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SYNTHESIS OF [2,3,3,2'3',5',6'-$^2H_7$]-L-TYROSINE FROM PHENOL-d_6

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SUMMARY

An enantioselective synthesis of [2,3,3,2'3',5',6'-$^2H_7$]-L-tyrosine is described. *Z*-3-(4-acetoxy-2,3,5,6-$^2H_4$-phenyl)-3-^{2}H-2-acetylamino acrylic acid was prepared in five steps from phenol-d_6. Chiral reduction of the olefin followed by removal of the acetate protecting groups furnished the d_7-amino acid in high ee.

Key Words: L-tyrosine-d_7, deuterium labelling, enantioselective reduction, formylation.

INTRODUCTION

A synthesis suitable for the preparation of gram quantities of [2,3,3,2'3',5',6'-$^2H_7$]-L-tyrosine was necessary for the production of a series of mass spectrometry internal standards. The pioneering work of Knowles and coworkers (1) indicated that this amino acid could be prepared in nonracemic form by enantioselective reduction of the appropriate α-amidoacrylic acid. It was envisioned that this

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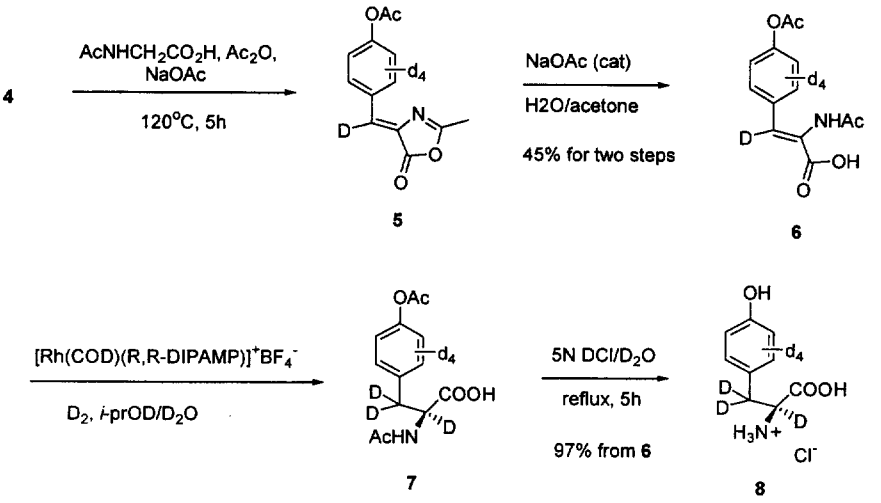
for the reduction of **6** is consistent with those reported by Knowles *et al* for similar substrates (1d).



The salt **8** was neutralized, and the crude tyrosine was recrystallized from water. The ee of the final material was enriched to 98% by the above operation (99.6% d_7 by mass spectrometry). The overall yield (seven steps) from phenol-d_6 was 12.1 %.

