# SYNTHESIS OF [$^3$H]ZOLPIDEM

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**SUMMARY**

*Zolpidem is a novel hypnotic agent possessing a substituted imidazo[1,2-a] pyridine structure. The synthesis of the tritium labelled compound with a specific activity of 60.5 Ci/mmol is described. This new radioligand was initially used to establish and characterize the binding properties of Zolpidem in the rat brain.*

*Key words : Zolpidem, Imidazo[1,2-a]pyridine, Tritium, Radioligand.*

## INTRODUCTION

Zolpidem (1), N,N,6-trimethyl-2-(4-methylphenyl)-imidazo[1,2-a]pyridine-3-acetamide (R,R)-(+)-hemitartrate, is a benzodiazepine receptor agonist, being chemically unrelated to the benzodiazepines and possessing pharmacological and therapeutic advantages over them. It binds preferentially to central benzodiazepine receptors and has a higher affinity for cerebellar than for hippocampal benzodiazepine sites[1]. Its biochemical and pharmacological profile and its chemistry have been described[1-6]. In a previous paper[7] we have described the synthesis of the $^{14}$C-labelled compound which was used for pharmacokinetic and drug metabolism studies during the development of this drug.

We now report the synthesis of the tritium labelled compound at high specific activity which was used to establish the presence and characterize the

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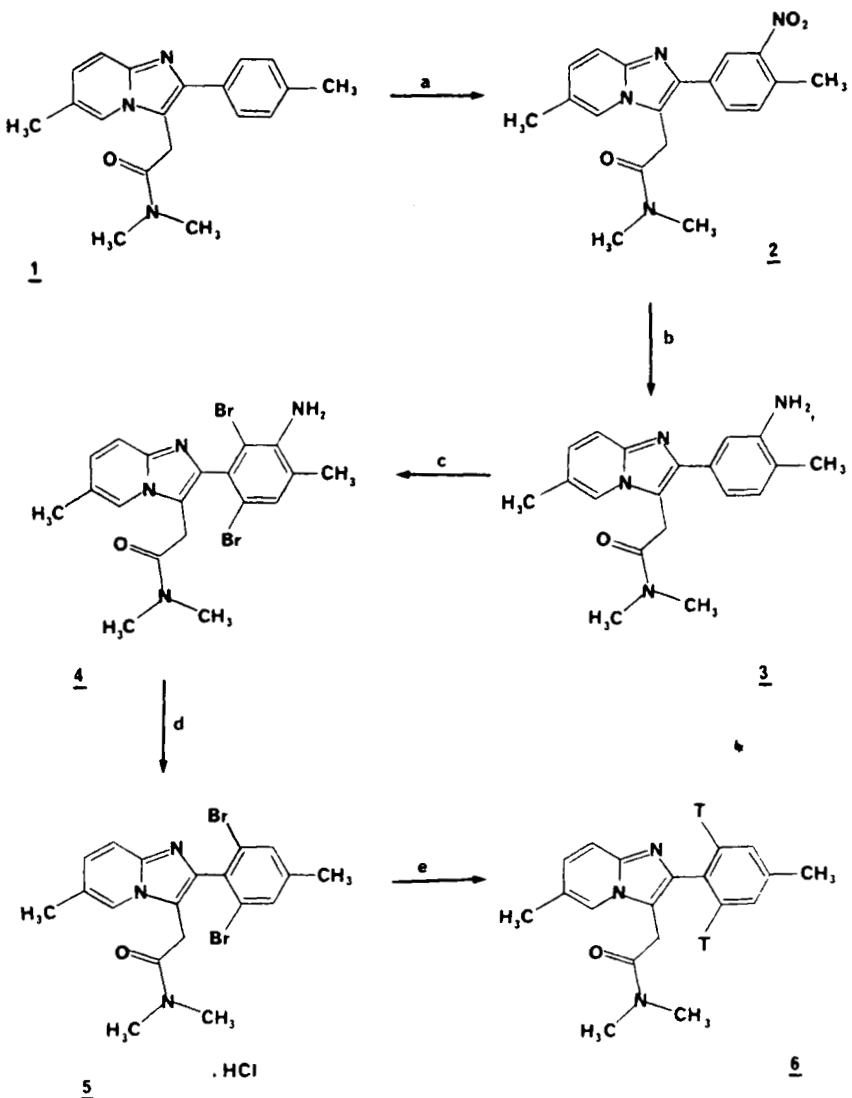
binding properties of this compound in the rat brain. The usefulness of this new radioligand in high affinity binding in the rat brain has been recently described[8].

