Synthesis and Authentication of Iodoazidophenpyramine, a Photoaffinity Reporter Ligand Previously used for Histamine H_1-Receptor Labelling

Giovanni Sorba[1], Wasyl Tertiuk, and C. Robin Ganellin*

Department of Chemistry, University College London, The Christopher Ingold Laboratories, 20 Gordon Street, London WC1H 0AJ, England

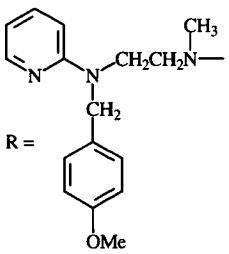
Key Words: iodine, photoaffinity, ligand, azide, histamine H_1-receptor

Summary

The synthesis is described of aminophenpyramine (**7**) (*N*-{5-[2-(4-aminophenyl)ethanamido]pentanyl}-*N'*-(4-methoxybenzyl)-*N*-methyl-*N'*-(2-pyridinyl)-1,2-ethandiamine), its monoiodo- and diiodo-derivatives (**8** and **9**), and iodoazidophenpyramine (**1**). The last compound is synthesised by two different routes to confirm the identity of the [^{125}I]iodinated ligand previously made only in solution and used for characterisation of the histamine H_1-receptor protein. The procedures employ the novel intermediates 4-amino-3-iodo-phenylacetic acid (**11**) and 4-azido-3-iodo-phenylacetic acid (**13**). They have general applicability to the synthesis of non-radioactive iodinated photoaffinity receptor ligands which may be required for chemical authentication of the corresponding radiolabelled compounds.

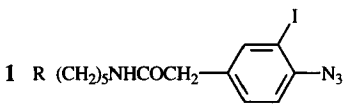
Introduction

Radiolabelled photoaffinity ligands constitute valuable tools with which to study the structure of protein receptors. They are light-activatable moieties that form reactive intermediates e.g. carbenes or nitrenes, which insert into the side chains of appropriate amino acids of a receptor site and radioactively tag the receptor for isolation and structural studies[1]. Aromatic azides are often used since they are synthetically readily incorporated in biologically active molecules and form a reactive nitrene upon irradiation with light. Iodine ^{125}I is a very useful label since it has a very high specific activity and, when incorporated into suitable ligands, can detect very low concentrations of receptor protein.



The anilino group is commonly used to generate radiolabelled photoaffinity ligands since it is sufficiently activated towards electrophilic substitution by iodine and diazotization then yields the required azido compound. It is quite usual, however, to see reports of the generation of such radiolabelled ligands without any chemical characterisation or supporting evidence for their chemical structure. Surprisingly, the appropriate reagents (such as 4-amino or 4-azido-3-iodophenylacetic acid) required for synthesis of the non-radiolabelled material, needed for comparison to chemically authenticate the radiolabelled ligands, have not even been properly described before. It is important to chemically characterise the labelled products because they may consist of mixtures of mono-, di- and non-iodo labelled materials, and this should be determined. Even if separated by HPLC into a single radiochemically labelled iodinated product it is important to know whether one or two iodine atoms have been introduced.

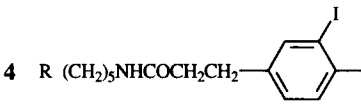
Previously, we reported jointly with others that [^{125}I]iodoazidophenpyramine **1**,*N*-{5-[2-(3-iodo-4-azidophenyl)-ethanamido]pentanyl}-*N'*-(4-methoxybenzyl)-*N*-methyl-*N'*-(2-pyridinyl)-1,2-ethandiamine, is a highly specific and extremely potent reversible histamine H_1-receptor antagonist which, upon ultra-violet irradiation, is covalently incorporated into solubilised H_1 receptors from guinea-pig brain [2,3]



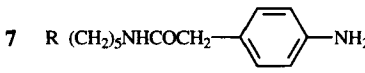
1 R $(CH_2)_5NHCOCH_2$— Iodoazido-phenpyramine

