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THE FIRST SELECTIVE ANTAGONIST FOR A $GABA_C$ RECEPTOR

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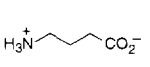
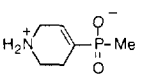
Abstract. (1,2,5,6-Tetrahydropyridine-4-yl)methylphosphinic acid (TPMPA, **1**), which is synthesized in six steps from 3-butyn-1-ol, is the first γ-aminobutyric acid (GABA) receptor antagonist that shows strong selectivity for mammalian $GABA_C$ receptors. Copyright © 1996 Elsevier Science Ltd

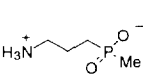
γ-Aminobutyric acid (GABA) is the most widely distributed inhibitory neurotransmitter in the central nervous system of vertebrates. Agents that influence GABA receptors have pronounced effects on brain function and show demonstrated or potential utility for the treatment of a variety of CNS disorders.[3] Three classes of mammalian GABA receptors, denoted $GABA_A$, $GABA_B$, and $GABA_C$, have been well characterized so far. $GABA_A$ receptors are ligand-gated Cl^- channels, while $GABA_B$ receptors regulate K^+ and Ca^{2+} channels through GTP-binding proteins and intracellular messenger pathways.[4,5] $GABA_C$ receptors were initially defined pharmacologically by their insensitiviy to the specific $GABA_A$ receptor antagonist bicuculline and the $GABA_B$ receptor agonist baclofen.[6] Only recently have $GABA_C$ receptors been studied at the molecular level and, at present, the singular $GABA_C$ receptors with known molecular composition are those found in neurons of the retina. Retinal $GABA_C$ receptors are ligand-gated Cl^- channels comprised of homo- or heterodimeric assemblies of ρ1 and ρ2 subunits.[6] At present, the physiological functions of $GABA_C$ receptors are largely unknown. In this paper we report the synthesis and initial pharmacological characterization of the first selective antagonist for a mammalian $GABA_C$ receptor, (1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid (TPMPA, **1**).



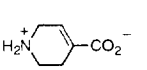
GABA



TPMPA (**1**)



3-APMPA (**2**)



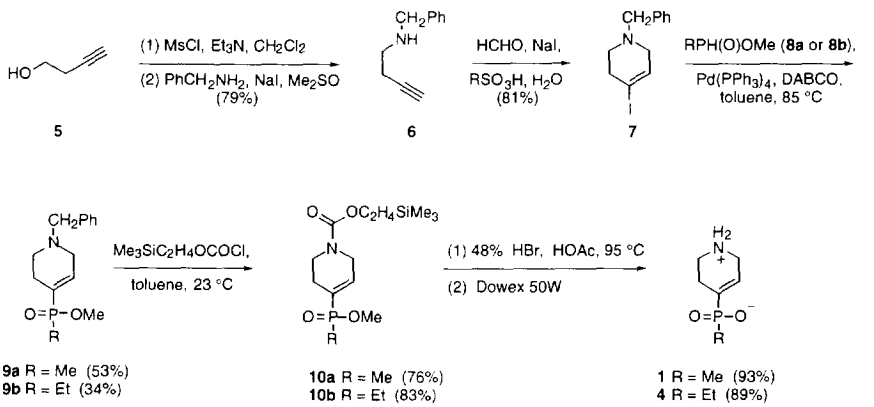
isoguvacine (**3**)

The rationale behind the design of TPMPA was as follows. Previous studies had shown that the flexible GABA analogue 3-aminopropyl(methyl)phosphinic acid (3-APMPA, **2**) is inactive at $GABA_A$ receptors, is a high potency agonist for $GABA_B$ receptors, and is a potent competitive antagonist of retinal $GABA_C$ receptors (Table 1).[7,8] In contrast, the semi-rigid GABA analogue isoguvacine (**3**) is an agonist for $GABA_A$ receptors, is inactive at $GABA_B$ receptors, and is a partial agonist for retinal $GABA_C$ receptors (Table 1).[7,9] Therefore, we reasoned that the methylphosphinic acid analog of isoguvacine, TPMPA (**1**), might retain activity as an antagonist for $GABA_C$ receptors, but be weak, or inactive, as a ligand for $GABA_A$ and $GABA_B$ receptors.

