

## **SYNTHESIS OF $^{14}\text{C}$ -LABELLED BROMOCRESOL GREEN AND METACRESOL PURPLE**

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### **SUMMARY**

[methyl- $^{14}\text{C}$ ]-Bromocresol green **1** was synthesized from [ $^{14}\text{C}$ ] - methyl iodide in four steps via 3-[methyl- $^{14}\text{C}$ ]-methylanisole **3**, [methyl- $^{14}\text{C}$ ]-metacresol **4** and [methyl- $^{14}\text{C}$ ]-metacresol purple **6**. The overall yield of **1** from [ $^{14}\text{C}$ ] methyl iodide was 5.3 % and the specific activity 112 mCi/mmol.

Key Words =  $^{14}\text{C}$ -Bromocresol Green,  $^{14}\text{C}$ -Metacresol Purple.

### **INTRODUCTION**

Bromocresol Green or 4,4-(3H-2,1-benzoxathiol-3-ylidene) bis [2,6-dibromo-3-methylphenol]-S,S-dioxide **1** is used as an indicator in volumetric assays ; yellow at pH 3.8 and blue-green at pH 5.4. It is commonly prepared by adding bromine to a suspension of m-cresolsulfonphthalein in glacial acetic acid (1-8).

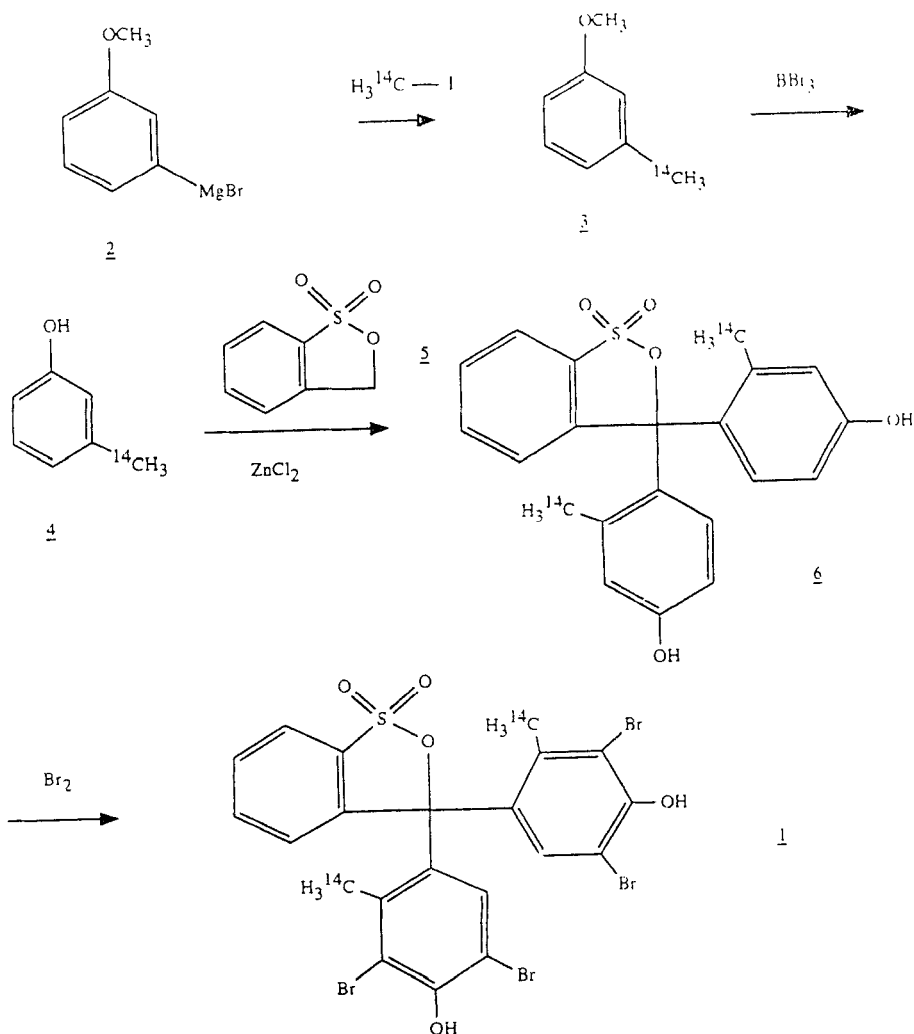
In view of new therapeutic uses, we synthesized [ $^{14}\text{C}$ ]-labelled Bromocresol Green **1** according to scheme 1 with a radiochemical purity higher than 99 %.

