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# SYNTHESIS OF 2-AMINO-5-(1-METHYL-5-NITRO-[4-$^3$H]-2-IMIDAZOLYL)-1,3,4-THIADIAZOLE

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## SUMMARY

2-Amino-5-(1-methyl-5-nitro-4-iodo-2-imidazoyl)-1,3,4-thiadiazole (**5**) was prepared from imidazole *via* a 4-steps sequence. Reductive desiodination of **5** using a tetrahydrofuran solution of $NaBH_4$ in the presence of tritiated water provided the title compound (specific radioactivity 12.6 Ci/mmol) in one step.

**Key words**: Megazol - CL 64855 - 5-nitro imidazoles - reductive desiodination - tritiated water.

## INTRODUCTION

"Megazol": 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole, **6** first synthesized by the American Cyanamid Company (1) has a broad spectrum of antiprotozoan and antibacterial activities (2). Despite the mutagenicity risk associated with the presence of the nitro group, this drug has gained renewed interest due to its high *in vivo* efficiency against *Trypanosoma cruzi* strains which are usually resistant to Nifurtimox and Benznidazole, the two drugs currently used for Chagas disease therapy (3,4). In collaboration with other research groups, we also recently demonstrated its high efficiency against *Trypanosoma brucei*, the causative agent of African trypanosomiasis, sleeping sickness (5,6). Megazol has therefore become a lead structure for the design of novel compounds and for the identification of biological targets (7) involved in both types of trypanosomes. Thus, the synthesis of labelled Megazol was required for the study of its uptake into the parasite, and for pharmacokinetic studies. For these purposes tritium labelling of the 4-position of the imidazolyl group was chosen.

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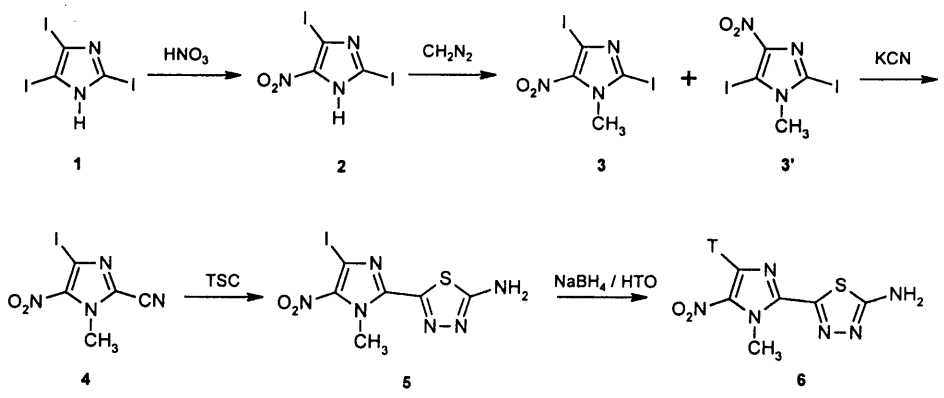
## SYNTHESIS

The sequence which we developed is depicted in Scheme 1. It was designed so that tritium was introduced in the last step thereby optimising the experimental procedure and providing the compound at low cost.




Scheme 1

Triiodoimidazole **1** was prepared by iodination of imidazole in alkaline medium (10). Subsequent nitration of the most reactive position 5 furnished compound **2** (8,9,10). Reaction of **2** with diazomethane (13) led to a mixture of **3** and **3'** in a 93/7 ratio. Both products were easily separated by flash chromatography. Substitution of the iodo group of **3** by the cyanide anion allowed the formation of compound **4**. It was previously shown that this reaction is regiospecific for position 2 (11). Nucleophilic addition of thiosemicarbazide on the nitrile group of **4** under acidic conditions led to an imonium intermediate which rapidly undergoes ring closure to afford **5**. Reduction of the latter by sodium borohydride in $H_2O$ or in HTO gave respectively Megazol and 4-tritiated Megazol. Introduction of tritium from the solvent and not from the reducing agent can be ascribed to the formation of a stabilized carbanion next to the nitro group (scheme 2). A similar effect has already been observed with other aromatic compounds (12). Megazol was then purified by HPLC and obtained at 12.6 Ci/mmol specific radioactivity.

$$BH_4^- + ArX \longrightarrow BH_3 + HX + Ar^-$$
$$Ar^- + T_2O \longrightarrow ArT + OT^-$$

Scheme 2

## EXPERIMENTAL SECTION

TLC analyses were performed using Merck GDF254 silica gel plates. Compounds were purified by flash chromatography over silica gel (60 Merck ; 0.04-0.06 mesh). Melting points were measured on an electrothermal apparatus and were corrected. Infra-red spectra were measured on a Perkin-Elmer 1610 FTIR spectrophotometer. NMR spectra were run on a Brucker AC 50 or AC 80 spectrometer. Megazol and tritiated Megazol were purified by HPLC (Nucleosil OD5, eluent : $H_2O$ 70/$CH_3CN$ 30). The total radioactivity was determined by liquid scintillation counting after an appropriate dilution .

