SYNTHESES OF ^{14}C-LABELLED PRIZIDILOL DIHYDROCHLORIDE

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SUMMARY

Two syntheses of radiolabelled prizidilol dihydrochloride (DL)3-[2-(3-t-butylamino -2-hydroxypropoxy)phenyl]-6-hydrazino-pyridazine dihydrochloride) are described.

1. A ten stage synthesis (Scheme 1) which gave [3,6-$^{14}C_2$] prizidilol dihydrochloride (10) in an overall yield of 0.91%.
2. A later, alternative procedure (scheme 2) leading to [4,5-$^{14}C_2$] prizidilol dihydrochloride (16) with an overall radiochemical yield of 8%.

Keywords: Prizidilol dihydrochloride, [1,4-$^{14}C_2$] succinic acid, [2,3-$^{14}C_2$] succinic anhydride, preparative layer chromatography, reverse isotope dilution, ^{14}C-labelled.

INTRODUCTION

Prizidilol (SK&F 92657)[1] is an antihypertensive drug which produces a sustained fall in blood pressure in both animals[2,3,4] and man[5,6] as a result of precapillary vasodilatation, together with competitive β-adrenoreceptor blockade. Many β-blockers have a characteristic 2-hydroxy-3-alkylaminopropoxy side chain coupled to

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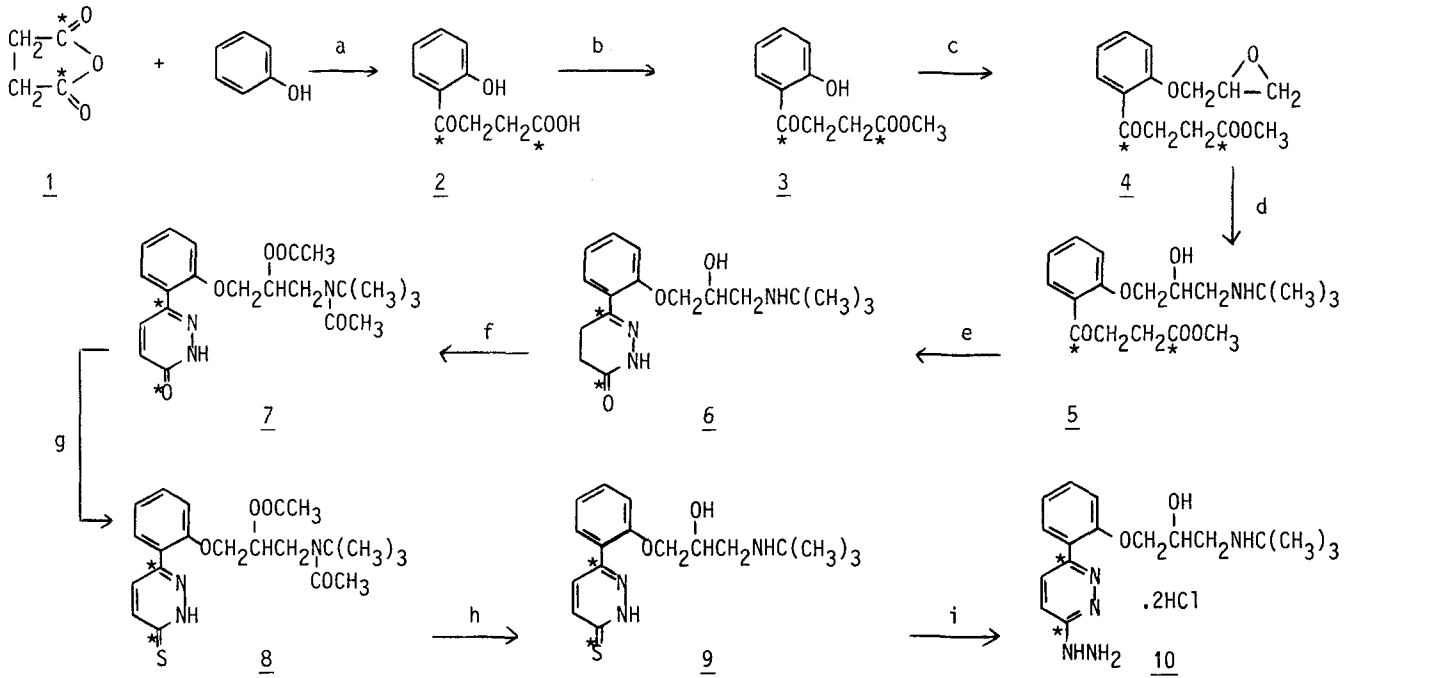
Scheme 1



