Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 3 September 2010, Revised 2 October 2010, Accepted 13 October 2010 Published online 30 November 2010 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.1849

An efficient synthesis of [D_6]leucocrystal violet

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This report presents an efficient synthesis of [D_6]leucocrystal violet. Esterification of *p*-toluenesulphonyl chloride with [D_4]methanol provided [D_3]methyl *p*-toluenesulphonate, which was used to methylate aniline to give [D_6]*N,N*-dimethylaniline. Condensation of [D_6]*N,N*-dimethylaniline with *bis*[4-(dimethylamino)phenyl]methanol afforded [D_6]leucocrystal violet in an overall yield of 30% in three steps.

Keywords: [D_6]leucocrystal violet; synthesis; labelled internal standards

Introduction

Crystal violet (CV) is a well known and inexpensive dye effective against fungal and parasite infection in fish.[1] Similar to malachite, crystal violet is readily absorbed into fish tissue in an aquatic environment and is reduced metabolically by fish to its persistent leuco moiety, leucocrystal violet (LCV).[2,3] Owing to its potential carcinogenic, genotoxic, mutagenic and teratogenic properties in many animal species and cell lines,[4] CV has never been registered as a veterinary drug in any nation. Numerous methods have been developed for the determination of CV and its metabolite LCV in fish.[1,5–8] Liquid chromatography isotope diluted tandem mass spectrometry (LC-IDMS/MS) methods proved sensitive and reliable among various analytical approaches.[9]

Reports of the synthesis of labelled leucocrystal violet are very rare. In this study, we describe an efficient synthesis of [D_6]leucocrystal violet starting from commercially available [D_4]methanol. The target compound, with excellent isotopic and chemical purities, was synthesized in acceptable yield and could be used as an internal standard in the determination of LCV in aquatic products.

Experiment

Materials and instruments

[D_4]Methanol (99.8 atom% D) was provided by J&K Chemical. All other chemicals were of analytical grade. ^{1}H-NMR (500.13 MHz) and ^{13}C-NMR (125.70 MHz) spectra were recorded on a Bruker DRX500 spectrometer in CD_3Cl (TMS as internal standard). FT-IR spectra were recorded on a Nicolet FT-IR 6700 spectrometer using KBr pellets. EI-MS spectra were obtained with TSQ Quantum Access spectrometer.

Synthesis

[D_3]Methyl p-*toluenesulphonate* ***1***

[D_3]methyl *p*-toluenesulphonate (1) was obtained by the modification of a previous procedure.[10] To a mixture of NaOH (17.46 g, 0.44 mol) and [D_4]methanol (4.52 g, 0.125 mol) in a two-phase system of water (50 mL) and THF (50 mL) was added dropwise a solution with *p*-toluenesulphonyl chloride (47.60 g, 0.25 mol) in tetrahydrofuran (80 mL) at ice bath temperature. The reaction solution was kept at −5 to 0°C for another 6.5 h and then poured into ice-water. The mixture was extracted with CH_2Cl_2 (500 ml × 3) and the combined organic layers were washed with water. After drying over $MgSO_4$, the solvent was evaporated *in vacuo* to give 23.2 g of pure product as a colourless oil in 98% yield based on labelled substrate consumed. LC-MS-ESI$^+$: $[M+H]^+ = 190.2$ (100%).

[D_6]N,N-Dimethylaniline ***2***

To a mixture of [D_3]methyl *p*-toluenesulphonate (22.65 g, 0.12 mol) and aniline (5.18 g, 0.056 mol) was added a solution of 30% NaOH solution (30 mL), followed by the addition of phase transfer catalyst, tetrabutylammonium bromide (0.75 g, 2.3 mmol). The reaction proceeded for 4 h at 70°C, then the reaction mixture was cooled to room temperature, the mixture was extracted with diethyl ether (50 ml × 3) and the combined extracts were dried over $MgSO_4$ and evaporated. The crude product was purified by chromatography on silica gel column (*n*-hexane: ethyl acetate: triethylamine = 25:1:0.05) to give 4.73 g of yellow oil [D_6]*N,N*-dimethylaniline 2 in 62% yield. LC-MS-ESI$^+$: $[M+H]^+ = 128.37$(100%).

[D_6]Leucocrystal violet ***3***

[D_6]Leucocrystal violet (3) was obtained by the modification of a known procedure.[11] *Bis*[4-(dimethylamino)phenyl]methanol (13.52 g, 0.05 mol), [D_6]*N,N*-dimethylaniline (3.18 g, 0.025 mol)

