PHOSPHINIC ACID ANALOGUES OF γ-AMINOBUTYRIC ACID (GABA). SYNTHESIS OF A NEW RADIOLIGAND

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SUMMARY

The synthesis of a new radioligand, which binds with high selectivity and affinity (K_D=7.4 nM) to the GABA-B receptor, is described. A Wittig-Horner approach was employed to prepare an unsaturated protected intermediate (6), suitable for tritiation and deprotection.

Key words : tritium, γ-aminophosphinic acids, GABA-B, synthesis

INTRODUCTION

During our studies on phosphorus analogues of biologically important carboxylic acids, we prepared [1] a number of substituted γ-aminophosphinic acids, as mimics of the inhibitory neurotransmitter GABA. A number of these compounds exhibited extremely high affinities to the GABA-B receptor (TABLE 1), together with a high selectivity for the GABA-B receptor; these facts led us to postulate that a radioligand based on this chemistry might prove useful in the further study of this receptor. This paper reports the synthesis of such a radioligand, and confirms the predicted utility [2].

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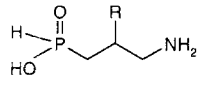
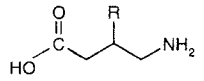
TABLE 1 Binding to GABA-B receptor (IC_{50})

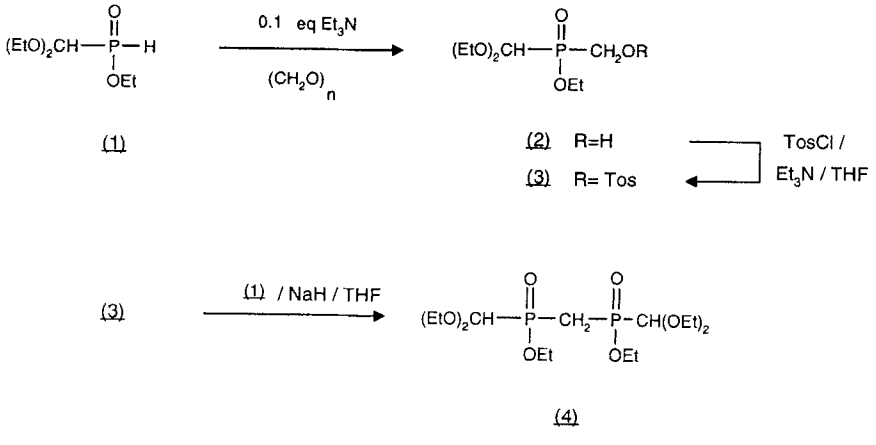
	R=H	R=OH	R=4-ClC_6H_4
	$1x10^{-9}$M	$1.6x10^{-8}$M	$3.5x10^{-8}$M
	$8x10^{-8}$M	$1.5x10^{-6}$M	$4.5x10^{-8}$M

IC_{50} values quoted are concentrations required to displace 50% of tritiated baclofen bound to cat cerebellar membranes.

RESULTS AND DISCUSSION

Our previous syntheses of functional phosphinic acids rely on the use of synthons of phosphinic acid. Such synthons contain the diethoxymethyl group as a stable, easily introduced but acid labile protecting group for the P-H functionality [3]. This present work uses one of these building blocks, ethyl diethoxymethylphosphinate (1). This compound is readily prepared [4] in large quantities, from commercially available starting materials.

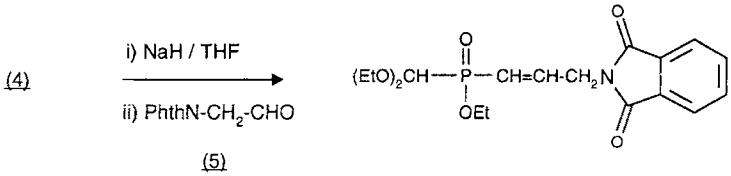
The route envisaged for the synthesis of a protected, unsaturated γ-aminophosphinate, suitable for tritiation, utilizes a Wittig-Horner reaction. The preparation of the required bis-phosphinate, diethoxymethyl-(diethoxymethyl-ethoxyphosphinoylmethyl)-phosphinic acid ethyl ester (4) ,[5] is shown in Scheme 1.



SCHEME 1

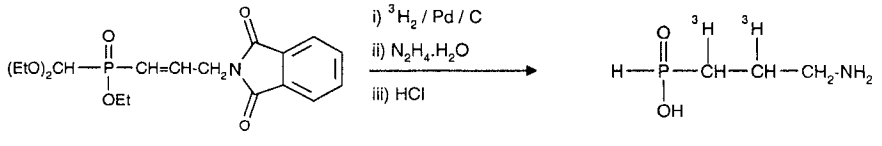
Base- catalysed addition of (1) to paraformaldehyde gave ethyl diethoxymethyl (hydroxymethyl)phosphinate (2) in 52% yield. Treatment with p-toluenesulphonyl chloride (TosCl) in the presence of base led to ethyl diethoxymethyl(tosyloxymethyl)phosphinate (3) in 46% yield. Displacement of the tosyl group by the anion derived from (1) gave the bis-phosphinate (4) , isolated in 60% yield after chromatography.