The synthesis of TPMPA (**1**) and its ethylphosphinate analog **4** (TPEPA) is summarized in Scheme 1. 3-Butyn-1-ol (**5**) was converted to *N*-benzyl-3-butyn-1-amine (**6**),[10] which undergoes efficient iodide-promoted intramolecular Mannich cyclization to give 1-benzyl-4-iodo-1,2,5,6-tetrahydropyridine (**7**).[10,11] Coupling of **7** at elevated temperature with methyl methylphosphinate (**8a**, R = Me)[12] or methyl

ethylphosphinate (**8b**, R = Et)[12] in the presence of 4 mol % of $Pd(PPh_3)_4$ and 1,4-diazobicyclo[2.2.2]octane (DABCO) afforded phosphinate derivatives **9a** (53%) and **9b** (34%).[13] If 1 mol % of the palladium catalyst was used, coupling did not proceed to an appreciable extent. After considerable experimentation, we found that the benzyl group could be removed and the phosphinic ester cleaved in ~70% overall yield by sequential treatment of **9a** or **9b** with 2-(trimethylsilyl)ethyl chloroformate, followed by cleavage of the resulting carbamate esters **10** with HBr and HOAc.[14,15] Purification of the derived amino acids by ion exchange chromatography then provided pure samples of TPMPA (**1**) and TPEPA (**4**).

Scheme 1. Synthesis of TPMPA (**5a**) and TPEPA (**5b**).



Effects of TPMPA on retinal $GABA_C$ receptors were measured using electrical recording techniques in *Xenopus* oocytes expressing cloned human ρ1 GABA receptor subunits.[16,17] TPMPA was a potent competitive inhibitor of ρ1 $GABA_C$ receptors (Table 1).[18] Unlike imidazole-4-acetic acid, a compound previously reported to selectively interact with retinal $GABA_C$ receptors,[19] TPMPA did not itself activate currents, indicating that it is not a partial agonist/antagonist. TPMPA was >100-fold weaker as an inhibitor of rat brain $GABA_A$ receptors expressed in oocytes, and displayed only weak agonist activity for $GABA_B$

Table 1. Pharmacology of 3-APMPA, isoguvacine and TPMPA at the three major classes of mammalian GABA receptors.[a]

Drug	$GABA_A$	$GABA_B$	$GABA_C$
3-APMPA	Inactive	Potent agonist (EC_{50} ~ 0.2 μM)	Potent antagonist (K_b = 0.8 μM)
Isoguvacine	Agonist (EC_{50} = 310 μM)	Inactive	Partial agonist (EC_{50} = 99 μM)
TPMPA	Weak antagonist (K_b = 320 μM)	Weak agonist (EC_{50} ~ 500 μM)	Potent antagonist (K_b = 2.1 μM)

[a] Values for 3-APMPA and isoguvacine are taken from references 7-9.

receptors, as assayed by whole cell patch clamp recordings in rat hippocampal slices (Table 1).[20] Details of the pharmacological characterization of TPMPA are reported elsewhere.[21] TPEPA, the ethyl analogue of TPMPA, was also a competitive inhibitor of ρ1 $GABA_C$ receptors, but was ~2-fold less potent than TPMPA. Like TPMPA, TPEPA had only weak inhibitory effects on $GABA_A$ receptors. TPEPA was not tested for actions on $GABA_B$ receptors.

Conclusion. TPMPA is the first GABA receptor antagonist that shows strong selectivity for homomeric human $GABA_C$ receptors composed of ρ1 subunits. Since TPMPA is readily prepared in six steps from commercially available 3-butyn-1-ol, it should be a useful tool for investigating the function of $GABA_C$ receptors in the retina and in other parts of the central nervous system.

Experimental Details for the Synthesis of TPMPA.