2,4,5 Triiodoimazole : **1**

Treatment of imidazole with iodine according to Groziack's procedure (10) afforded **1.**

2,4-Diiodo-5-nitroimidazole : **2** (8,9,10)

To a mixture of 10 mL of conc. nitric acid and 10mL of conc. sulfuric acid, maintained at 0°C, were carefully added 13.35 g of triiodoimidazole **1** (30 mmol). After one hour the mixture was poured onto 50 g crushed ice and neutralised to pH 3 with sodium hydroxide. The brown solid was filtered, washed with an aqueous solution of sodium iodide and water. After drying under vacuum at 40°C for 15 hours, 4.9 g of **2** were obtained.
m.p.= 202-203 (45% Yield) $^{13}C$ NMR : (DMSO D6) δ 92.3 and 77.6.

2,4-Diiodo-1-methyl-5-nitroimidazole : **3** (13)

To an ethereal solution (20 mL) of 12mM diazomethane prepared from "Diazald" were added 3.68 g (10 mmol) of **2** dissolved in 30 mL THF. The solution was stirred at room temperature for one hour. Solvents were then evaporated under vacuum and the residue was purified by flash chromatography to yield 3.3 g of **3** (90% Yield) m.p = 189-190°C.
$^{13}C$ NMR (DMSO D6) δ = 39 ; 93.8 ; 107.7 ; 141.9 and δ = 39.4 ; 84 ; 97.5 for **3'**.

2-Cyano-4-iodo-1-methyl-5nitro imidazole : **4**

A mixture of 378 mg (1 mmol) of **3** , 80 mg (1.2 mmol) KCN and 10 mg of "Crown ether 18C6" was heated in 3 mL DMF at 80°C for two hours ; DMF was then distilled off under vacuum. The residue was diluted with water (5 mL) and extracted with dichloromethane (10 mL). After evaporation of the solvent, the residue was purified by flash chromatography to yield 120 mg of **4** (43% Yield)
$^{1}H$ NMR ($CDCl_3$) δ = 4.18 $^{13}C$ NMR ($CDCl_3$) δ = 37.3 ; 89.2 ; 108.5 ; 126.7
IR (KBr) CN 2245cm-1 m.p. = 111-112°C.

4 Iodo megazol : **5**

A mixture of 278 mg (1 mmol) of **4** and 100 mg of thiosemicarbazide in 2 mL trifluoroacetic acid was heated for 15 hours at 60°C. After evaporation of trifluoroacetic acid under vacuum the oily residue was neutralized using 10 % sodium bicarbonate aqueous solution. The solid, thus formed, was washed with 3 mL water and then 5 mL dichloromethane to give 250 mg of a powder which was recrystallised from Ethanol/Acetone.
$^{13}C$ NMR (DMSO D6) δ 36.2 ; 93 ; 133 ; 141 ; 147 ; 170.
MS/CI($NH_3$):m/e = 353($(M+1)^+$ ;100), 297(29),227(18)

Unlabelled "megazol": **6**

50 Mg (0.14mmol) of **5**, and 10 mg of sodium borohydride (0.25 mM) in 10 mL of THF/$H_2O$ (9/1) were stirred for 15 hours at room temperature. After evaporation of the solvents under vacuum, the residue was washed three times with 4mL water and then 4mL dichloromethane. After flash chromatography (ethyl acetate/petroleum ether 3/1) 25 mg of megazol were obtained (Yield: 60%).
$^1H$ NMR (DMSO D6) δ=4.32 (NCH3); 7.8 (NH2); 8.2 (4-H). MS/CI($NH_3$):227($(M+1)^+$; 100); 244 (75)

Labelled "megazol": **6**

The isotopic labelling conditions were optimized by working first in $D_2O$. 5 Mg (0.013 mM) of iodomegazole **5**, 4 mg of sodium borohydride and 2 mg of $CH_3COONa$ were suspended in 5 mL THF. 10 µL of $D_2O$ (99% isotopic purity) were then added. The solution was stirred for 15 hours at room temperature. THF was then evaporated and the solid residue taken up in 4mL $H_2O$. The aqueous phase was extracted with 4x3 mL dichloromethane and purified by HPLC, on a Nucleosil C18 column using water/acetonitrile, 70/30 as eluant, (flow rate 1mL/min, λ =260 nm.). 1.1Mg (35% yield) of 4-D megazol of 65% isotopic enrichment (determined by mass spectrometry) were obtained.
The same procedure was applied for tritium labelling. A solution of tritiated water in THF (prepared from PdO and 50 Ci of tritium gas) was vacuum transferred to a solution of iodomegazole (9 mg), sodium borohydride (7 mg) and sodium acetate (4 mg). Purification using HPLC yielded [$^3$H-4]-megazol (100 mCi). Radiochemical purity (>99%) was checked by HPLC and by thin layer radiochromatography (silicagel ethyl acetate/petroleum ether 3/1). Specific radioactivity of 12.6 Ci/mmole was determined by mass spectrometry (C.I., $NH_3$ )
MS/CI($NH_3$):m/e=227 (m+1+ 100%), 228(18), 229(83), 244( 75), 246(49)

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