2 R CH_3 Mepyramine

3 R $(CH_2)_5NH_2$ SK&F 94461



4 R $(CH_2)_5NHCOCH_2CH_2$— Iodobol-pyramine



7 R $(CH_2)_5NHCOCH_2$— Aminophen-pyramine

and atrial [4] membranes. Gel electrophoresis of the labelled membranes afforded an estimate of the molecular mass of the ligand binding domain of the H_1 receptor (56 kDa for the brain and 68 kDa for the heart). The H_1 receptor from bovine adrenal medulla has since been cloned and isolated and shown [5] to be a protein containing 491 aminoacids (M_r 55,954). Radiolabelled photoaffinity ligands such as **1** provide very important tools with which to elucidate the receptor binding sites for both agonist and antagonist molecules.

Iodoazidophenpyramine (**1**) belongs to a series of compounds modelled on the potent H_1-receptor antagonist mepyramine (**2**) in which an iodinated reporter group is

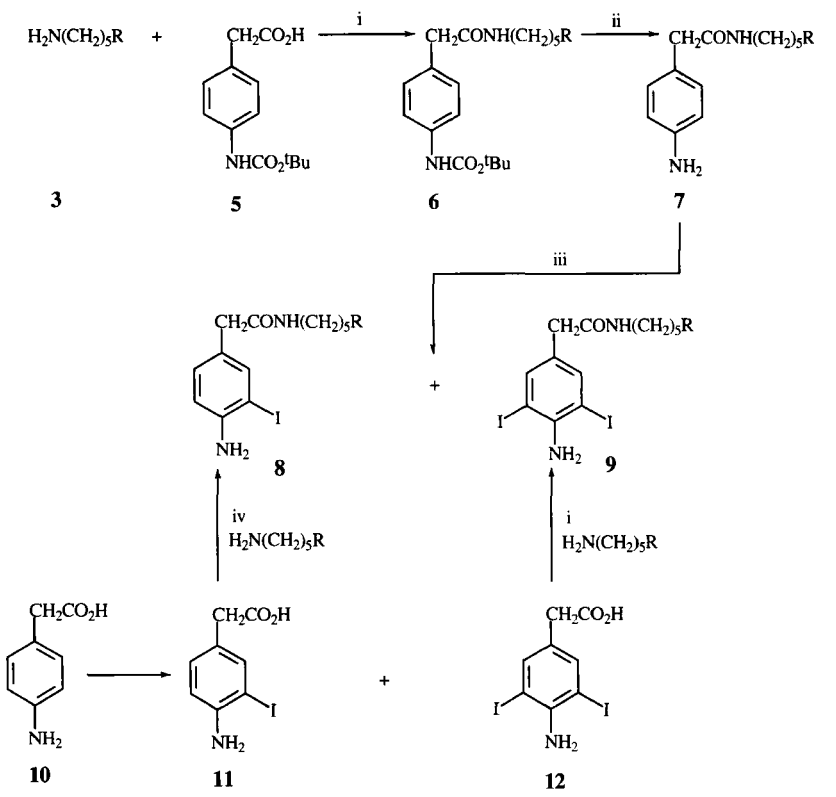
[1] On leave from the Department of Pharmaceutical Chemistry and Toxicology, University of Turin, Italy.

  0365-6233/95/0909-0677 $5.00 + .25/0

attached via a short alkylene chain to a position in the mepyramine molecule which does not interfere with receptor binding. In this series, an amino group, optimally separated by a chain of five carbon atoms from the tertiary amino group of mepyramine to give the compound[6] SK&F 94461 (**3**), serves for the introduction of various functional groups via amidification. The first such example was [^{125}I]iodobolpyramine **4** which was developed as a highly specific probe to provide a sensitive means of detecting H_1 receptors[6,7].