a) $AlCl_3$/dichloromethane b) methanol/HCl gas c) epibromohydrin, K_2CO_3, MEK d) t-butylamine

e) hydrazine hydrate f) acetic anhydride, bromine g) phosphorus pentasulphide h) sodium hydroxide, methanol

i) hydrazine hydrate

an aromatic system; prizidilol incorporates this moiety with the characteristic vasodilating structure of a hydrazinopyridazine and possesses both modes of action.

Prizidilol is used as the dihydrochloride monohydrate, a pale yellow crystalline solid, $C_{17}H_{25}N_5O_2.2HCl.H_2O$ of molecular weight 422.383 and melting point 163-6°C.

DISCUSSION

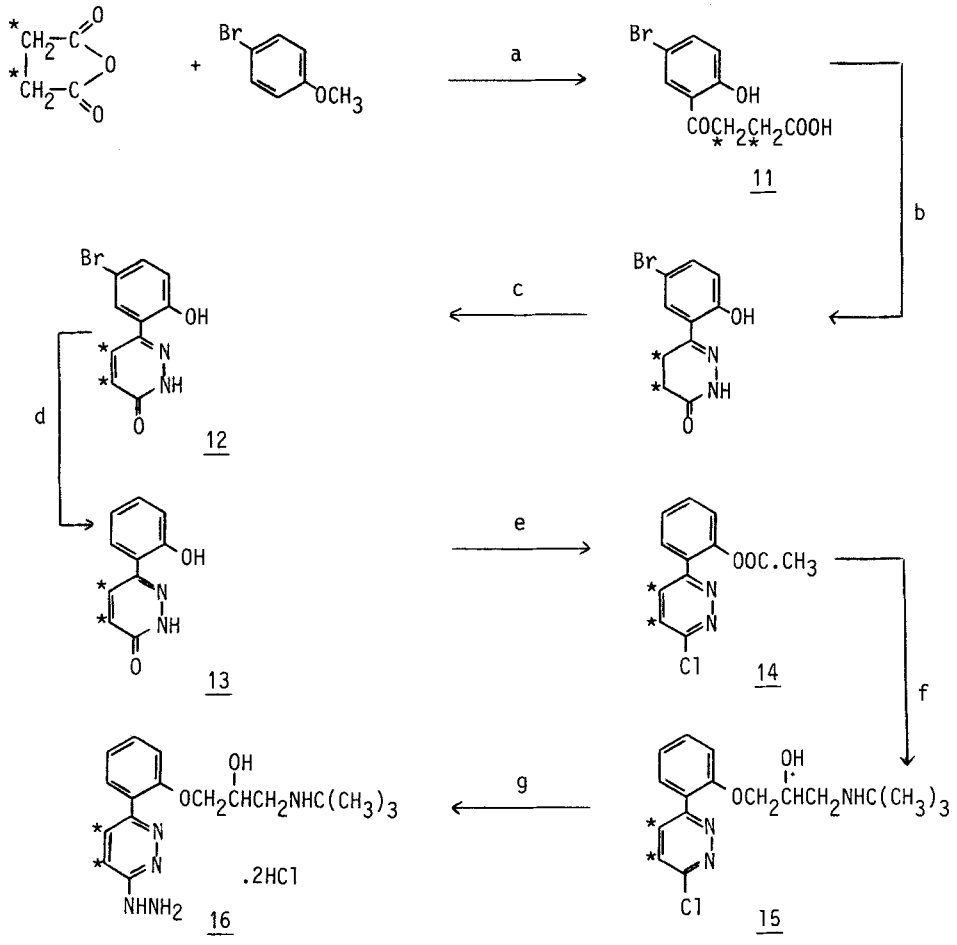
Prizidilol dihydrochloride was originally synthesised by the process shown in Scheme 1 in an overall yield of 0.91%. The principal deficiencies of this route were its length (10 stages), the large loss of activity encountered during preparation of the benzoylpropionic acid (2) due to formation of a p-succinoylated by-product, and the need for extensive use of plc for purification of several intermediates exhibiting poor crystallinity.