Methyl (1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)methylphosphinate (9a). A mixture of **7**[10,22] (4.50 g, 15 mmol), methyl methylphosphinate **8a**[12] (2.0 g, 21 mmol), 1,4-diazobicyclo[2.2.2]octane (DABCO, 5.1 g, 46 mmol), $Pd(PPh_3)_4$ (500 mg, 0.5 mmol) and toluene (150 mL) was stirred at 80-90 °C under a N_2 atmosphere for 1 h. The reaction mixture was then concentrated and the residue was purified by column chromatography on silica gel (5:3 to 2:1 CH_2Cl_2:hexane containing 5 % Et_3N) to give phosphinate **9a** (2.1 g, 53 %) as a viscous oil: ^1H NMR (300 MHz, $CDCl_3$) δ 7.20-7.40 (m, 5H, Ph), 6.67 (dd, J = 19.2, 1.2 Hz, 1H, CHC=C), 3.58 (d, J = 5.1 Hz, 3H, OCH_3), 3.55 (d, J = 1.2 Hz, 2H, CH_2Ph), 3.01-3.22 (m, 2H, NCH_2C=C), 2.47-2.68 (m, 2H, CH_2CH_2N), 2.22 (broad s, 2H, NCH_2CH_2), 1.43 (dd, J = 14.4, 0.6 Hz, 3H, CH_3P); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.6 (d, J = 8.6 Hz), 137.4, 128.8, 128.2 (d, J = 122 Hz), 128.1, 127.0, 62.2, 52.9 (d, J = 14.0 Hz), 50.4 (d, J = 4.5 Hz), 48.6 (d, J = 9.3 Hz), 25.3 (d, J = 9.4 Hz), 12.7 (d, J = 100 Hz); IR (film) 2917, 1645, 1456, 1208, 1038, 894, 700 cm^{-1}; HRMS (CI) m/z 266.1322 (266.1310 calcd for $C_{14}H_{21}NO_2P$).

(1,2,5,6-Tetrahydropyridine-4-yl)methylphosphinic acid (TPMPA, 1). A solution of 2-(trimethysilyl)-ethyl chloroformate (6.68 g, 37.0 mmol), **9a** (6.98 g, 26.4 mmol, previously dried by azeotroping twice with toluene) and dry toluene (30 mL) was maintained under a N_2 atmosphere at room temperature for 12 h. After concentration the resulting residue was purified by column chromatography on silica gel (1:1 to 1.5:1 CH_2Cl_2:hexane containing 5 % Et_3N) to give carbamate **10a** (6.39 g, 76%) as a viscous oil.[23]

A solution of **10a** (6.3 g, 19.8 mmol), 48% HBr (160 mL) and glacial acetic acid (160 mL) was heated at reflux (bath temperature 90-100 °C) for 1 day and then concentrated Toluene (20 mL) was added and the resulting mixture was concentrated to remove residual acid, and this procedure was repeated twice. Saturated aqueous NH_4OH then was added slowly until the solution was basic. The resulting solution was concentrated, additional toluene (20 mL) was added and the solution was again concentrated. The resulting residue was purified by ion exchange chromatography on Dowex 50W (pre-washed 5-6 times with 1 M HCl and then with distilled water until the eluent was neutral). The column was eluted with distilled water (500 mL) and then with a 1:50 to 1:20 mixture of 12 M NH_4OH and distilled water The early ninhydrin-negative fractions were rejected, and the later ninhydrin-positive fractions were collected and concentrated to give pure **1** (2.96 g, 93%) as a colorless solid: R_f = 0.52 (6:6:3:5 BuOH:CH_3COOH:pyridine:H_2O); ^1H NMR (300 MHz, CD_3OD) δ 6.33 (d, J = 17.1 Hz, 1H, CHC=C), 3.30 (t, J = 1.4 Hz, 2H, C=CCH_2N), 3.20 (t, J = 5.9 Hz, 2H, CH_2CH_2N), 2.49 (broad s, 2H, NCH_2CH_2), 1.24 (d, J = 13.8 Hz, 3H, CH_3P); ^{13}C NMR (75 MHz, CD_3OD) δ138.3 (d, J = 121 Hz), 128.6 (d, J = 8.2 Hz), 43.4 (d, J = 13.9 Hz), 41.8 (d, J = 8.4 Hz), 23.3 (d, J = 9.8 Hz), 16.1 (d, J = 97.8 Hz); HRMS (EI) m/z 161.0601 (161.0606 calcd for $C_6H_{12}NO_2P$).[24]