Results and Discussion

We describe herein, iodoazidophenpyramine (**1**) which also serves as an example for the general synthesis of a cold iodinated photoaffinity ligand. Originally this was produced by acylation of the amino chain of SK&F 94461 (**3**) to introduce a *p*-aminophenylacetyl residue giving aminophenpyramine [2,3](**7**); a radioactive ^{125}I atom was then introduced into the molecule and the aromatic amino group was converted into azido. These last two stages were carried out in solution without isolating the reaction products, although they were purified by HPLC; hence the assignment of the structure as [^{125}I]iodoazidophenpyramine was entirely presumptive and the compound was not characterised chemically. *p*-Toluidine, a partial structure of aminophenpyramine readily undergoes mono- or di-iodination and mepyramine, another partial structure, may undergo iodination in the 5-position of the pyridine ring. We therefore set out to use cold material to verify the chemistry and authenticate the products. However, this turned out to be more difficult and time consuming than we had anticipated.

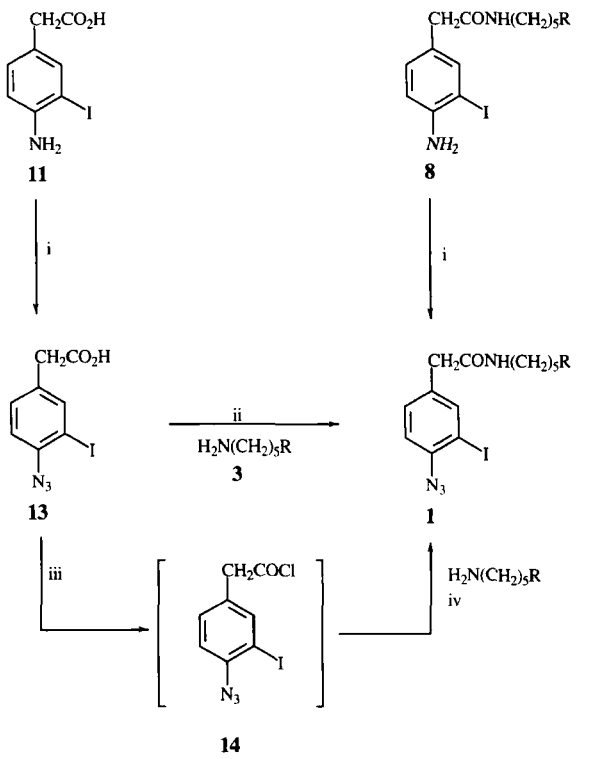


Scheme 1. Reagents and conditions: i, *N*-hydroxysuccinimide (NHS), dicyclohexylcarbodiimide (DCC), THF; ii, 3N HCl, CH_2Cl_2; iii, NaI, chloramine T, aq AcOH; iv, NHS, DCC, CH_2Cl_2. For R, see structures **1–4.**

Although previously[2] the amine SK&F 94461 (**3**) had been coupled directly with 4-aminophenylacetic acid to give aminophenpyramine (**7**), the reaction produced mixtures; therefore reaction control was improved by employing *tert*-butyloxycarbonyl to protect the anilino p-amino group (**5**) (Scheme 1) and this gave a much cleaner product. Coupling was effected with *N*-hydroxysuccinimide (NHS) and dicyclohexylcarbodiimide (DDC) in tetrahydrofuran and the product **6** was hydrolysed *in situ* with dilute HCl to afford aminophenpyramine as the oxalate hemihydrate (**7**) (Scheme 1), in good yield and high purity.

Iodination of aminophenpyramine base (**7**) in dilute AcOH with NaI in the presence of chloramine T as described in ref. [2] gave a mixture which was proven to be the mono and diiodo derivatives (**8** and **9** respectively) in an approximate ratio of 6:1 (by HPLC areas). Therefore these two compounds had to be synthesised separately by unambiguous routes.

The monoiodo compound, iodoaminophenpyramine (**8**), was synthesised directly by coupling the amine SK&F 94461 (**3**) and 4-amino-3-iodo-phenylacetic acid (**11**) in the pres-



Scheme 2. Reagents and conditions: i, $NaNO_2$, 4N HCl; NaN_3; ii, NHS, DCC, CH_2Cl_2; iii, $SOCl_2$; iv, N NaOH, CH_2Cl_2. For R, see structures **1–4.**

ence of NHS and DCC (Scheme 1). The acid (**11**) was prepared in 56% yield by iodination of 4-aminophenylacetic acid (**10**) with iodine monochloride and isolated after separation from some 4-amino-3,5-diiodo-phenylacetic acid[8] (**12**). The diiodo-acid **12** was utilized to prepare diiodoaminophenpyramine (**9**) from the amine SK&F 94461 (**3**).

