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SYNTHESIS OF (R)-(2,3,4,5,6-PENTADEUTEROPHENYL)OXIRANE

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Summary: (R)-(2,3,4,5,6-Pentadeuterophenyl)oxirane, ***5*** was synthesised with an overall yield of 6.3% from benzene-d_6. Friedel-Crafts acylation of benzene-d_6 with methyl oxalylchloride gave methyl 2-oxo-2-(2,3,4,5,6-pentadeuterophenyl)acetate, ***1*** that was chirally reduced (*Saccharomyces cerevisiae*) to methyl (R)-2-hydroxy-2-(2,3,4,5,6-pentadeuterophenyl)acetate (methyl (R)-d_5-mandelic acid), ***2***. Reduction of ***2*** with lithium aluminium hydride gave (R)-(2,3,4,5,6-pentadeuterophenyl)ethane-1,2-diol, ***3***. Tosylation of ***3*** and subsequent treatment with potassium hydroxide assisted cyclisation to ***5***.

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1. INTRODUCTION

Exposure of man to an electrophilic genotoxin may be monitored by measurement of the covalent adduct that these agents form with haemoglobin (Hb).[1] Adducts to the N-terminal valine of Hb have been established as a suitable bio-marker for exposure and exploited as a surrogate marker of DNA damage. [1, 2] It therefore follows that Hb adduct formation can give a measure of the carcinogenic potential of a chemical. For an achiral electrophilic genotoxin a direct correlation can be established between the frequency of Hb adduct formation and damage to DNA. With a chiral electrophilic genotoxin this frequency may be perturbed due to an enantioselective preference to one epimer. The low frequency of adduct formation in the reaction of chiral electrophilic genotoxins with Hb, or DNA, precludes the determination of any enantioselectivity in these reactions by conventional techniques (ie NMR and hplc) and therefore this information is presently unavailable. Our approach to understanding this important area of biological chiral recognition has been to utilise mass spectroscopy (detection limit 10 pmol adduct/g globin) and a synthetic racemic mixture of a chiral electrophilic genotoxin that is specifically deuterated in one enantiomer. Phenyloxirane (PO) was chosen as a representative chiral electrophilic genotoxin for this research. We here describe our synthetic approach to (R)-(2,3,4,5,6-pentadeuterophenyl)oxirane [d_5-(R)-PO] that was stoichiometrically diluted with (S)-phenyloxirane [(S)-PO] to give the required d_5-(R)-PO/(S)-PO racemate.

2. RESULTS AND DISCUSSION

The introduction of maiden chirality by the reduction of substituted 2-ketoacid esters to yield 2-hydroxyacid esters in high enantiomeric excess has been described.[3-5] In particular, methyl 2-oxo-phenylacetate was converted to methyl (R)-mandelic acid (ee ~ 100 %) by actively fermenting yeast (*Saccharomyces cerevisiae*).[3] The reported synthesis of (R)-(phenyl)oxirane from this natural chiral precursor affords a convenient route to (R)-(2,3,4,5,6-pentadeuterophenyl)oxirane.[6-8] Friedel-Crafts