## DISCUSSION

Our approach to the synthesis of Zolpidem at high specific activity was to synthesise a suitable dihalogenated precursor and then to incorporate the tritium into the molecule by catalytic reductive dehalogenation in the presence of tritium gas. Attempts at the synthesis of such a precursor by direct iodination or bromination of Zolpidem were unsuccessful, only complex mixtures were obtained which were difficult to purify. A successful synthesis of dibromo-Zolpidem and [$^3$H]Zolpidem with a specific radioactivity of 60.5 Ci/mmol was achieved as outlined in scheme 1. Under carefully controlled conditions Zolpidem (1) could be nitrated to yield the mononitro compound (2) as the major product. Dinitro- and trinitro-compounds were isolated as by-products. Reduction of the nitro compound (2) to the corresponding amine (3) was readily achieved using tin and hydrochloric acid. This amine (3) was brominated with bromine in chloroform to yield the dibromo compound (4). Diazotisation of (4) and reduction of the diazo compound with hypophosphorous acid yielded the dibromo-Zolpidem analogue (5). Debromination of dibromo-Zolpidem (5) using a 10% Pd/$BaSO_4$ catalyst in the presence of tritium gas afforded on purification by preparative layer chromatography [$^3$H]Zolpidem (6) with a specific radioactivity of 60.5 Ci/mmol and a radiochemical purity of 98%. It was necessary in the debromination stage to use a 10% Pd/$BaSO_4$ catalyst since it was difficult to avoid saturating the pyridine ring of the imidazo[1,2-a]pyridine nucleus using a Pd/C catalyst.

Mass spectral analysis of the product indicates the presence of a small amount of the tritritio-labelled compound which probably accounts for the specific activity being slightly higher than the theoretical maximum. This third tritium probably being situated in the acetamido side chain methylene function although this has not been confirmed by proton decoupled tritium nmr studies.

## SCHEME 1




a) $HNO_3$, TFA ; b) Sn, HCl ; c) $Br_2$, $CHCl_3$ ;

d) $HNO_2$, $H_2PO_3$ ; e) $T_2$, $Pd/BaSO_4$

## EXPERIMENTAL

Melting points were obtained on a Büchi SMP 20 (Tottoli) melting point apparatus and are uncorrected. $^1H$ NMR spectra were recorded on a Bruker WP 200 SY spectrometer. Chemical shifts are reported in ppm. Mass spectra were recorded on a VG Micromass 7070 instrument in the electron impact mode (70 eV). Elemental analysis were determined using a Perkin Elmer 240 analyser, linked to a Tektronix 31 calculator and are within $\pm$ 0.3% of theoretical values. The tritiation step was carried out by Amersham International plc, UK. Radiochemical purity was determined by radiochromatography using a Berthold LB 2832 TLC Linear Analyser.

**N,N,6-Trimethyl-2-(4-methyl-3-nitrophenyl)imidazo[1,2-a]pyridine-3-acetamide (2)**

A solution of Zolpidem (1) (7.5 g, 24.4 mmol) in trifluoroacetic acid (60 ml) was cooled to -10°C and concentrated nitric acid (15 ml) was added dropwise and the reaction mixture stirred at -10°C for 1 hour. The reaction mixture was neutralised at 0°C with sodium hydroxide solution and the product which was a yellow solid was recovered by filtration to afford the desired nitro compound (2) (5.9 g, 16.74 mmol, 68.6%) mp = 226-7°C, $^1H$-NMR (200 MHz, $CDCl_3$, TMS) $\delta$ 8.2-7.06(m, 6H, Py$\underline{H}_3$ + Ar$\underline{H}_3$), 4.09 (S, 2H, -C$\underline{H}_2$-), 3.08 and 3.02 (S, 3H,NC$\underline{H}_3$), 2.65 and 2.37 (S, 3H, PyC$\underline{H}_3$ and ArC$\underline{H}_3$). IR$\gamma_{max}$ (KBr) 2920, 1640, 1521, 1345 and 810 $cm^{-1}$. MS, m/z 352 ($M^+$), 280 (base peak), 235, 233, 219, 92 and 72. Anal. Calcd for $C_{19}H_{20}N_4O_3$ : C, 64.76 ; H, 5.72 ; N, 15.9%. Found : C, 64.56 ; H, 5.74 ; N, 15.95%.