### **RESULTS AND DISCUSSION**

Condensation of 3-methoxyphenylmagnesium bromide **2** with [ $^{14}\text{C}$ ] - methyl iodide in THF (**9**) gave 3-(methyl -  $^{14}\text{C}$ ) methylanisole **3** in 75 % yield. O-demethylation of **3** by boron tribromide in dichloromethane (**9**) led to (methyl- $^{14}\text{C}$ ) -metacresol **4** (93 % yield) with a radiochemical purity of 99 %. However, **4** contained inactive phenol formed by successive hydrolysis and O-demethylation of **2**. Therefore, inactive phenol was removed by reverse phase liquid chromatography to give chemically and radiochemically pure **4** with a purification yield of 89 %.

Condensation of **4** with 2-sulfobenzoic anhydride **5** in presence of zinc dichloride as condensing agent gave metacresol purple **6** in 12 % overall yield.

Different methods were investigated for this condensation. All gave low yields with **4**, compared to these obtained with 2-methylphenol or phenol (8, 11).

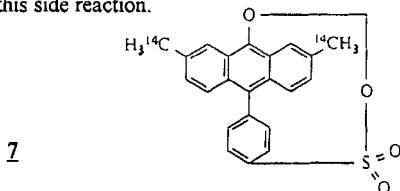


SCHEME 1

## SYNTHESIS OF METACRESOL PURPLE 6 AND BROMOCRESOL GREEN 1

Various condensation agents were tried in order to improve the yield : phosphoric anhydride (10), zinc chloride (8), perchloric acid and phosphoryl chloride (12,13). In most cases, the yield was not improved (4-5 %) and only the reaction rate increased. A 15 % yield was obtained with the latter condensing agent (12,13) but this method was discarded because of explosion hazards.

Therefore, we used a modification of the zinc chloride method. In all publications, metacresol was considered as a solvent for 5. With the radioactive compound 4, we only used a 100 % excess of 4 compared with 5. As condensation temperatures of 125-145°C gave rise to the formation of dimethylsulfone fluorane 7, the condensation was performed at 105°C to avoid this side reaction.



At this temperature, 4 was vaporized and reacted with 5 with difficulty. Consequently, this reaction was carried out in a sealed tube to give 6 (yield 12 %) and 51 % of unreacted 4. After column chromatography purification, 6 contained salts which were eliminated on a Dowex 50 WX 12 ion-exchange resin to yield pure 6 in an over all yield of 12 %.

Bromination of metacresol purple 6 with 80 % yield gave 1 which was purified by paper chromatography. Its chemical and radiochemical purity checked by TLC and HPLC were higher than 99 %. The specific radioactivity determined by mass spectrometry was 112 mCi/mmol and was also checked by UV spectrophotometry measurement and liquid scintillation counting.

The overall yield of 1 was 5.3 % from [<sup>14</sup>C] - methyl iodide and 10.4 % considering unreacted 4.

### EXPERIMENTAL

Reaction progress and radiochemical purities were determined by high performance liquid chromatography (HPLC) using a Du Pont system, serie 8000 ; a Rheodyne 7125 injector ; a Schoeffel SF 770 UV detector and a Berthold radioactive detector LB 505. Thin layer chromatography (TLC) was performed on Merck 60F 250 silicagel or Whatman ODS-2 for the reserved phase. U.V. measurements were performed on a Kontron Uvicon 860 U.V. spectrophotometer. Mass spectra were obtained on a Finnigan 4600 quadrupole mass spectrometer and NMR spectra on a Bruker AC 300. Radioactivity was counted on a LKB 211 liquid scintillation counter.

#### 3-[methyl-<sup>14</sup>C]-methylanisole 3

To a suspension of magnesium turnings (0.9 g, 36 mmol) in tetrahydrofuran (10 mL) were added dropwise 3.5 mL of a solution of 3-bromoanisole (27.7 mmol) in tetrahydrofuran (5 mL). The mixture was refluxed for 2 h. After titration 19 mmol of the resulting solution were reacted with [<sup>14</sup>C] - methyl iodide (10 mmol ; 569 mCi), diethyl ether was added and the mixture was heated under reflux for 8 h.

After acidification by dilute HCl, 3 was continuously extracted by diethyl ether. The organic layer was washed first with sodium thiosulfate solution and then with water, dried over sodium sulfate, and filtered to give 429 mCi of 3 (yield = 75 %).