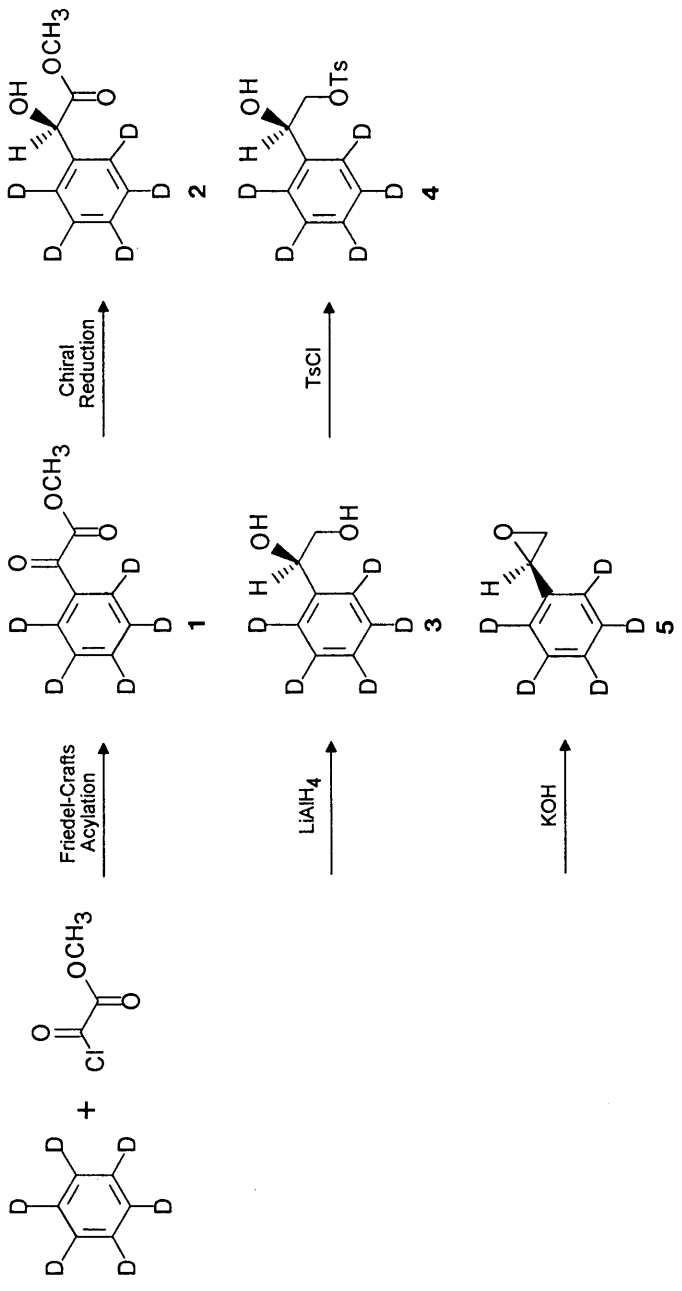
acylation of benzene-d_6 with methyl oxalylchloride gave the required achiral precursor, methyl 2-oxo-2-(2,3,4,5,6-pentadeuterophenyl)acetate, ***1*** (90% yield) that was reduced (*Saccharomyces cerevisiae*) to methyl (R)-2-hydroxy-2-(2,3,4,5,6-pentadeuterophenyl)acetate, ***2*** (ee ~ 100%). Reduction of ***2*** with $LiAlH_4$ gave (R)-(2,3,4,5,6-pentadeuterophenyl)ethane-1,2-diol, ***3***. Tosylation of ***3*** and subsequent treatment with potassium hydroxide assisted cyclisation to (R)-(2,3,4,5,6-pentadeuterophenyl)oxirane, ***5*** (ee > 99%, overall yield 6.3% from benzene-d_6). The synthetic route to ***5*** is given in Scheme 1.

3. EXPERIMENTAL

All of the following procedures were performed with unlabelled compounds. Yields at least as good as those quoted below were obtained and all organic compounds were authenticated by spectroscopic analysis. NMR spectra were acquired with a Bruker ARX250 instrument operating at frequencies of 250 MHz (^1H) and 62.89 MHz (^{13}C). Positive ion electron impact (EI^+) and fast atom bombardment (FAB^+) mass spectra were acquired by direct insertion of sample into a VG AutospecQ and VG 70-SEQ instrument, respectively.