Iodoaminophenpyramine (**8**) was converted into iodoazidophenpyramine (**1**) by diazotization and addition of sodium azide, which was isolated as the oxalate salt after purification by column chromatography (Scheme 2). In order to circumvent the need to diazotize the intermediate aniline **8**, a more direct procedure was devized of coupling the amine **3** with 4-azido-3-iodophenylacetic acid (**13**). The latter was prepared from 4-amino-3-iodophenylacetic acid (**11**) by diazotization and addition of sodium azide, and proved to be stable. The azido acid (**13**) was converted into the acid chloride **14** and coupled with the amine **3** under Schotten Baumann conditions to yield **1**. Even more conveniently, and in better yield, **13** was coupled directly with the amine **3** in the presence of NHS and DCC. The product was identical with **1** prepared via **8**.

In conclusion, the synthesis is described of 4-amino- and 4-azido-3-iodophenylacetic acids (**11**) and (**13**) needed for preparing cold iodinated analogues of labelled ligands. The above procedures should find a general application in the synthesis of other cold iodinated photoaffinity receptor ligands which may be required for authentication of radiolabelled compounds.

Experimental

General Methods

Melting points (open capillaries in Electrothermal® Cu block) are uncorrected. The IR spectra were obtained on a Perkin-Elmer 983 spectrophotometer.– ^{1}H NMR spectra were recorded on a Varian XL-200 (200 MHz) spectrometer on the δ scale relative to TMS as internal reference.– All compounds gave satisfactory elemental analyses (C,H,N,I figures within 0.4% of the calculated values) determined by A.A.T. Stones in the Department of Chemistry, University College London.– All organic solutions were dried over $MgSO_4$.– Column chromatography on Merck Silica gel 60.– Analytical HPLC on a Gilson binary gradient apparatus with UV detector at 254 nm and a (4 × 4 mm + 250 × 4 mm) Lichrosorb RP Select B 5 mm column. Solvent mixtures were used with a flow rate of 1 cm^3 min^{-1}.

*N-[5-[2-(4-Aminophenyl)ethanamidopentanyl]]-N'-(4-methoxybenzyl)-N-methyl-N'-(2-pyridinyl)-1,2-ethandiamine dioxalate hemihydrate (Aminophenpyramine) (**7**)*

A solution of DCC (0.87 g, 4.23 mmol) in dry THF (15 cm^3) was added to a stirred and cooled (ice-water bath) solution of [4-(*N-tert*-butyloxycarbamido)-phenyl]acetic acid (**5**) (1.06 g, 4.23 mmol) and NHS (0.486 g, 4.23 mmol). Stirring with cooling was continued for 2 h and then a solution of the amine **3** (1.50 g, 4.21 mmol) in dry THF (15 cm^3) was added. The mixture was stirred for 2 h at 20 °C, then filtered from *N,N*-dicyclohexylurea, followed by concentration under reduced pressure.

The resulting residue of compound **6** was dissolved in CH_2Cl_2 (60 cm^3) and shaken with 3N HCl (40 cm^3) for 30 min. The acidic phase was separated off, neutralised with solid K_2CO_3 until alkaline, and then extracted with CH_2Cl_2. The organic extract was dried, filtered and evaporated and the resulting oily residue was purified by chromatography using MeOH:EtOAc (2:3) as eluent to afford the free base of **7** as a pale yellow oil (1.62 g). The latter was converted into the oxalate salt in MeOH; the solvent was evaporated, and the residue was crystallised from the minimum of propan-2-ol and washed with ether to afford the *aminophenpyramine dioxalate hemihydrate* (**7**) as a colourless solid (1.85 g, 65% yield) which, after recrystallisation from propan-2-ol, had mp 74 – 75 °C; Anal. ($C_{29}H_{39}N_5O_2$, $2C_2H_2O_4$, $0.5H_2O$) C,H,N; IR (KBr): ν_{max} = 1639 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6/D_2O,): δ = 1.24 – 1.58 (m, 6H), 2.82 (s, 3H), 3.04 – 3.25, (m, 8H), 3.71 (s, 3H), 3.91 (m, 2H) 4.60 (s, 2H), 6.70 – 7.17 (m, 10H), 7.52 (m, 1H), 8.12 (m, 1H). HPLC: using 4:1 mixture of 0.1% trifluoroacetic acid (TFA) and MeCN containing 5% water and 0.1% TFA gave the major component at 7 min representing 98.9% of total area of elution curve.