TLC(SiO<sub>2</sub>) ; toluene-dioxane (100-25) ; R<sub>f</sub> = 0.78.

#### [methyl-<sup>14</sup>C] - metacresol 4

The product 3 (9.15 mmol ; 421 mCi) from the previous reaction was evaporated to dryness, taken up with dichloromethane and the resulting solution cooled to -60°C. Then, boron tribromide (7.85 mL ; 75.5 mmol) was added with stirring at -60°C for 5 min, at 0°C for 2 h and finally at room temperature for 1 h.

After hydrolysis with ice at 0°C and extraction with diethyl ether (3 x 30 mL), the organic layers were treated with 10 % NaOH solution (3 x 30 mL). Then, the aqueous layers were acidified with HCl, extracted with diethyl ether (3 x 30 mL), dried over sodium sulfate and filtered to give 392 mCi (6.98 mmol) of **4** (yield : 93 %). This **4** was purified by medium pressure liquid chromatography (elution by methanol-water (25-75)) to give 350 mCi (6.23 mmol) of radiochemically pure **4** (98 %).

- TLC (ODS phase) ; water-methanol (30-70) ;  $R_f = 0.58$
- $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ) ;  $\delta$  2.3 ppm (s,  $\text{CH}_3$ ) ; 6.63 (d, 4H) ; 6.65 (s, 2H) ; 6.69 (d, 6H) ; 7.15 (t, 5H).
- MS (E.I.) :  $m/z$  96-98,  $\text{M}^+$  (specific activity = 56 mCi/mmol).

#### [methyl- $^{14}\text{C}_2$ ] - metacresol purple **6**

To **4** (350 mCi ; 6.23 mmol) transferred to a Carius tube were added successively o-sulfobenzoic anhydride **5** (0.57 g ; 3.10 mmol) and zinc chloride (0.68 g ; 5 mmol). After freezing with liquid nitrogen, the tube was evacuated, sealed and then heated at 105 °C for 15 h in an autoclave.

After cooling and dissolution of the purple residue in ethyl acetate, the mixture was purified by liquid chromatography (Lichoprep Si60 ; ethyl acetate-methanol, 1.5 % ammonia solution (80-20-8)) to give **6** (43 mCi ; 0.77 mmol) contaminated by non radioactive impurities (mineral salts). This product was purified on a Dowex 50WX 12 column to give **6** (43 mCi ; 0.77 mmol) (green crystals ; chemical and radiochemical purities greater than 99 %).

- TLC ( $\text{SiO}_2$ ) : ethyl acetate-methanol-1.5 %-ammonia (80-20-8)

#### [methyl- $^{14}\text{C}_2$ ] - Bromocresol Green **1**

To **6** (43 mCi ; 0.77 mmol) in 40 mL of ethanol was added dropwise a solution of bromine (992 mg, 12.4 mmol) in 5 mL of ethanol with stirring at room temperature during 1.5 h. After evaporation under vacuum, the product was purified as follows :

- 1) preparative paper chromatography (Schleicher-Schüll) in tert-amyl alcohol-1.5%-ammonia (100-20) then elution of the product with methanol.
- 2) ion-exchange chromatography on Dowex 50WX 12 ; elution with methanol.

[methyl- $^{14}\text{C}_2$ ] - Bromocresol Green **1** (30 mCi ; 0.27 mmol) was obtained with a chemical and radiochemical purities greater than 99 %.

- TLC ( $\text{SiO}_2$ ) :

- 1) ethyl acetate-methanol-1.5 % ammonia solution (80-20-8) ;  $R_f = 0.44$ .
- 2) dichloromethane-ethanol-ethyl acetate-triethylamine (75-18-7-0.4) ;  $R_f = 0.23$ .

- HPLC (Zorbax Sil) :

dichloromethane-ethanol-ethyl acetate-triethylamine (75-18-7-0.4) ;

$T_r = 14.2$  min.

- UV (methanol) : Specific activity = 112 mCi/mmol

- MS (C.I.- $\text{NH}_3$ ) :  $m/z$  : 697-699  $[\text{M}+1]^+$  ; 714-716  $[\text{M}+\text{NH}_4]^+$  (Specific activity = 112 mCi/mmol)

- $^1\text{H-NMR}$  (methanol  $\text{D}_4$ ) :  $\delta$  2.0 ppm (s) ; 2.13 (s) ; 7.09 (d) ; 7.12 (s) ; 7.2 (s) ; 7.59 (t) ; 7.62 (t) ; 8.02 (d).

### ACKNOWLEDGEMENTS

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