The first attempt to couple the bis phosphinate (4) with the aldehyde derived from phthaloyl glycine, (1,3-dioxo-1,3-dihydro-isoindol-2-yl)acetaldehyde (5) met with little success. The desired product (6) rearranged under the basic reaction conditions to the corresponding enamine. However, by an inverse addition of the pre-formed anion derived from (4) to the aldehyde (5) at low temperature, a low yield of ethyl diethoxymethyl - (3-phthalimidoprop-1-ene)phosphinate (6) was obtained, Scheme 2. The double bond in compound (6) has *trans* configuration. Although the yield is low, unreacted bis - phosphinate (4) is readily recovered by chromatography.



SCHEME 2

It is well known in the literature that the P-H function is an efficient poison towards hydrogenation catalysts, rendering such reduction processes completely useless. However, by employing the diethoxymethyl group as a protecting group for the P-H function, compound (6) was efficiently reduced with tritium gas in the prescence of palladium on charcoal. Removal of the protecting groups was achieved stepwise. Treatment first with hydrazine hydrate removed the phthaloyl group, and subsequent treatment with 2N Hydrochloric acid gave tritiated 3-aminopropylphosphinic acid (7) Compound (7) was produced with a high specific activity of 19.5 Ci / mmol and a radiochemical purity of > 97%, Scheme 3.



SCHEME 3

The tritiated compound (7) showed high affinity to GABA-B receptors in membranes obtained from rat cerebral cortex [2]. The dissociation constant K_D was 7.4 ± 1.7 nM and the number of binding sites B_{max} was 1072 ± 181 fmol/mg protein. It proved to be a radioligand superior to ^{3}H-GABA and to ^{3}H-baclofen as the specific binding was more than 90% and a filtration assay could be used [2]. Experimental details of the radioreceptor assay are described elsewhere [6].

CONCLUSIONS

We have shown that ethyl diethoxymethylphosphinate (1) may be readily transformed into new and useful synthons for the synthesis of functional phosphinic acids. In this instance we have prepared the new radioligand (7). Due to the potent and highly selective binding of such γ-aminophosphinic acids, compound (7) is now the radioligand of choice in GABA-B receptor binding studies.

EXPERIMENTAL

General

Proton NMR spectra were obtained on a Jeol FX-90 spectrometer operating at 89.55 MHz; Phosphorus NMR spectra were recorded on the same instrument at 36.21 MHz. Chemical shifts are relative to tetramethylsilane and 85% H_3PO_4 respectively. Column chromatography was performed on Merck silica kieselgel 60 (70-230 mesh). THF refers to tetrahydrofuran freshly distilled from sodium / benzophenone. Sodium hydride was obtained free from oil by repeated washing with hexane followed by drying. TLC analyses were performed on Merck silica-gel G plates (20x5). HPLC was carried out using a Nucleosil 10C18 semi-preparative column (50 x 0.7 cm), Orlita HPLC pump, Cecil variable wavelength UV detector. Radiochromatographic analyses were performed with a Berthold linear analyser. Radioactive quantitation was by scintillation counting using ^{3}H-hexadecane internal standard. Chemical concentrations were determined on a Beckman amino acid analyser by comparison with reference material.

Ethyl diethoxymethyl(hydroxymethyl)phosphinate (2)

A mixture of ethyl diethoxymethylphosphinate (19.6 g, 0.1 mol), paraformaldehyde (3.0 g, 0.1 mol) and triethylamine (1.0 g, 0.01 mol) was heated at 130 °C for a period of 2.5 hr. The mixture was allowed to cool and volatile material removed by evaporation. Distillation of the residue then gave (2) (11.7 g, 52%) as a clear liquid, b.p. 150°C / 0.01mmHg. ^{1}H NMR ($CDCl_3$): 1.3 (9H, m); 3.6-3.9 (4H, m); 4.0 (2H, m); 4.2 (2H, m); 4.8 (2H, m).

Ethyl diethoxymethyl(tosyloxymethyl)phosphinate (3)

To a mixture of (2) (4.0 g, 17.7 mmol) and p-toluenesulphonyl chloride (3.4 g, 17.7 mmol) in diethyl ether (25 ml) was added triethylamine (1.97 g, 19.5 mmol) at a temperature of 15°C under an atmosphere of nitrogen. The reaction was stirred 16 hr at room temperature, the solvent removed by evaporation and the crude material extracted into ethyl acetate. After washing with water, the organic layer was dried and evaporated, and the crude product purified on silica gel using an elaunt of hexane: ethyl acetate 1:1. Compound (3) was obtained as a pale-yellow liquid, (3.1 g, 46%); ^{1}H NMR ($CDCl_3$): 1.3 (9H, m); 2.5 (3H, s); 3.6-3.9 (4H, m); 4.0-4.2 (4H, m); 4.8 (1H, d, J=8 Hz); 7.6 (4H, ABq); Found: C 47.4%; H 6.7%; S 8.2%; P 8.3%; Calcd for $C_{15}H_{25}O_7PS$: C 47.36%; H 6.63%; S 8.43%; P 8.14%.

