monochromatic Mo-K_α radiation (λ = 0.71073 Å) in ω-scan mode in the range of $1.75° < \theta < 27.5°$.[46] $C_{14}H_9Cl_2F_3N_2O$ (349.14 g mol^{-1}) crystallizes in the triclinic system, space group $P\bar{1}$, with $a = 9.002$ (2), $b = 10.267$ (3), $c = 8.811$ (3) Å, $\alpha = 109.73$ (3)°, $\beta = 109.87$ (3)°, $\chi = 84.91$ (2)°, $V = 720.7$ (4) $Å^3$, $Z = 2$, $\mu(\text{Mo-}K_\alpha) = 0.49\ mm^{-1}$, and $D_{calcd} = 1.609\ g\ cm^{-3}$. Unit cell parameters were determined by least-squares refinement using 60 centered reflections within $8.6° < \theta < 14.4°$. A total of 3313 reflections were collected to $2\theta_{max} = 55°$ (*h*: −11→10, *k*: −13→12, *l*: 0→11) of which 3313 were unique. The structure was solved by direct phase determination and refined by full-matrix anisotropic least-squares with the aid of the program SHELXL-97.[47] In refinements, weights were used according to the scheme $w = 1/[\sigma^2(F_o)]$. The refinement converged to the final agreement factors $R = 0.044$, and $R_w = 0.042$, for 204 parameters and 2646 observed reflections with $F > 3\sigma(F)$; data-to-parameter ratio being 12.97. The electron density of the largest difference peak was found to be 0.29 $eÅ^{-3}$, while that of the largest difference hole was 0.22 $eÅ^{-3}$. All non-hydrogen atoms were refined anisotropically. The hydrogen positions were calculated using a riding model and were considered fixed with isotropic thermal parameters in all refinements. In the crystals of **7**, the molecules are connected by intermolecular hydrogen bonds H(12)···O(15) [2.17 Å]—direction [1$\bar{1}$0]—forming 'dimeric' arrays. Tables of bond distances and angles, atomic coordinates, and anisotropic thermal parameters for **7** (CCDC 171412) have been deposited with the Cambridge Crystallographic Data Centre.[48] Software used to prepare material for publication: SCHAKAL 88.[49]

Neurochemical assay for the determination of tyrosine hydroxylase (TH) activity

As described previously,[39] female Wistar rats (age: ca. 2 months, body weight: 150–200 g) were sacrificed by decapitation, the brains were immediately removed, and stored at −40 °C prior to preparation of homogenates from the nucleus accumbens serving as the TH source. The nucleus accumbens was identified according to De Groot.[50] For each experimental procedure, one nucleus accumbens of each of two different rats was used to reduce interindividual differences.[51] Brains were homogenized by 3×10 strokes with 10 μL of an ice-cold solution of 0.1 M sodium phosphate buffer (pH 7.4) per mg tissue weight. Protein concentrations were determined as reported by Bensadoun and Weinstein[52] using bovine γ-globulin as standard. TH activity was assayed according to Naoi et al.,[53] but modified in the following manner: The assay was composed of 20 μL of homogenate containing approximately 40 μg of protein, 2 mM of ferrous ammonium sulfate, 10 μg of bovine catalase, 50 μM of NSD 1015 as inhibitor of L-3,4-dihydroxy-