EXPERIMENTAL

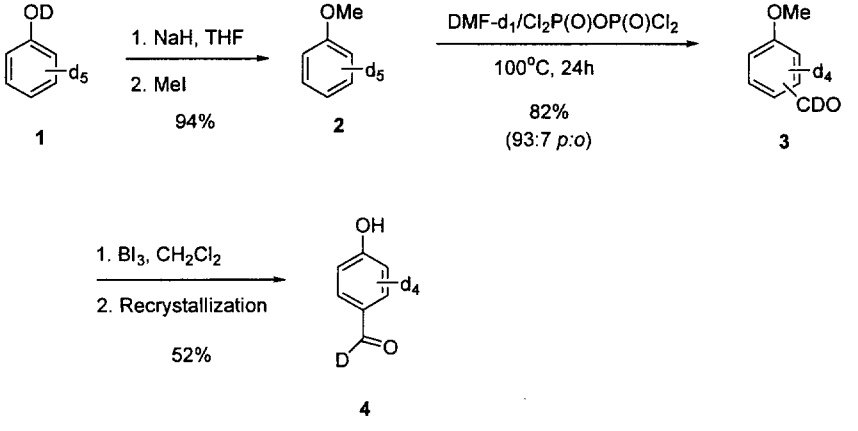
(R,R)-(-)-1,2-Bis[(*o*-methoxyphenyl)(phenyl)-phosphino]ethane(1,5-cycloocta-diene) rhodium (I) tetrafluoroborate ("Knowles/Monsanto catalyst") was purchased from Strem Chemical. Deuterium (D_2, 99.8 atom % *d*) was purchased from Cambridge Isotope Laboratories. All other reagents and labelled materials were obtained from Aldrich and used without further purification. Flash chromatography was performed on a Biotage FlashElute™ system with the same manufacturer's prepacked silica gel cartridges. Enantiomeric excesses were determined by HPLC (Waters) using a Phenomenex™ Chirex™ chiral column [250 x 4.5 mm, CSP 3126, (D)-penicillamine ligand exchange]. Melting points were obtained on a MelTemp

substrate could be obtained from perdeuterated phenol using the Vilsmeier reaction and Erlenmeyer azalactone synthesis.

RESULTS AND DISCUSSION

Phenol-d_6 **1** was converted to its sodium salt, then treated with methyl iodide to give the known (2) anisole **2** in 94% yield (Scheme 1). The latter was formylated using a recent modification (3) of the Vilsmeier reaction with N,N-dimethylformamide-1-*d* and diphosphoryl chloride to afford an 82% yield of *ortho* and *para* (7:93) anisaldehyde-d_5 **3** (97.8% d_5). Demethylation (4) with BI_3 gave a mixture of hydroxybenzaldehyde isomers from which the desired 4-isomer could then be obtained in pure form by recrystallization.

SCHEME 1



Olefin **6** was synthesized from **7** in two steps by the method of Wong (5) and coworkers (Scheme 3). The enantioselective reduction was carried out using deuterium gas and the Knowles/Monsanto (1) catalyst. The acetate protecting groups were removed upon exposure of **7** to refluxing 5N DCl/D_2O (6,7) for five hours. The enantiomeric excess of the perdeuterated tyrosine hydrochloride **8** was 91% (chiral HPLC) with no observed racemization in the process. The ee observed

(LabInstruments, Inc.) apparatus and are uncorrected. NMR spectra were measured on a Bruker DPX 300 spectrometer. Mass spectra were determined on a Finnegan MAT 95 (EI) and Micromass LCT (electrospray ionization). Deuterium content for the labelled compounds was evaluated by mass spectral analysis (EI for **2**; electrospray ionization for **3**, **4**, **6**, **8**) using the "average mass approach (***M***)" described by Blom (8) and Munson (9). The R_f's exhibited by compounds **2-4**, **6** and the HPLC R_T for **8** were identical to those of authentic, unlabelled samples in side by side comparisons and co-spotted or co-injected samples.

[2,3,4,5,6-$^2H_5$]-Anisole (2). A solution of **1** (10 g, 0.10 mol) in 30 mL of THF was added dropwise over 1 h to a precooled (0°C) suspension of sodium hydride (4.7 g of a 60 wt. % mineral oil dispersion, prewashed with hexanes) in 20 mL of THF. The resulting yellow solution was stirred for an additional 10 minutes, then iodomethane (20 mL, 0.32 mol) was added rapidly. The reaction was warmed to room temperature, then refluxed for 19 h. The cooled reaction mixture was diluted with water (200 mL) and extracted with pentane (1 x 150 mL, 1 x 100 mL). The extracts were dried (Na_2SO_4), filtered, and the solvents removed by simple distillation at atmospheric pressure. The concentrate was distilled through a vacuum-jacketed, short path distillation apparatus to afford **2** (10.2 g, 94%) as a colorless liquid [bp 153°C/760 Torr; lit. (1) 155°C/760 Torr]. M = 113.02 (determined from the M^+ cluster); M_{d0} = 108.07; 99.1% d_5.