*Iodination of aminophenpyramine (**7**)*

Aminophenpyramine base (**7**, 0.55 g, 1.12 mmol obtained from the dioxalate) and NaI (0.25 g, 1.68 mmol) were dissolved in water (30 cm^3) and AcOH (5 cm^3), cooled in an ice bath, and treated with a solution of chloramine T (0.47 g, 1.68 mmol) in water (5 cm^3) which was slowly added dropwise with vigorous stirring and cooling. A pale orange precipitate formed and the mixture was stirred for a further 10 min and then sodium metabisulphite (0.64 g, 3.37 mmol) was added portionwise. The resulting solution was basified (solid Na_2CO_3) and extracted with CH_2Cl_2. The combined exracts were dried ($MgSO_4$) and concentrated and the resulting residue was chromatographed using EtOAc: MeOH (2:1) as eluent, and discarding the first fraction. Evaporation of the eluent gave a pale yellow partially solid residue (0.30 g) which was shown by tlc (in MeOH, NH_4OH, EtOAc, 1:1:2) and HPLC to contain three main components, identified by comparison with authentic materials (below). HPLC used a gradient solvent mixture of 4:1 0.1% TFA in water : 0.1% TFA in MeCN which was programmed to change to a 1:1 ratio during 20 min at a flow rate of 1 cm^3 min^{-1}. Three main peaks were obtained with respective retention times of 6.7, 14.3 and 20.1 min (peak areas in the approximate ratios of 1:6:1) corresponding to compounds **7**, **8**, and **9** respectively.

*4-Amino-3-iodophenylacetic acid (**11**) and 4-amino-3,5-diiodophenylacetic acid (**12**)*

A solution of 4-aminophenylacetic acid (**10**) (12.0 g, 0.079 mol) in dil. HCl (110 cm^3 of 0.8 N) was cooled (ice) and to it was added a solution of iodine monochloride (13.1 g, 0.081 mol) in dil HCl (64 cm^3 of 2N) dropwise with stirring and cooling during 2 h. The mixture was stirred and cooled for a further 5 h and then left at 4 °C for 14 h. The resulting precipitate was collected, washed with water, and dried to afford *4-amino-3,5-diiodophenylacetic acid* (**12**); yield 6.2 g (19%) which was crystallised from DMF and had mp 215–216 °C (dec.) (ref.[8] mp 220-221 °C); Anal. ($C_8H_7I_2NO_2$) C,H,N,I; ^{1}H NMR (DMSO-d_6): δ = 3.62 (s, 2H), 5.00 (s, 2H), 7.52 (s, 2H).

The above reaction mixture filtrate (from **12**) was neutralised to pH 5 with NaOH (13.1 g, 0.332 mol in 100 cm^3 water) and the resulting white precipitate was collected, washed with water, and dried to afford *4-amino-3-iodophenylacetic acid* (**11**); yield 12.6 g (56%). A sample, crystallised from propan-2-ol had mp 127–128 °C (dec.) Anal. ($C_8H_8INO_2$) C,H,N,I; ^{1}H NMR (DMSO-d_6,): δ = 3.32 (s, 2H), 5.12 (s, 2H), 6.69 (d, H), 6.96 (d, H), 7.43 (s, H), 12.2 (br, 1H).