phenylalanine (L-DOPA)-decarboxylase (aromatic L-amino acid decarboxylase, EC 4.1.1.28), 10 mM of sodium acetate buffer (pH 6.0), previously gassed with oxygen for 10 min, and the indicated THβCs **2**–**4**. After a preincubation period of 5 min at 37 °C, the assay was started by addition of 0.1 mM of L-tyrosine and 10 μM of 6,7-dimethyl-5,6,7,8-tetrahydropterin ($DMPH_4$) as cofactor. The artificial $DMPH_4$ was employed for these incubation experiments because of its enhanced chemical stability as compared to the natural cofactor tetrahydropterin and due to the fact that no interfering metabolites of the cofactor were monitored by the high-performance liquid chromatography (HPLC) measurements described below. After 10 min at 37 °C, incubation was terminated by addition of 33 mL/L perchloric acid containing 150 nM L-α-methyl-DOPA as the internal standard for analysis by means of HPLC coupled to a electrochemical detector.[39] L-DOPA, the product of enzymatic hydroxylation of L-tyrosine, was monitored on a RP-18 column (Eurospher RP 18, 250×4.0 mm, particle size: 5 μm) using a degassed solution of 0.3 mM of disodium-EDTA, 0.52 mM of 1-sodium-octanesulfonate, 115 mL/L of methanol and 0.1 M of citrate buffer (pH 3.0) as the mobile phase under isocratic conditions.[39] The identity of L-DOPA was assessed in each of the samples by retention times analysis using an authentic solution of L-DOPA. Non-enzymatic formation of L-DOPA was determined applying 0.1 mM D-tyrosine as substrate and 0.1 mM α-methyl-L-para-tyrosine (α-M*p*T) as known inhibitor of TH. Enzymatic L-DOPA formation was calculated by total L-DOPA synthesis minus non-enzymatic formation. All data given in this paper reflect enzymatic L-DOPA synthesis. Values were expressed in picomoles (pmol) L-DOPA/min/mg protein. For statistics, the Superior Performing Software System (SPSS) for Windows, version 3.0 (Microsoft Corporation, Redmond, WA, USA) was used to calculate means ± standard deviations. Results were compared by analysis of variance (ANOVA) or Student's *t*-test where applicable. Results were considered to be of statistical significance, if the calculated *p* value was lower than 0.05.

Acknowledgements

This investigation was supported by the Fonds der Chemischen Industrie. One of us (R.G.) thanks the Freistaat Bayern for a graduate research fellowship according to the 'Graduiertenförderungsgesetz'. Moreover, we wish to thank Dr. H. Friedrich and Dr. R. Brückner for synthetic contributions to this field, and we gratefully acknowledge engaged experimental assistance by M. Michel. Furthermore, we are indebted to Dipl.-Chem. M. Münchbach and Dipl.-Chem. C. Rummey for skillful technical help.