[2,3,5,6,7-$^2H_5$]-Anisaldehyde Mixture (3). To a stirred solution of N,N-dimethylformamide-1-*d* (6.0 g, 81 mmol) and **2** (6.1 g, 54 mmol) at 0°C was added diphosphoryl chloride (11.2 mL, 81.0 mmol) dropwise over a 10 minutes. The reaction mixture was then heated in an oil bath maintained at 105°C for 24 h. The cooled reaction mixture was poured onto 100g of crushed ice, and the pH adjusted to *ca.*10 with 2N NaOH (240 mL) at 0°C. The mixture was extracted with methylene chloride (4 x 50 mL); the extracts were dried ($MgSO_4$), filtered, and concentrated (aspirator) to give an amber oil (9 g). The crude product was purified by Kugelrhor distillation (60-100°C/0.3 Torr) to afford **3** (6.08 g, 81%) as a light

yellow oil. The *o:p* ratio was determined to be 7:93 by integration of the OCH_3 singlets for each isomer: (300 MHz, $CDCl_3$): δ *ortho*: 3.93; *para*: 3.89. Analytical TLC on silica gel, 3:2 hexane:EtOAc, $R_f = 0.50$. $\boldsymbol{M} = 141.99$ [determined from the $(M + H)^+$ cluster]; $M_{d0} = 137.10$; 97.8% d_5.

4-Hydroxy-[2,3,5,6,7-$^2H_5$]-Benzaldehyde (4). To a well stirred solution of **3** (5.01 g, 35.6 mmol) in 320 mL of methylene chloride was added 14.4 g (36.8 mmol) of boron triiodide. The mixture was stirred vigorously for 2 min, then 40 mL of water was added. The supernatant liquid was decanted, and the remaining solid dissolved in ether (200 mL). The ether solution was washed with the original aqueous layer, then the aqueous layer was extracted with additional ether (3 x 200 mL). All of the organic layers were combined and concentrated under reduced pressure (aspirator). The residue was taken up in ether (400 mL), washed with 5% aqueous $Na_2S_2O_3$ (5 x 20 mL), dried ($MgSO_4$) and concentrated (aspirator) to give a dark colored solid (4.1 g, 98%). The crude product was adsorbed onto 20 g of silica gel, and the isomeric hydroxybenzaldehyde-d_5 mixture eluted with 3:1 hexane:EtOAc (8 x 175 mL), then 7:3 hexane:EtOAc (3 x 150 mL). Fractions 4-11 were concentrated, and the resultant yellow solid (2.35 g) was recrystallized from hexane/EtOAc (15mL/5mL) to give 1.82 g (43%) of **4** as light yellow needles. Concentration of the mother liquors afforded an additional 0.36 g (9%) of **4**. Analytical data for **4**: mp 113-114°C [lit. (10) 112-114°C for the unlabelled compound]; analytical TLC on silica gel, 3:2 hexane:EtOAc, $R_f = 0.32$; $\boldsymbol{M} = 125.99$ [determined from the $(M - H)^-$ cluster]; $M_{d0} = 121.09$; 98.1 % d_5.

***Z*-3-(4-acetoxy-2,3,5,6-$^2H_4$-phenyl)-3-^{2}H-2-acetylamino acrylic acid (6).** A mixture of **4** (1.8 g, 14 mmol), N- acetyl glycine (2.0 g, 17 mmol), sodium acetate (1.5 g, 19 mmol) in 6.7 mL of acetic anhydride was heated in an oil bath maintained at 120°C for 5 h. The cooled reaction mixture was diluted with 7 mL of ice water, and the resultant yellow solid filtered and washed with cold 50% aqueous ethanol. The crude azalactone (2.42 g) was suspended in 100 mL of 4:1 water:acetone and treated with 43 mg (0.52 mmol) of sodium acetate. The reaction mixture was