*N-[5-[2-(4-Amino-3-iodophenyl)ethanamidopentanyl]]-N'-(4-methoxybenzyl)-N-methyl-N'-(2-pyridinyl)-1,2-ethanediamine oxalate hydrate (Iodoaminophenpyramine)(**8**)*

To a solution of 4-amino-3-iodophenylacetic acid (**11**) (0.39 g, 1.41 mmol) and NHS (0.16 g, 1.41 mmol) in dry CH_2Cl_2 (25 cm^3) was added DCC (0.29 g, 1.41 mmol) in dry CH_2Cl_2 (5 cm^3) with stirring and cooling (ice bath). The cooled mixture was stirred for 1 h, then amine **3** (0.50 g, 1.40 mmol) in dry CH_2Cl_2 (5 cm^3) was added, and the mixture was stirred with cooling for a further 3 h; it was then filtered from the precipitated *N,N*-dicyclohexylurea, washed with aq Na_2CO_3 (x2) and then with water, dried, and concentrated to yield a pale yellow oil. The latter was purified by chromatography using MeOH:EtOAC (2:3) as eluent to furnish the product as an oily base (0.25 g) which was treated with oxalic acid (0.48 g, 5.3 mmol) in MeOH. The methanolic solution was concentrated and the resulting residue was crystallised from the minimum of propan-2-ol and washed with dry ether to provide the product **8** as oxalate hydrate (yield 0.80 g, 79%) which, after crystallisation from propan-2-ol, had mp 53–54 °C; Anal. ($C_{29}H_{38}IN_5O_2$, $C_2H_2O_4$,H_2O) C,H,N,I; ^{1}H NMR (DMSO-d_6/D_2O): δ = 1.23 – 1.58 (m, 6H), 2.83 (s, 3H), 3.06 – 3.23 (m, 8H), 3.70 (s, 3H), 3.91 (m, 2H), 4.62 (s, 2H), 6.69 – 7.18, (m, 8H), 7.52 (m, 2H) 8.11 (m, 1H). HPLC: using 7:3 mixture of 0.1% aq TFA and MeCN containing 5% water and 0.1% TFA

gave a major component at 10.1 min representing 97.9% of total area of elution curve.

N-[5-[2-(4-Amino-3,5-diiodophenyl)ethanamidopentanyl]]-N'-(4-methoxybenzyl)-N-methyl-N'-(2-pyridinyl)-1,2-ethanediamine (Diiodoaminophenpyramine)(**9**)

In a similar manner to that for compound **8**, 4-amino-3,5-diiodophenylacetic acid (**12**) (0.57 g, 1.41 mmol) and NHS (0.16 g, 1.41 mmol) in dry THF (25 cm^3) were treated with DCC (0.29 g, 1.41 mmol) in THF (5 cm^3), and then with amine **3**. The resulting product was similarly purified by column chromatography to afford the required product as a colourless solid (yield 0.080 g, 77%) which, after crystallisation from EtOAc with the addition of Et_2O, had mp 116–117 °C; Anal. ($C_{29}H_{37}I_2N_5O_2$) C,H,N,I; ^1H NMR ($CDCl_3$): δ = 1.25, 1.42 (m, 6H), 2.25 (s, 3H), 2.36, (t, 2H), 2.56 (t, 2H), 3.18 (m, 2H), 3.32 (s, 2H), 3.63 (t, 2H), 3.77 (s, 3H), 4.57 (s, 2H), 4.67 (s, 2H), 5.70 (s, 1H), 6.47 – 7.38 (m, 7H), 7.51 (s, 2H), 8.13 (m, 1H). HPLC: using a 3:2 mixture of 0.1% TFA and MeCN containing 5% water and 0.1% TFA gave the major component at 8.6 min representing 97.9% of total area of elution curve.