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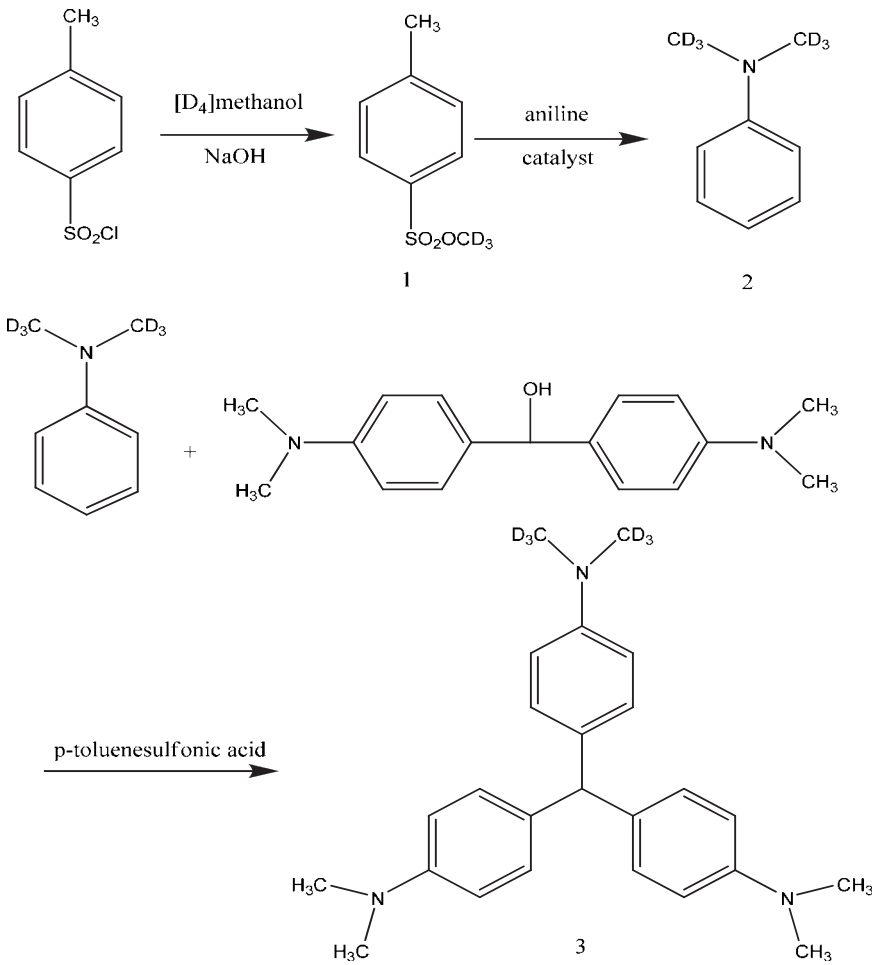

Scheme 1. Synthesis of [D_6]leucocrystal violet.

and *p*-toluenesulphonic acid (0.75 g, 4.35 mmol) were heated under reflux in toluene (60 mL) for 12 h. After washing with water and removal of toluene *in vacuo*, the residue was treated with acetone to give 4.754 g of colourless crystals in 50% yield. The product obtained showed 99.4% chemically pure and more than 99% isotopically enrichment on the basis of HPLC ananlysis and mass spectrometry, respectively. ^{1}H NMR ($CDCl_3$)δ: 2.89 (s,6H); 5.28 (s,1H); 6.64–7.23 (*m*,12H); ^{13}C NMR ($CDCl_3$)δ: 40.1(s, 6C); 54.1(s, 1C); 112.6(s, 4C); 112.5(s, 2C); 129.9(*m*, 6C); 133.8 (s, 3C); 148.8(s, 3C); FT-IR (KBr) cm^{-1}: 2879.8, 2792.6, 1612.8, 1563.5, 1479.6, 1442.5, 809.9; LC-MS-ESI$^+$:$[M+H]^+$ = 380.3 (100%).

Results and discussion

Although the manufacture of leucocrystal violet is fairly common on an industrial scale, reports of the preparation of isotopically labelled leucocrystal violet and its analogues have not been found in the literature. Herein, we report a new synthetic method with satisfying labelling yields, starting from [D_4]methanol.

N,*N*-dimethylaniline is a versatile and important building block for many triphenylmethane dyes. The synthesis of labelled *N*,*N*-dimethylaniline is of importance for corresponding labelled analogues. Our approach to the synthesis of [D_6]leucocrystal violet (3) is summarized in Scheme 1. (2), as the key intermediate in this synthesis, was obtained in two steps. The isotopic label was introduced at the first stage by the treatment of [D_4]methanol with *p*-toluenesulphonyl chloride in which the yield of [D_3]methyl *p*-toluenesulphonate (1) was 98% based on [D_4]methanol consumed. The alkylation reactions of amines were often carried out under high temperature in the previous literature.[12–14] We found that [D_6]*N*,*N*-dimethylaniline (2) could be synthesized in a satisfying 62% yield under mild reaction conditions by using phase transfer catalysis. The as-synthesized [D_6]*N*,*N*-dimethylaniline (2) could then be converted to [D_6]leucocrystal violet (3) with *bis*[4-(dimethylamino)- phenyl]methanol in the presence of *p*-toluenesulphonic acid in an overall yield of 30% in three steps. The above syntheses could be easily modified for the preparation of labelled triphenylmethane dyes, e.g. [D_6]crystal violet in which [D_6]*N*,*N*-dimethylaniline reacts with *bis*(4-dimethylamino)benzophenone.[15]

Acknowledgement

This research was supported by the research grant (2009IK134) from General Administration of Quality Supervision, Inspection and Quarantine, People's Republic of China.

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