References and Notes

1. Davis, G. C.; Williams, A. C.; Markey, S. P.; Ebert, M. H.; Caine, E. D.; Reichert, C. M.; Kopin, I. J. *J. Psychiatr. Res.* **1979**, *1*, 249.
2. Langston, J. W.; Ballard, P.; Tetrud, J. W.; Irwin, I. *Science* **1983**, *219*, 979.
3. Singer, T. P.; Trevor, A. J.; Castagnoli, N., Jr. *Trends Biochem. Sci.* **1987**, *12*, 266.
4. Pezzoli, G.; Strada, O.; Silani, V.; Zecchinelli, A.; Perbellini, L.; Javoy-Agid, F.; Ghidoni, P.; Motti, E. D.; Masini, T.; Scarlato, G.; Agid, Y.; Hirsch, E. C. *Ann. Neurol.* **1996**, *40*, 922.
5. Pezzoli, G.; Canesi, M.; Antonini, A.; Righini, A.; Perbellini, L.; Barichella, M.; Mariani, C. B.; Tenconi, F.; Tesei, S.; Zecchinelli, A.; Leenders, K. L. *Neurology* **2000**, *55*, 667.
6. Rybicki, B. A.; Johnson, C. C.; Uman, J.; Gorell, J. M. *Mov. Dis.* **1993**, *8*, 87.
7. Montgomery, E. B. *Toxicology* **1995**, *97*, 3.
8. Gorell, J. M.; Johnson, C. C.; Rybicki, B. A.; Peterson, E. L.; Richardson, R. J. *Neurology* **1998**, *50*, 1346.
9. Betarbet, R.; Sherer, T. B.; MacKenzie, G.; Garcia-Osuna, M.; Panov, A. V.; Greenamyre, J. T. *Nat. Neurosci.* **2000**, *3*, 1301.
10. Yoshida, M.; Ogawa, M.; Suzuki, K.; Nagatsu, T. *Neurosci. Lett.* **1990**, *119*, 109.
11. Nagatsu, T. *Neurosci. Res.* **1997**, *29*, 99.
12. McNaught, K. S. P.; Carrupt, P.-A.; Altomare, C.; Cellamare, S.; Carotti, A.; Testa, B.; Jenner, P.; Marsden, C. D. *Biochem. Pharmacol.* **1998**, *56*, 921.
13. Collins, M. A.; Neafsey, E. J.; Cheng, B. Y.; Hurley-Gius, K.; Ung-Chhun, N. A.; Pronger, D. A.; Christensen, M. A.; Hurley-Gius, D. *Adv. Neurol.* **1986**, *45*, 179.
14. Matsubara, K.; Gonda, T.; Sawada, H.; Uezono, T.; Kobayashi, Y.; Kawamura, T.; Ohtaki, K.; Kimura, K.; Akaike, A. *J. Neurochem.* **1998**, *70*, 727.
15. Collins, M. A.; Neafsey, E. J. In *Neurotoxic Factors in Parkinson's Disease and Related Disorders*; Storch, A., Collins, M. A., Eds.; Kluwer Academic/Plenum: New York, Boston, Dordrecht, London, Moscow, 2000; p 115.
16. Bringmann, G.; Feineis, D.; Grote, C.; God, R.; Clement, H.-W.; Sontag, K.-H.; Janetzky, B.; Reichmann, H.; Rausch, W.-D.; Riederer, P.; Wesemann, W. In *Pharmacology of Endogenous Neurotoxins*; Moser, A., Ed.; Birkhäuser: Boston, Basel, Berlin, 1998; p 151.
17. Bringmann, G.; Feineis, D.; Brückner, R.; Blank, M.; Peters, K.; Peters, E.-M.; Reichmann, H.; Janetzky, B.; Grote, C.; Clement, H.-W.; Wesemann, W. *Bioorg. Med. Chem.* **2000**, *8*, 1467.
18. Bringmann, G.; Hille, A. *Arch. Pharm.* **1990**, *323*, 567.
19. Bringmann, G.; God, R.; Feineis, D.; Wesemann, W.; Riederer, P.; Rausch, W.-D.; Reichmann, H.; Sontag, K.-H. *J. Neural Transm.* **1995**, *46* (Suppl), 235.
20. Bringmann, G.; Feineis, D.; God, R.; Fähr, S.; Wesemann, W.; Clement, H.-W.; Grote, C.; Kolasiewicz, W.; Sontag, K.-H.; Heim, C.; Sontag, T. A.; Reichmann, H.; Janetzky, B.; Rausch, W.-D.; Abdel-mohsen, M.; Koutsilieri, E.; Götz, E. M.; Gsell, W.; Zielke, B.; Riederer, P. *Biog. Amines* **1996**, *12*, 83.
21. Sontag, K.-H.; Heim, C.; Sontag, T. A.; Kolasiewicz, W.; Clement, H.-W.; Grote, C.; Wesemann, W.; Janetzky, B.; Reichmann, H.; Feineis, D.; God, R.; Bringmann, G.; Rausch, D.; Abdel-mohsen, M.; Abdel-moneim, M.; Chan, W. W.; Koutsilieri, E.; Zielke, B.; Götz, M.; Gsell, W.; Riederer, P. In *The Basal Ganglia V*; Ohye, C., Kimura, M., McKenzie, J.S., Eds.; Plenum: New York, London, 1996; p 387.
22. Heim, C.; Sontag, K.-H. *J. Neural Transm.* **1997**, *50* (Suppl), 107.
23. Bringmann, G.; God, R.; Fähr, S.; Feineis, D.; Fornadi, K.; Fornadi, F. *Anal. Biochem.* **1999**, *270*, 167.
24. Bringmann, G.; Münchbach, M.; Feineis, D.; Messer, K.; Diem, S.; Herderich, M.; Clement, H.-W.; Stichel-Gunkel, C.; Kuhn, W. *J. Chromatogr. B* **2002**, *767*, 321.