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22. Intermediates **6** and **7** were prepared using the general procedure detailed in reference 10. Characterization data for **7**: a yellow oil that rapidly darkened on standing and was used immediately in the next step; IR (film) 2780, 1545, 1384, 1341, 1054, 981, 729, 698 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.20-7.40 (m, 5H, ArH), 6.26 (t, J = 3.3 Hz, 1H, CHC=C), 3.56 (s, 2H, CH_2Ph), 3.02 (d, J = 3.0 Hz, 2H, NCH_2CH), 2.61 (s, 4H, NCH_2CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.8, 135.2, 129.1, 128.3, 127.2, 93.1, 62.2, 55.6, 51.6, 39.7; MS (CI) *m/z* 301 (9), 300 (MH, 100), 174 (34); HRMS (CI) *m/z* 300.0251 (300.0254 calcd for $C_{12}H_{15}NI$).
23. Characterization data for **10a**: ^1H NMR (300 MHz, $CDCl_3$) δ 6.73 (dd, J = 19.2, 1.2 Hz, 1H, CHC=C), 4.18-4.25 (m, 2H, OCH_2), 4.07-4.17 (m, 2H, $C{=}CCH_2N$), 3.62 (d, J = 11.1 Hz, 3H, OCH_3), 3.47-3.68 (m, 2H, CH_2CH_2N), 2.21-2.30 (m, 2H, NCH_2CH_2), 1.48 (d, J = 14.1 Hz, 3H, CH_3P), 0.97-1.06 (m, 2H, CH_2CH_2O), 0.04 (s, 9H, $(CH_3)_3Si$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.4, 138.9 (d J = 4.4 Hz), 129.1 (d, J = 124 Hz), 63.7, 50.6 (d, J = 5.8 Hz), 43.9 (d, J = 13.7 Hz), 39.6, 24.4 (d, J = 6.8 Hz), 17.7, 12.7 (d, J = 101 Hz), −1.6; IR (film) 2954, 1699, 1457, 1282, 1236, 1038 cm^{-1}; HRMS (CI) *m/z* 320.1431 (320.1447 calcd for $C_{13}H_{27}NO_4PSi$).
24. TPEPA (**4**) was prepared in identical fashion. Characterization data for **4**: a colorless solid: R_f = 0.52 (6:6:3:5 BuOH-CH_3COOH-pyridine-H_2O): ^1H NMR (300 MHz, CD_3OD) δ 6.34 (d, J = 17.1 Hz, 1H, CHC=C), 3.58 (t, J = 2.4 Hz, 2H, $C{=}CCH_2N$), 3.11 (t, J = 5.7 Hz, 2H, CH_2CH_2N), 2.40 (t, J = 2.1 Hz, 2H, NCH_2CH_2), 1.50 (qd, J = 15.0, 7.5 Hz, 2H, CH_3CH_2P), 1.03 (dt, J = 17.4, 7.7 Hz, 3H, CH_3CH_2P); ^{13}C NMR (75 MHz, CD_3OD) δ 136.7 (d, J = 117 Hz), 131.3 (d, J = 6.5 Hz), 44.0 (d, J = 13.0 Hz), 42.1 (d, J = 8.1 Hz), 24.3 (d, J = 9.4 Hz), 23.4 (d, J = 97.9 Hz), 7.3 (d, J = 3.8 Hz); HRMS (EI) *m/z* 175.0759 (175.0762 calcd for $C_7H_{14}NO_2P$).

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