Ethyl (diethoxymethyl) methylene bis-phosphinate (4)

Ethyl diethoxymethylphosphinate (1) (5.9 g, 30 mmol) was dissolved in dry THF (50 ml) and cooled to -15 °C under an atmosphere of nitrogen. Sodium hydride (0.86 g, 36 mmol) was added and the mixture stirred until gas evolution ceased. Ethyl diethoxymethyl(tosyloxymethyl)phosphinate (3) (11.4 g, 30 mmol) in dry THF (50 ml) was added, and the mixture stirred at room temperature for a period of 12 hr. The solvent was removed by evaporation and the product extracted into ethyl acetate and washed with water. The organic layer was dried and stripped, and the crude product purified by distillation in a Kugelrohr apparatus. Compound (4) was obtained as a viscous oil (7.3g, 60%), b.p. 170-175°C / 0.1 mmHg; ^{1}H NMR ($CDCl_3$); 1.3 (18H, m); 3.0-2.2 (2H, m); 4.5-3.6 (12H, m); 5.0 (2H, m); Found: C 44.2%; H 8.6%; P 15.2%; Calcd for $C_{15}H_{24}O_8P_2$: C 44.56%; H 8.48%; P 15.32%.

Ethyl diethoxymethyl-(3-phthalimidoprop-1-ene)phosphinate (6)

Sodium hydride (0.03 g, 1.25 mmol) was suspended in dry THF (15 ml) under an atmosphere of nitrogen. The bis-phosphinate (4) (0.5 g, 1.24 mmol) in dry THF (10 ml) was added and the solution stirred at room temperature for 15 min. This clear yellow solution was then added, via syringe, to a solution of formyl methylphthalimide (5) (0.25 g, 1.32 mmol) in dry THF (10 ml) at a temperature of -15 °C. After 15 min at this temperature, the red solution was quenched with sat. ammonium chloride solution and extracted into diethyl ether. After drying and evaporation, the crude product was chromatographed on silica gel using ethyl acetate as eluant, to give compound (6) as a clear oil, (0.13 g, 27%). ^{31}P NMR ($CDCl_3$): +30.0 p.p.m.; ^{1}H NMR ($CDCl_3$): 1.2 (9H, m); 3.7 (4H, m); 4.1 (2H, m); 4.4 (2H, m); 4.6 (1H, d, J=8 Hz); 5.8 (1H, t of m); 6.8 (1H, t of m); 7.8 (4H, m).

(1, 2-^{3}H)-3-Aminopropanephosphinic acid (7)

Ethyl diethoxymethyl-(3-phthalimidoprop-1-ene)phosphinate (3.9 mg, 0.00987 mmol) was combined with 10% Palladium on charcoal (5.2 mg) in THF (0.5 ml) and stirred under an atmosphere of

tritium gas for 30 min (total uptake=0.36 ml). The reaction was filtered through a membrane filter (ACRO LC13), which was washed with ethanol (2 x 0.5 ml), and the filtrate evaporated to dryness immediately prior to HPLC purification. The eluant used for HPLC purification was acetonitrile / water / acetic acid (450 / 550 / 1), UV detection being at 260 nm, with a pressure of 200 bar. Fractions 14-16 were combined, concentrated to dryness, and the residue dissolved in ethanol and the solution re-evaporated to dryness. The residue was suspended in ethanol (0.3 ml), hydrazine hydrate (0.1 ml) added, and the mixture heated at 80 ^{o}C for 1 hr. The mixture was allowed to cool, evaporated and the residue treated consecutively with 2N HCl (0.25 ml) and 2N NaOH (0.25 ml), each at 50 ^{o}C for 30 min, with filtration and evaporation to dryness prior to NaOH treatment. The basic solution was cooled, made acidic with 2N HCl, evaporated to dryness, ethanol (5 ml) evaporated from the residue which was then re-suspended in ethanol (2 ml). The residual sodium chloride was filtered off (ACRO LC13), washed with a small volume of ethanol, the combined filtrate evaporated to dryness and the residue taken up in pH 3 triethylammonium dihydrogen phosphate buffer (0.5 ml: 0.001 M) prior to HPLC purification.

HPLC eluent : 0.001 M phosphate buffer (pH 3)

Detection : Radioactive detection only

Fractions (25 x 4 ml) were collected, the main radioactive peak was associated with fractions 19 to 23, these were combined, evaporated to small volume and the solution applied to a column (5 x 0.5 cm) of Dowex anion exchange resin (acetate form), eluting with water. The eluate was evaporated to dryness, the residue dissolved in constant boiling HCl (0.5 ml) and heated at 100 ^{o}C for 2.5 hr to give a clear colourless solution. This was evaporated to dryness, ethanol (5 ml) was evaporated from the residue which was dissolved in a small volume of water. The solution was applied to a column (5 x 0.5 cm) of Dowex anion exchange resin (acetate form) and eluted with water. The radioactive eluent was evaporated to dryness, ethanol (5 ml) was evaporated from the residue which was then dissolved in distilled water(5 ml).

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