**2-(3-Amino-4-methylphenyl)-N,N,6-trimethylimidazo[1,2-a]pyridine-3-acetamide (3).**

The nitro compound (2) (5.7 g, 16.18 mmol) obtained from the previous reaction was suspended in 2M hydrochloric acid (100 ml) and was treated with 4 g of tin, 2M hydrochloric acid (20 ml) was added and the mixture heated for 3½ hours at 110°C. The reaction mixture was cooled to 0°C and then neutralised with sodium hydroxide and the product extracted with dichloromethane. The white solid obtained on evaporation of the organic phase was recrystallised from

a mixture of methanol : acetone to yield the desired amino compound (3) (4 g, 12.41 mmol, 76.7%) ; mp = 253-4°C. $^1$H-NMR (200 MHz, DMSO-$d_6$, TMS) $\delta$ 7.93 (S, 1H, ArH), 7.43-6.69 (m, 5H, PyH$_2$ + ArH$_3$), 4.84 (S, 2H, -NH$_2$), 4.12 (S, 2H,- CH$_2$-), 3.10 and 2.89 (S, 3H, NCH$_3$), 2.29 (S, 3H, PyCH$_3$) and 2.09 (S, 3H, ArCH$_3$). IR$_{\gamma\,max}$ (KBr) 3310, 1635, 1500, 1390, 1135 and 795 cm$^{-1}$.MS, m/z 322 ($M^+$), 250 (base peak), 234 and 92. Anal. Calcd for $C_{19}H_{22}N_4O$ 0.1 $H_2O$ : C, 70.46 ; H, 6.90 ; N, 17.30%. Found : C, 70.32 ; H, 6.87 ; N, 17.06%.

**2-(3-Amino-2,6-dibromo-4-methylphenyl)-N,N,6-trimethylimidazo[1,2-a]pyridine-3-acetamide (4)**

The amine (3) (4 g, 12.41 mmol) in chloroform (100 ml) was treated dropwise with a solution of bromine (3.5 ml, 10.9 g) in methanol (50 ml) and the mixture stirred for 15 minutes at 25°C. The solvent was evaporated to dryness and the residue redissolved in dichloromethane (30 ml), and washed with a 10% solution of sodium thiosulphate(20 ml). The product was extracted from the organic phase with 2M hydrochloric acid solution (20 ml), the acidic extracts were neutralised with sodium hydroxide and the product extracted into dichloromethane. The combined organic extracts were washed with water (3 x 25 ml), dried ($Na_2SO_4$) and evaporated to dryness to yield the 2,6-dibromo-3-amino compound (4) (4.7 g, 9.8 mmol, 79%) mp : 266-8°C. $^1$H-NMR (200 MHz, DMSO-$D_6$, TMS) $\delta$ 8.18 (S, 1H, PyH), 7.47 (d, 1H, J = 10 Hz, PyH), 7.33 (S, 1H, ArH), 7.11 (d, 1H, J = 10 Hz, PyH), 5.20 (S, 2H, -NH$_2$), 3.75 (S, 2H, -CH$_2$-), 2.85 and 2.77 (S, 3H, NCH$_3$), 2.33 (S, 3H, PyCH$_3$) and 2.23 (S, 3H, ArCH$_3$). IR$_{\gamma\,max}$ (KBr) 3440, 3290, 3150, 1635, 1418, 1270 and 802 cm$^{-1}$. MS, m/z 480 ($M^+$), 408 (base peak), 327, 248 and 124. Anal. Calcd for $C_{19}H_{20}Br_2N_4O$. 0.1 $H_2O$ : C, 47.52 ; H, 4.22 ; N, 11.67%. Found : C, 47.36 ; H, 4.32 ; N, 11.54%.