An improved, 7-stage synthesis (Scheme 2) was later developed which gave, generally, more readily crystallised intermediates and specifically avoided the formation of isomers during succinoylation by utilising p-bromoanisole as substrate. The bromine was removed at a convenient stage by hydrogenolysis. These improvements resulted in a higher overall yield of 8%.

EXPERIMENTAL

Radioactivity measurements were carried out using a Searle Mark III Liquid Scintillation Counter. Preparative layer chromatography purification was carried out on Analtech 02013 Silica gel GF 254 1000μ plates. Radiochemical purities were determined by reverse isotope dilution and analytical HPLC using a Waters C_{18} μ Bondapak column, a Perkin-Elmer LC55 uv/vis Spectrophotometer at 254nm. [The mobile phase used was acetonitrile:citric acid (10^{-2}M) (15:85, v/v) and at a flow rate of 1.5ml/min prizidilol had a

Scheme 2



a) $AlCl_3$, dichloromethane b) hydrazine hydrate

c) sodium m-nitrobenzene sulphonate d) H_2 gas, 10% Pd/C

e) acetic anhydride, phosphorus oxychloride f) epibromohydrin in sodium methoxide/DMF, t-butylamine

g) hydrazine hydrate

retention time of 3 mins. The total column eluant fraction was collected manually every 30 secs. To the fractions was added Pico-Fluor 15 (10ml) and the radioactivity determined by liquid scintillation counting.]

[1,4-$^{14}C_2$] Succinic acid (25mCi,21.92mCi.mmol^{-1}) was purchased from Amersham International plc for use in Scheme 1, and [2,3-$^{14}C_2$] succinic anhydride (201.4 mCi, 47.4 mCi.mmol^{-1}) was supplied by ICI, Physics and Radioisotope Services, Billingham for use in Scheme 2.

SYNTHESIS OF [3,6-$^{14}C_2$] PRIZIDILOL DIHYDROCHLORIDE (10)

SUCCINIC ANHYDRIDE -1,4-$^{14}C_2$ (1)

A solution of [1,4-$^{14}C_2$] succinic anhydride (135mg; 25mCi; 1.14 mmol) in thionyl chloride (1.5ml) was refluxed for 1h then evaporated to dryness to give succinic anhydride (113.8mg, 99.4%).

3-(2-HYDROXYBENZOYL)-[1,4-$^{14}C_2$]PROPIONIC ACID (2)

A stirred solution of [1,4-$^{14}C_2$] succinic anhydride (113.8mg; 1.138mmol) and phenol (106.0mg; 1.12mmol) in sym-tetrachloroethane (0.8ml) was maintained at 40° for 1h, treated with anhydrous aluminium chloride (320mg; 2.39mmol), heated to 135°, then, after 1h, cooled and treated with hydrochloric acid (6M; 2.0ml) to give a biphasic solution. The tetrachloroethane layer was removed and further extractions of the acidic aqueous layer were made using the same solvent (3 x 2.0ml). The combined tetrachloroethane solutions were shaken with 20% sodium carbonate solution (3 x 0.5ml) which was separated and acidified (HCl). The dark solid obtained was filtered off and recrystallised from water to give the required product (88.5mg; mp 140.1°) in 40.1% yield.

METHYL 3-(2-HYDROXYBENZOYL) [1,4-$^{14}C_2$]PROPIONATE (3)

A refluxing solution of the substituted propionic acid (2) (88.0mg; 0.45mmol) in methanol (2.0ml) was saturated with HCl gas for 30 mins. The methanol was then removed under reduced pressure and the residue distributed between water (1.0ml) and dichloromethane (5.0ml). The organic layer was washed with 10% sodium carbonate solution (0.75ml), dried (Na_2SO_4) and evaporated to give the required product (90.0mg; 95.4%) as a pale brown oil.