25. Bringmann, G.; Friedrich, H.; Birner, G.; Koob, M.; Sontag, K.-H.; Heim, C.; Kolasiewicz, W.; Fähr, S.; Stäblein, M.; God, R.; Feineis, D. *J. Chromatogr. B* **1996**, *687*, 337.
26. Grote, C.; Clement, H.-W.; Wesemann, W.; Bringmann, G.; Feineis, D.; Riederer, P.; Sontag, K.-H. *J. Neural Transm.* **1995**, *46* (Suppl), 275.
27. Gerlach, M.; Xiao, A.-Y.; Heim, C.; Lan, J.; God, R.; Feineis, D.; Bringmann, G.; Riederer, P.; Sontag, K.-H. *Neurosci. Lett.* **1998**, *257*, 17.
28. Rausch, W.-D.; Abdel-mohsen, M.; Koutsilieri, E.; Chan, W. W.; Bringmann, G. *J. Neural Transm.* **1995**, *46* (Suppl), 255.
29. Janetzky, B.; Gille, G.; Abdel-mohsen, M.; God, R.; Rausch, W.-D.; Bringmann, G.; Reichmann, H. *Drug Dev. Res.* **1999**, *46*, 51.
30. Nagatsu, T.; Levitt, M.; Udenfried, S. *J. Biol. Chem.* **1964**, *239*, 2910.
31. Levitt, M.; Spector, S.; Sjoerdsma, A.; Udenfried, S. *J. Pharmacol. Exp. Ther.* **1965**, *148*, 1.
32. Hirata, Y.; Togari, A.; Nagatsu, T. *J. Neurochem.* **1983**, *40*, 1585.
33. Maruyama, W.; Naoi, M. *Life Sci.* **1994**, *55*, 207.
34. Minami, M.; Takahashi, T.; Maruyama, W.; Takahashi, A.; Dostert, P.; Nagatsu, T.; Naoi, M. *J. Neurochem.* **1992**, *58*, 2097.
35. Maruyama, W.; Takahashi, T.; Minami, M.; Takahashi, P.; Dostert, P.; Nagatsu, T.; Naoi, M. *Adv. Neurol.* **1993**, *60*, 224.
36. Scholz, J.; Moser, A. In *Pharmacology of Endogenous Neurotoxins*; Moser, A., Ed.; Birkhäuser: Boston, Basel, Berlin, 1998; p 221.
37. Hirata, Y.; Nagatsu, T. *Brain Res.* **1985**, *337*, 193.
38. Mogi, M.; Harada, M.; Kojima, K.; Kiuchi, K.; Nagatsu, I.; Nagatsu, T. *Neurosci. Lett.* **1987**, *80*, 213.
39. Scholz, J.; Bamberg, H.; Moser, A. *Neurochem. Int.* **1997**, *31*, 845.
40. Fukuda, T. *Brain Res.* **1994**, *639*, 325.
41. Matsubara, K.; Idzu, T.; Kobayashi, Y.; Nakahara, D.; Maruyama, W.; Kobayashi, S.; Kimura, K.; Naoi, M. *Neurosci. Lett.* **1995**, *199*, 199.
42. Knorr, A. M.; Deutch, A. Y.; Roth, R. H. *Brain Res.* **1989**, *495*, 355.
43. Moser, A.; Scholz, J.; Gänsle, A. *Neuropeptides* **1999**, *33*, 492.
44. Kumer, S. C.; Vraner, K. E. *J. Neurochem.* **1996**, *67*, 443.
45. Almas, B.; le Bourdelles, B.; Flatmark, T.; Mallet, J.; Haavik, J. *Eur. J. Biochem.* **1992**, *209*, 249.
46. Siemens. *P3 X-Ray Program System*; Analytical X-Ray Instruments Inc.: Madison, WI, USA, 1997.
47. Sheldrick, G. M. Program Package *SHELXL-97, Bruker AXS*; Analytical X-Ray Instruments Inc.: Madison, WI 53719, USA, 1997.
48. A complete listing of the atomic coordinates for **7** can be obtained free of charge, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44–1223–336–033; e-mail: deposit@ccdc.com.ac.uk), on quoting the depository number CCDC-171412, the names of the authors, and the journal citation.
49. Keller, E. *SCHAKAL 88. A FORTRAN Program for the Graphic Representation of Molecules and Crystallographic Models*; Kristallographisches Institut der Universität: Freiburg, Germany, 1990.
50. De Groot, J. In *The Rat Forebrain in Stereotaxic Coordinates*, 2nd ed.; N.V. Noord-Hollandsche Uitgevers Maatschappij: Amsterdam, 1963
51. Moser, A.; Reavill, C.; Jenner, P.; Marsden, C. D.; Cramer, H. *Exp. Brain Res.* **1986**, *62*, 567.
52. Bensadoun, A.; Weinstein, D. *Anal. Biochem.* **1976**, *70*, 241.
53. Naoi, M.; Takahashi, T.; Nagatsu, T. *J. Chromatogr.* **1988**, *427*, 229.