**2-(2,6-Dibromo-4-methylphenyl)-N,N,6-trimethylimidazo[1,2-a]pyridine-3-acetamide(5)**

The 2,6-dibromo-3-amino compound (4) (2.32 g, 4.83 mmol) was dissolved in a mixture of distilled water (10 ml) and 2 M hydrochloric acid (10 ml). The

reaction mixture was treated with concentrated hydrochloric acid (0.9 ml) and cooled to 0°C. A solution of sodium nitrite (1.1 g, 15.86 mmol) in water (2.6 ml) was added. After 15 minutes hypophosphorous acid (7.3 ml) was added and the reaction mixture stirred at room-temperature overnight. This was extracted with dichloromethane (2 x 25 ml). The organic phase was washed with 10% sodium bicarbonate (20 ml), water (2 x 20 ml), dried ($Na_2SO_4$) and evaporated to dryness. The residue was taken up in ethanol and treated with ethanolic hydrogen chloride to form the hydrochloride salt which was precipitated with ethyl acetate to yield the dibromo hydrochloride salt (5) (1.9 g, 3.78 mmol, 78.3%) as a white solid, mp : 272-5°C. $^1$H-NMR (80 MHz, DMSO-$d_6$, TMS) $\delta$8.62 (1S, 1H, PyH) 7.90 and 7.73 (S, 2H, $PyH_2$ and $ArH_2$), 4.03 (S, 2H, $-CH_2-$), 2.97 and 2.82 (S, 3H, $NCH_3$), 2.45 and 2.40 (S, 3H, $PyCH_3$ and $ArCH_3$). IR $\gamma_{max}$ (KBr)1610, 1520, 1402, 921, 822, 750 and 595 $cm^{-1}$. MS, m/z 465 ($M^+$), 393 (base peak), 313, 261, 233 and 36. Anal. Calcd for $C_{19}H_{19}Br_2N_3O.HCl$ : C, 45.49 ; H, 4.02 ; Cl, 7.06 ; N, 8.37%. Found : C, 45.32 ; H, 4.09 ; Cl, 6.96 ; N, 8.28%.

**N,N,6-Trimethyl-2-(4-methyl-[2,6-$^3H_2$]phenyl)imidazo[1,2-a]pyridine-3-acetamide, [$^3$H]Zolpidem (6)**

The dibromo intermediate (5)(20.5 mg, 0.041 mmol) in dry methanol (1 ml) was stirred at room-temperature for 3½ hours in the presence of 10% Pd/$BaSO_4$ (67 mg), triethylamine (100 µl) and tritium gas (10 Ci). The catalyst was filtered off, washed with ethanol (10 ml) and benzene (10 ml). The labile tritium was removed by repeated evaporation with ethanol (3 x 5 ml). The residue was dissolved in ethanol and counted. This crude product (2.2 Ci) was found to be 90% radiochemically pure by t.l.c. (Whatman KC 18 reverse phase), elution with methanol : water : acetic acid (80:20:0.5). This crude product was purified by preparative layer chromatography on $SiO_2$ plates (3 x 1 mm) eluting in chloroform: acetone : triethylamine (35:15:2) to yield [$^3$H]Zolpidem (6) (1.7 Ci) with a specific activity of 60.5 Ci/mmol which was determined by scintillation counting

and UV (in 95% ethanol) measurements at $\lambda_{max}$ 243 nm.

The radiochemical purity was found to be 98% by thin-layer chromatography on silica gel in :

a) chloroform : acetone : triethylamine (35:15:2) Rf = 0.71

b) benzene : ethanol : triethylamine (80:20:0.25) Rf = 0.63

and by thin-layer chromatography on Whatman KC 18 reverse phase in :

c) methanol : water : acetic acid (80:20:0.5) Rf = 0.48

This was confirmed by HPLC using an ultrasphere 5 µm ODS column (25 x 1 cm) eluting with methanol : water (6:4) containing 0.2% triethylamine.

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