N-[5-[2-(4-Azido-3-iodophenyl)ethanamidopentanyl]]-N'-(4-methoxybenzyl)-N-methyl-N'(2'-pyridinyl)-1,2-ethanediamine oxalate (Iodoazidophenpyramine) (**1**)

Method A. A solution of sodium nitrite (55 mg, 1.04 mmol) in water (1 cm^3) was added dropwise during 5 min to a cooled (ice-salt bath) solution of iodoaminophenpyramine base (**8**) (400 mg, 0.65 mmol) in 4N HCl (5 cm^3) with stirring. After being stirred for a further 15 min, the mixture was treated with 4 drops of saturated aq urea. Ten minutes later, a solution of sodium azide (83 mg, 1.32 mmol) in water (1 cm^3) was slowly added dropwise with stirring and cooling. The mixture was stirred at 0 °C for 15 min, then stirred at 20 °C for 15 min, basified with solid Na_2CO_3, and extracted with CH_2Cl_2. The organic phase was dried and concentrated, and the resulting oil was chromatographed on a silica-gel column using MeOH:EtOAc (2:3) as eluent to afford the product as free base (334 mg). The latter was treated with oxalic acid (100 mg, 1.11 mmol) in MeOH; the mixture was evaporated and the residue crystallised from the minimum of propan-2-ol; the resulting crystals were washed with dry ether to give the product *iodoazidophenpyramine oxalate* (**1**) which, after recrystallisation from propan-2-ol, had mp 110–111 °C; yield 370mg, 78%. The NMR spectrum and HPLC characteristics were identical with authentic material made by a different route (below).

Method B. A solution of $NaNO_2$ (1.10 g, 20.8 mmol) in water (10 cm^3) was added dropwise to a stirred suspension of 4-amino-3-iodophenylacetic acid (**11**) (3.66 g, 13.2 mmol) in 6N HCl (20 cm^3) at 0 to –5 °C. The mixture was stirred for 30 min, then a few drops of saturated urea solution were added and, after *10* min, a solution of sodium azide (1.72 g, 26.5 mmol) in water (10 cm^3) was added very slowly with stirring and cooling. The mixture was stirred in an ice bath for 3 h, then kept at 4 °C overnight, and the purple coloured solid which had formed was collected and dissolved in aqueous Na_2CO_3. The solution was treated with decolourising charcoal, filtered and acidified with 2N HCl to precipitate *4-azido-3-iodophenylacetic acid* (**13**) as a pale grey solid which was collected, washed with water and dried in vacuo at 20 °C (yield 3.5 g, 88%). Crystallisation (×2) from petroleum spirit (bp 60–80 °C) furnished pure material mp 122–123 °C (dec.); Anal. ($C_8H_6IN_3O_2$) C,H,N,I; ^1H NMR (DMSO-d_6): δ = 3.54 (s, 2H), 7.32 (m, 2H), 7.73 (s, 1H); IR (KBr): ν_{max} = 2126 cm^{-1} (N_3), 1698 (C=O).

4-Azido-3-iodophenylacetic acid (**13**) (0.65 g, 2.15 mmol) was added portionwise with stirring to thionyl chloride (2.5 cm^3) at 20 °C and the resulting solution was boiled under reflux for 1 h. The excess thionyl chloride was distilled off under reduced pressure and the residue was taken into CH_2Cl_2 and evaporated; the treatment with CH_2Cl_2 was repeated and the residue was then dissolved in CH_2Cl_2 (2 cm^3) and added dropwise together with aq NaOH (86 mg, 2.15 mmol in 2 cm of water) to a vigorously stirred and cooled (ice bath) solution of the amine **3**(0.69 g, 1.94 mmol) in CH_2Cl_2 (6 cm^3). The mixture was stirred for a further 30 min and then the organic phase was separated off and the aq phase extracted twice with CH_2Cl_2. The combined organic phases were washed with aqueous Na_2CO_3, then with water, dried ($MgSO_4$) and evaporated. The residual oil was chromatographed in the dark using MeOH:CH_2Cl_2 (20:1) as eluent. The resulting product (0.91 g) was converted into the oxalate in propan-2-ol (using 0.28 g, 3.11 mmol of oxalic acid), induced to crystallise by the addition of dry ether, collected and washed with ether to give *iodoazidophenpyramine oxalate* (**1**) as a colourless solid (1.00 g, 71% yield) which, after recrystallisation from propan-2-ol, had mp 110-111 °C; Anal. ($C_{29}H_{36}IN_7O_2,C_2H_2O_4$) C,H,N,I; ^1H NMR (DMSO-d_6): δ = 1.25 – 1.61 (m, 6H), 2.85 (s, 3H), 3.07 – 3.28 (m, 6H), 3.39 (s, 2H), 3.72 (s, 3H), 3.91 (m, 2H), 4.63 (s, 2H), 6.69 – 7.55 (m, 9H), 7.75 (s, 1H), 8.09 (m, 1H); IR (KBr): ν_{max} = 2117 cm^{-1} (N_3), 1637 (C=O). HPLC: using 11:9 mixture of 0.1% TFA and MeCN containing 5% water and 0.1% TFA gave the major component at 6.9 min representing 97.9% of total area of elution curve.