3.1. Preparation of methyl 2-oxo-2-(2,3,4,5,6 pentadeuterophenyl)acetate (*1*).

To an ice cooled solution of benzene-d_6, (99.9 atom %, 25g, 0.3 mol) and methyl oxalylchloride (36.75g, 0.3 mol) in chloroform (50 ml) was added anhydrous aluminium chloride (44g, 0.33 mol) slowly with stirring over 15 min. Once effervescence had ceased the solvent was removed by evaporation and ice cold water (100 ml) was added cautiously, with stirring at 0 °C, to the remaining slurry. The product was extracted into diethyl ether (3 x 100 ml). The ether extracts were combined, dried ($MgSO_4$) and solvent evaporated to yield an orange oil that was purified by column chromatography (SiO_2, $CHCl_3$: CH_3OH, 9:1, v/v) to give **1** (45.5g, 90%); $\delta_H(CDCl_3)$: 3.95 (s, CH_3) ppm; $\delta_C(CDCl_3)$: 53.2 (CH_3), 129.7 (t, aromatic), 131.0 (t, aromatic), 132.6 (s, aromatic), 164.4 ($\underline{C}(O)OCH_3$), 186.4 (C=O) ppm; *m/z* (EI^+) 169 (M^+, 10%), 110 ($[M\text{-}CO_2CH_3]^+$, 100%), 82 ($[C_6D_5]^+$, 70%) and 54 ($[C_4D_3]^+$, 35%).



3.2. Preparation of methyl (R)-2-hydroxy-2-(2,3,4,5,6-pentadeuterophenyl)acetate (*2*).[3-5,9,10]

To a sterile 1L conical flask containing a solution of sucrose (30 g) in water (100 ml) was added yeast (*Saccharomyces cerevisiae*, 30 g) as a suspension in water (80 ml). Upon fermentation, about 1 h, methyl 2-oxo-2-(2,3,4,5,6-pentadeuterophenyl)acetate, *1* (1.5 g, 9.1 mmol) was added and the mixture incubated with shaking at 37 °C for 24 h. A further addition of sucrose (15 g), dissolved in water (50 ml), was made and the mixture fermented for a further 24 h. The reaction mixture was centrifuged (IEC Centra-8R centrifuge, 2,000 g, 10 °C, 15 min). The supernatant was decanted off and the pellet resuspended in distilled water (2 x 100 ml) and centrifuged. The aqueous fractions were combined, a saturated solution of sodium chloride (50 ml) was added and the product extracted into diethyl ether (3 x 200 ml). The combined extracts were dried ($MgSO_4$) and the solvent removed to give a crude product that was recrystalised from hexane : ether to give *2* (1.1g, 71%), m.p. 56 °C; $\delta_H(CDCl_3)$: 3.8 (s, 3H, CH_3), 3.5 (broad s, 1H, OH) and 5.2 (s, 1H, C(H)OH) ppm; $\delta_C(CDCl_3)$: 53.4 (CH_3), 73.2 (CHOH), 126.6 (t, aromatic), 128.5 (t, aromatic), 138.5 (s, aromatic), 174.6 (C(O)OCH_3), ppm; *m/z* (EI^+) 171 (M^+, 75%), 112 ($[M\text{-}CO_2CH_3]^+$, 100%) ; $[\alpha]_{20}^D$ -147° [c = 1, CH_3OH] (methyl (R)-2-hydroxy-2-phenylacetate, b.p. 135 °C / 12 mmHg, m.p. 56-58°C; $[\alpha]_{20}^D$ -144° [c = 1, CH_3OH]).

3.3. Preparation of (R)-(2,3,4,5,6-pentadeuterophenyl)ethane-1,2-diol (*3*).[11,12]

Methyl (R)-2-hydroxy-2-(2,3,4,5,6-pentadeuterophenyl)acetate, **2** (2.0 g, 12 mmol) in anhydrous diethyl ether (10 ml) was added dropwise over a period of 30 min with stirring at 20 °C to a suspension of lithium aluminium hydride (0.51 g, 13.4 mmol) in anhydrous diethyl ether (25 ml). After the reaction had subsided (4 h), water (60 ml) was slowly added and the biphasic mixture extracted with diethyl ether (2 x 100 ml). The combined extracts were dried ($MgSO_4$) and the solvent removed to give a crude orange crystalline product that was recrystallised (pentane/ether) to give 3 (1.1 g, 64%), m.p. 67 °C; δH($CDCl_3$): 2.1 (broad s, 1H, OH) 2.6 (broad

s, 1H, OH), 3.7 (m, 2H, CH_2) 4.8 (m, 1H, CH); *m/z* (EI^+) 143 (M^+, 15%), 112 ($[C_6D_5CHOH]^+$, 100%), 82 ($[C_6D_5]^+$, 75%), 54 ($[C_4D_3]^+$, 25%); $[\alpha]_{20}^{D}$ -70° [c = 0.5, $CHCl_3$] (methyl (R)-phenylethane-1,2-diol, yellow crystals, m.p. 67-69 °C; $[\alpha]_{20}^{D}$ -69° [c = 1, $CHCl_3$]).