METHYL 3-[2-(2,3-EPOXYPROPOXYBENZOYL)] [1,4-$^{14}C_2$]PROPIONATE (4)

A mixture of (3) (90.0mg; 0.43mmol), potassium carbonate (anhydrous; 63.0mg; 0.636mmol) and epibromohydrin (215mg; 1.58mmol) in dry MEK (3.0ml) was stirred and refluxed under an efficient condenser and calcium chloride tube for 48h. The cooled reaction mixture was filtered to remove inorganic salts and the filtrate was evaporated at 95°/1.0mm to remove both solvents and excess of epibromohydrin. The required product was contained in the residual oil (120mg) which was used without further purification for the preparation of (5).

DL-METHYL 3-[2-(2-HYDROXY-3-t-BUTYLAMINOPROPOXYBENZOYL)] [1,4-$^{14}C_2$]PROPIONATE (5)

The epoxide (4) (120mg) in methanol (1.0ml) was treated with t-butylamine (2.0ml) and the solution stirred and refluxed for 1h. Evaporation of the reaction mixture gave a brown gum (164.4mg) which was purified by plc using chloroform/methanol mixtures to give the required product (109.0mg; 75% overall from (3), mp 80°).

DL-3-[2-(2-HYDROXY-t-BUTYLAMINOPROPOXY)PHENYL]-4,5-DIHYDRO-[3,6-$^{14}C_2$]PYRIDAZIN-3[2H]-ONE (6)

A solution of (5) (109.0mg; 0.323mmol) in glacial acetic acid (0.5ml) and water (0.5ml) was treated with hydrazine hydrate (100mg;

1.92mmol), refluxed for 4h then evaporated to dryness. The residue was treated with saturated potassium carbonate solution and the resulting cloudy solution thoroughly extracted with dichloromethane Evaporation of the dried ($MgSO_4$) extract yielded a glassy solid (104mg), recrystallisation of which from 2-propanol gave the required, cyclised product (95.4mg; (92.5%); mp 135-40°).

6-[2-(2-ACETOXY-3-N-ACETYL-t-BUTYLAMINOPROPOXY)PHENYL] [3,6-$^{14}C_2$] PYRIDAZIN-3[2H]-ONE (7)

A mixture of the dihydropyridazinone (6) (55.4mg; 0.174mmol), anhydrous potassium carbonate (60mg; 0.429mmol) and acetic anhydride (0.5ml; 4.9mmol) were stirred and heated at 80° for 3h, diluted with acetic acid (1.0ml), then at 80° treated with bromine (30mg; 0.188mmol) in acetic acid (1.0ml) during 1h. Stirring and heating were continued for 20 mins after bromine addition was complete. The reaction mixture was evaporated and the residue distributed between water and dichloromethane. The dichloromethane layer was separated, washed with water, dried ($MgSO_4$), evaporated, and the residue purified by plc to give the required product (45.0mg: 64%) as a gum.

DL-6-[2-(2-ACETOXY-3-N-ACETYL-t-BUTYLAMINOPROPOXY)PHENYL]-[3,6-$^{14}C_2$]-PYRIDAZINE-3[2H]-THIONE (8)

A stirred solution of the pyridazinone (7) (93.1mg; 0.23mmol) in dry pyridine (1.5ml) was treated with phosphorus pentasulphide (100mg; 0.45mmol) then refluxed for 3h. Further phosphorus pentasulphide (20.0mg; 0.09mmol) was added and reflux continued for 1h. The upper (pyridine) layer was removed from the reaction mixture and the thick

oily residue washed with fresh pyridine (3 x 1.0ml). The combined washings were evaporated and the dark residue was azeotroped with ethanol, dissolved in dichloromethane and the solution washed with 0.1N hydrochloric acid (1.0ml). Evaporation of the dried ($MgSO_4$) dichloromethane solution gave a gum which was purified by preparative layer chromatography. Development of the plate was carried out in two stages: elution with chloroform alone, followed by chloroform-methanol (25:1, v/v) to give at high Rf the required product (46.4mg; 47.9%) and, at low Rf, starting pyridazinone (37.2mg; 40.0%).