Method C. A solution of DCC (0.29 g, 1.41 mmol) in dry CH_2Cl_2 (5 cm^3) was added to a stirred and cooled (ice-water bath solution of 4-azido-3-iodophenylacetic acid (**13**) (0.43 g, 1.41 mmol) and NHS (0.16 g, 1.41 mmol) in dry CH_2Cl_2 (25 cm^3). Stirring with cooling was continued for 1 h and then a solution of the amine **3** (0.50 g, 1.40 mmol) in dry CH_2Cl_2 (5 cm^3) was added. The mixture was stirred with cooling for 3 h, then filtered from the precipitate of *N,N'*-dicyclohexylurea, washed with aq Na_2CO_3, then washed twice with water, dried, and concentrated to afford a pale yellow oil. The latter was chromatographed using MeOH:EtOAc (2:3) as eluent to afford the product **3** as an oily base (0.75 g); this was treated with oxalic acid (0.23 g, 2.55 mmol) in MeOH. The solvent was evaporated and the residue was crystallised from the minimum of propan-2-ol. The colourless solid product as the *oxalate* **1** was collected and washed with ether (yield 0.82 g, 80%); after being recrystallised from propan-2-ol it had mp 110 – 111 °C and IR and NMR spectra identical with those of the material made by Methods A and B above.

References

[1] a) A.H. Newman, *Annu. Rep. Med. Chem.* **1990** *25*, 271–280; b) M.A. Buck, C.M. Fraser, J.G. Venter, in *Comprehensive Medicinal Chemistry, Vol. 3*, ed. J.C. Emmett, Pergamon Press, Oxford, **1990** 91–92.

[2] M. Ruat, M. Körner, M. Garbarg, C. Gros, J–C. Schwartz, W. Tertiuk, C.R. Ganellin, *Proc. Natl. Acad. Sci. USA*, **1988** *85*, 2743–2747.

[3] C.R. Ganellin, M.L. Bouthenet, M. Garbarg, C. Gros, R.J. Ife, M. Körner, M. Ruat, J-C. Schwartz, W. Tertiuk, C.J. Theobald in *Trends in Medicinal Chemistry*, eds. H. van der Goot, G. Domany, L. Pallos, H. Timmerman, Elsevier, Amsterdam, **1989** 333–350.

[4] M. Ruat, M.L. Bouthenet, J-C. Schwartz, C.R. Ganellin, *J. Neurochem.* **1990** *55*, 379–385.

[5] M. Yamashita, H. Fukui, K. Sugama, Y. Horio, S. Ito, H. Mizuguchi, H. Wada, *Proc. Natl. Acad. Sci. USA*, **1991** *88*, 11515–11519.

[6] R.C. Blakemore, C.R. Ganellin, M. Garbarg, C. Gros, R.J. Ife, M. Körner, M. Ruat, J-C. Schwartz, W. Tertiuk, C.J. Theobald, *Eur. J. Med. Chem.* **1987** *22*, 91–100.

[7] M. Körner, M.L. Bouthenet, C.R. Ganellin, M. Garbarg, C. Gros, R.J. Ife, N. Sales, J-C. Schwartz, *Eur. J. Pharmacol.* **1986** *120*, 151–160.

[8] J.W. Barnett, F.A. Robinson, B.M. Wilson, *J. Chem. Soc.* **1946** 202–204.

Received: June 9, 1995 [FP027]