3.4. Preparation of (R)-2-hydroxy-2-(2,3,4,5,6-pentadeuterophenyl)ethyltoluenesulphonate (*4*).[13-15]

To a stirred solution of (R)-(2,3,4,5,6-pentadeuterophenyl)ethane-1,2-diol, *3* (1 g, 7.0 mmol) in a mixture of anhydrous pyridine (1 ml) and toluene (2 ml) was added with stirring at 0 °C a solution of p-toluenesulphonyl chloride (1.5 g, 7.1 mmol) in anhydrous pyridine (2 ml) over 1 h. After the reaction had subsided (48 h), water (2 ml) was added and the product extracted into chloroform (3 x 3 ml). The chloroform extracts were pooled, dried ($MgSO_4$) and evaporated to yield a white crystalline solid that tainted with prolonged storage at room temperature (21-23 °C) (0.62g, 30%), m.p. 75 °C; $\delta_H(CDCl_3)$: 2.4 (s, 3H, CH_3), 3.4 (broad s, 1H, OH), 4.2 (ddd, 2H, CH_2) and 4.9 (dd, 1H, CH), 7.45 (d, 2H, aromatics), 7.85 (d, 2H, aromatics) ppm; *m/z* (EI^+): 297 (M^+, 10%), 267 ($[M\text{-}CH_2O]^+$, 85%), 155 ($[CH_3(C_6H_4)SO_2]^+$, 40%), 91 ($[C_7H_7]^+$, 100%), (FAB^+): 298 ($[MH]^+$, 62%), 280 ($[M\text{-}H_2O\text{+}H]^+$, 100%); $[\alpha]^{20}_D$ +34° [c = 0.2, CH_3OH] (2-hydroxy-(2-phenyl)-ethyltoluenesulphonate, white crystals, m.p. 74-76 °C; $[\alpha]^{20}_D$ +34° [c = 2, C_2H_5OH]).

3.5 Preparation of (R)-(2,3,4,5,6-pentadeuterophenyl)oxirane (*5*).[13-15]

0.98 M Sodium methoxide in methanol (2 ml) was added dropwise over a period of 10 min to a stirred solution of (R)-2-hydroxy-2-(2,3,4,5,6-pentadeuterophenyl)ethyltoluenesulphonate, *4* (0.7 g, 2.2 mmol) in chloroform (3 ml) at 4 °C. A white precipitate of sodium p-toluenesulphonic acid was observed. The reaction was allowed to continue for a further 30 min after which water (2 ml) was slowly added and the product was extracted into chloroform (3 x 3 ml). The combined extracts were dried ($MgSO_4$) and the solvent removed to give crude (R)-(2,3,4,5,6-pentadeuterophenyl)oxirane, *5*

that was purified by flash column chromatography (SiO_2, $CHCl_3$:CH_3OH, 9:1, v/v) to yield a colourless liquid, 0.51 g, 51%; b.p. 62 °C / 10 mm Hg; $\delta_H(CDCl_3)$: 2.95 (dd, 1H, one of CH_2), 3.3 (dd, 1H, one of CH_2) and 4.0 (m, 1H, CH) ppm; *m/z* (EI^+): 125 (M^+, 30%), 110 ($[C_7D_5O]^+$, 35%), 96 ($[C_7D_5H_2]^+$, 100%), 82 ($[C_6D_5]^+$, 30%), 70 ($[C_5D_5]^+$, 30%), 54 ($[C_4D_3]^+$, 20%); $[\alpha]^{20}_D$ +30° [c = 0.1, CH_3OH] ((R)-phenyloxirane, b.p. 194 °C; $[\alpha]^{18}_D$ +33° [neat]).

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