DL-6-[2-(2-HYDROXY-3-t-BUTYLAMINOPROPOXY)PHENYL]-[3,6-$^{14}C_2$]-PYRIDAZINE-3[2H]-THIONE (9)

The protected thione (8) (74.2mg; 0.177mmol) in methanol (1.0ml) containing 0.6N sodium hydroxide solution (1.3ml; 0.78mmol) was stirred and refluxed for 1.5h then the cooled reaction mixture was acidified to pH4 using dilute acetic acid. The clear supernatant liquor was decanted from the black tar and adjusted to pH 8.5 using 0.6N sodium hydroxide solution to give an off-white solid (28mg; mp 178°) which on recrystallisation from aqueous ethanol gave the required product (13.0mg; (22%); mp 186°) with specific activity 21.4mCi.mmol^{-1}

DL-3-[2-(3-t-BUTYLAMINO-2-HYDROXYPROPOXY)PHENYL]-[3,6-$^{14}C_2$]-6-HYDRAZINOPYRIDAZINE DIHYDROCHLORIDE (10)

The thione (9) (13.0mg; 0.039mmol) was refluxed with stirring in hydrazine hydrate (0.5ml) for 1h. The oil which separated on cooling was extracted into dichloromethane (3x0.5ml) which was then washed with water (0.2ml), dried (MgSO4) and evaporated to give the free base of prizidilol (10mg) as a light oil. To this was added 1N hydrochloric acid (0.1ml) and the solution was evaporated under

reduced pressure. Trituration of the residue with n-propanol gave ^{14}C-prizidilol (8.0mg; 61%, 65μCi/mg, 523μCi).
Radiochemical purity was determined by reverse isotope dilution and found to be 97.6%.

SYNTHESIS OF [4,5-$^{14}C_2$] PRIZIDILOL DIHYDROCHLORIDE (16)

3-(5-BROMO-2-HYDROXYBENZOYL)-[2,3-$^{14}C_2$]PROPIONIC ACID (11)

[2,3-$^{14}C_2$]Succinic anhydride (424.9mg, 201.4mCi, 4.25mmol) 4-bromoanisole (782mg, 5.18mmol) and aluminium trichloride (1.79gm, 13.4mmol) were stirred together in dichloromethane (4ml) for 1h. The reaction mixture was then slowly heated to reflux and maintained at this temperature with vigorous stirring for 6h then allow to stand at room temperature overnight. The reaction mixture was cooled in ice, then water (10ml) and concentrated hydrochloric acid (2ml) added with vigorous stirring. The dichloromethane was removed under reduced pressure from the two phase system to leave a buff solid, which was filtered off, washed with water and dried to give the required product (696mg) in a 60% yield.

6-(5-BROMO-2-HYDROXYPHENYL)-3(2H)-[4,5-$^{14}C_2$]PYRIDAZINONE (12)

The substituted propionic acid (11) (696mg, 2.55mmol) was stirred in water (5ml) containing hydrazine hydrate (0.5ml) for 2.5h at 80°C and a buff solid was precipitated. To the cooled stirred slurry from the hydrazinolysis was added solid sodium hydroxide (320mg, 8mmol) and sodium 3-nitrobenzenesulphonate (700mg, 3.11mmol). The resulting dark brown solution was heated under reflux for 3.5h. The reaction mixture was cooled, the pH adjusted to 8.5 with 1N hydrochloric acid and the buff precipitate obtained was collected, washed with water then dried ($MgSO_4$) to give the required product

(12) (291mg) in a 42.7% yield. NMR analysis confirmed the presence of the required pyridazinone (12) and the absence of any unoxidised dihydropyridazinone.

6-(2-HYDROXYPHENYL)-3(2H)-[4,5-$^{14}C_2$]PYRIDAZINONE (13)

The bromophenyl pyridazinone (12) (291mg, 1.09mmol) was dissolved in a solution of sodium hydroxide (300mg, 7.5mmol) in water (5ml) and hydrogenated at room temperature at 50psi in the presence of 10% Pd/C catalyst (25mg) for 5h after which time hydrogen uptake was complete. The catalyst was removed by filtration and washed with water (2 x 2ml). The combined filtrates were then adjusted to pH 8.5 with 1N hydrochloric acid and the off-white solid which precipitated was collected, washed with water and dried to give the required debrominated material (13) (182mg; (89%); mp 295-6°); mixed mp showed no depression with an authentic sample.