refluxed for 2.5 h, then filtered hot. The filtrate was cooled slowly to room temperature, then left at 4°C overnight. The mixture was filtered to give **6** (1.9 g, 50% overall) as yellow crystals. Analytical data for **6**: mp 223°C (dec.) [lit. (5) 233°C (dec.) for the unlabelled compound], ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.7 (br s, 1H), 9.47 (s, 1H), 2.28 (s, 3H), 1.98 (s, 3H); analytical TLC on reverse phase (C18), 85:15 MeCN:MeOH, R_f = 0.46; M = 269.07 [determined from the $(M + H)^+$ cluster]; M_{d0} = 264.18; 97.8% d_5.

L-(2,3,3,2',3',4',5'-$^2H_7$)-tyrosine. A 250 mL Parr Bottle was evacuated (30 Torr) and filled with nitrogen (7 cycles) then placed inside a nitrogen filled glove bag. *It is critical to purge rigorously the reaction vessel and solvents with nitrogen as the rhodium catalyst is poisoned by oxygen.* The bottle was charged with 35 mL of a deoxygenated solution of 2-propan(ol-*d*):D_2O (88:12), **6** (1.9 g, 7.1 mmol), and 15 mg (0.020 mmol) of [Rh(COD)(R,R-DIPAMP)]$^+BF_4^-$. The apparatus was evacuated (45 Torr) and pressurized (45 psi) with deuterium gas (6 cycles), then the slurry was shaken at ambient temperature for 16 h under a pressure of 49 psi of D_2. The yellow, *homogeneous* solution was diluted with methanol and concentrated under reduced pressure (aspirator) to give 1.92 g (99%) of a yellow/brown foam. The crude reduction product **7** was suspended in 36 mL of 5N DCl in D_2O (prepared from 29 mL of 20% DCl in D_2O and 7 mL of D_2O) and refluxed for 5 h. The reaction mixture was filtered into a 250 mL round bottomed flask and 200 mL of benzene was added. The aqueous portion of the reaction mixture was removed by azeotropic distillation at atmospheric pressure leaving behind 1.51 g (98%) of hydrochloride salt **8** as a golden brown solid. Chiral HPLC analysis: sample concentration 0.35 mg/mL in 4:1 H_2O:MeOH, 30 μL injection; isocratic run (65.0 min): mobile phase 85:15 2 mM aqueous $CuSO_4$:MeOH @ 1 mL/min, UV detection (230 nm); R_T = 37.2 min (R_T = 54.2 min for D-isomer based on assay of authentic racemic mixture); enantiomeric excess: 91%. The crude salt was dissolved in 36 mL of 3N DCl in D_2O (prepared from 18 mL of 20% DCl in D_2O and 18 mL of D_2O). The amber solution was cooled in an ice water bath and neutralized with

NaOD in D_2O (40 wt. %). The resultant solid (1.22 g) was isolated by filtration, then decolorized with 60 mg of Norit in 210 mL of boiling water (5 minutes). The mixture was filtered hot, and the clear and colorless filtrate was allowed to stand at room temperature overnight. Filtration afforded silky, fine white needles (0.80 g, 60 % overall from **6**). HPLC sample preparation: a 3.30 mg sample was suspended in 8.8 mL of 4:1 H_2O:MeOH and 0.17 mL of 0.100 N HCl was added; the mixture was sonicated until homogeneous. Chiral HPLC analysis: 30 μL injection; isocratic run (65.0 min): mobile phase 85:15 2 mM aqueous $CuSO_4$:MeOH @ 1 mL/min, UV detection (230 nm); R_T = 37.2 min; enantiomeric excess: 98%. $\boldsymbol{M}$ = 189.09 [determined from the $(M + H)^+$ cluster]; $\boldsymbol{M}_{d0}$ = 182.12; 99.6% d_7.

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