3-(2-ACETOXYPHENYL)-CHLORO-[4,5-$^{14}C_2$]-6-PYRIDAZINE (14)

The hydroxyphenylpyridazinone (13) (182mg, 0.968mmol) was refluxed with stirring in acetic anhydride (2ml) and pyridine (100µL) for 1.5h. The mixture was cooled, evaporated to dryness under reduced pressure and the residual oil triturated with methanol (2ml) and water (500µl) to give a pale brown solid.

The aqueous methanol was evaporated off under reduced pressure and the residue heated at 60°C with stirring in phosphoryl chloride (5ml) for 1.5h. The mixture was evaporated to dryness under reduced pressure and the residue triturated with iced water (20ml) then extracted with ethyl acetate (4 x 10ml). The combined extracts were dried ($MgSO_4$) and evaporated to dryness under reduced pressure to give the required chloropyridazine (14) (209mg) in a yield of 87%. Confirmation of the required compound was shown by tlc (chloroform methanol (25:1, v/v) on Merck silica 5735) compared to authentic sample.

3-[2-(3-t-BUTYLAMINO-2-HYDROXYPROPOXY)PHENYL]-6-CHLORO-[4,5-$^{14}C_2$]PYRIDAZINE (15)

To a stirred suspension of sodium hydride (50% dispersion in mineral oil) (87.5mg, 1.83mmol) in dimethylformamide (1ml) at room temperature was added methanol (38µl) followed by the acetoxyphenylchloropyridazine (14) (209mg, 0.874mmol). The mixture was stirred for 30 min then epibromohydrin (500µl) was added and stirring was continued for 4h. Water was carefully added dropwise until effervescence ceased, then the reaction mixture was evaporated under reduced pressure to a low bulk. Further water (20ml) was added then the mixture was extracted with ethyl acetate (4 x 5ml). The extracts were combined, dried ($MgSO_4$) and evaporated under reduced pressure to leave a waxy solid, which was refluxed with stirring in t-butylamine (10ml) and toluene (5ml) for 20h.
The reaction mixture was evaporated under reduced pressure and the residue purified by preparative layer chromatography (8 x Analtech 02013 Silicagel GF254 1000µ plates) using ethyl acetate:methanol: ammonia (5:1:1, by vol) as the developing solvent. The required zones were removed, combined and eluted with ethanol to give the required intermediate (15) (239.5mg) in an overall yield of 85% for 2 stages.

DL-3-[2-(3-t-BUTYLAMINO-2-HYDROXYPROPOXY)PHENYL]-[4,5-$^{14}C_2$]6-HYDRAZINOPYRIDAZINE DIHYDROCHLORIDE (16)

The chloropyridazine intermediate (239.5mg 0.714mmol) was refluxed with stirring in hydrazine hydrate (1ml) for 1h. The oil which separated on cooling was extracted into dichloromethane (3 x 2ml) and the combined extracts were washed with water (0.5ml), dried (MgSO4), then evaporated to leave a light oil (177mg) - the free

base of prizidilol. To this was added 1N HCl (1.07ml, 1.07mmol) and the solution evaporated under reduced pressure. The residue was combined with unlabelled prizidilol (500mg) and recrystallisation from n-propanol:water (9:1 v/v. 2.5ml) gave ^{14}C-prizidilol. (475mg, 19.7 μCi/mg, 9357μCi). Further unlabelled prizidilol (5g) was added to the mother liquor and recrystallisation from the above solvent mixture (20ml) gave ^{14}C-prizidilol (5.29gm, 1.21μCi/mg, 6401μCi).

Radiochemical purities were determined on both batches of material by reverse isotope dilution and found to be 97.5% in both cases. HPLC analyses on both batches gave the radiochemical purities of 97.3% and 97.8% respectively.

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