

CHAPTER 4

EXPERIMENTAL

4.1 General Experimental

4.1.1 Nuclear Magnetic Resonance Spectroscopy

^1H NMR spectra

All ^1H NMR spectra were obtained at either 300 MHz or 500 MHz on a Varian spectrometer. Peak frequencies were referenced relative to the 0.00 ppm chemical shift signal of TMS internal standard in deuterated solvent used. Resonances were assigned as follows: Chemical shift (number of protons, multiplicity, coupling constant(s), assigned proton(s)). Multiplicities are reported by the convention: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). Uncertainties: Chemical shift (± 0.01 ppm), coupling constants (± 0.1 Hz).

^{13}C NMR spectra

All ^{13}C NMR spectra were obtained at either 75 MHz or 125 MHz on a Varian spectrometer. Peak frequencies were referenced relative to the 77.0 ppm chemical shift signal of CDCl_3 or the carbon signal of the deuterated solvent used. Resonances were assigned as follows: Chemical shift (carbon type, assigned carbon(s)). ^{13}C NMR assignments were made from DEPT and/or gHSQC experiments. Uncertainties: Chemical shift (± 0.1 ppm)

4.1.2 Chromatography

Column Chromatography

Column chromatography was performed using Merck GF 254 flash silica gel (40-63 μm) packed by slurry method. Small scale separations (< 2.0 g) were performed using either a 10 mm or a 20 mm diameter column, and large scale separations (> 2.0 g) were performed using a 50 mm diameter column, each with the stated solvent system. Most column were developed using gradient elution of the solvent mixtures defined in the experimental section.

Preparative Thin Layer Chromatography (PTLC)

PTLC was performed using hand prepared glass-backed plates (20 \times 20 cm) using Merck 60 PF 254 silica gel. Compounds were detected under a 254 nm ultraviolet lamp.

Thin Layer Chromatography (TLC)

TLC was performed using aluminium-backed Merck sorbent silica gel. Compounds were detected under a 254 nm ultraviolet lamp if applicable, or by staining with an acidified, aqueous solution of ammonium molybdate and cerium (IV) sulfate, followed by development with a 1400 W heat gun.

Acidic Ion-Exchange Chromatography

Acidic ion-exchange chromatography was performed using DOWEX 50WX8-50 acidic cation exchange resin, packed by the slurry method in a 10 mm diameter column. In all cases the compounds were applied as their HCl salts dissolved in

distilled water. The column was first eluted with water and then eluted with 14% ammonia solution (w/w).

4.1.3 Melting Points

Melting points were obtained using a Gallenkamp MF-370 capillary tube melting point apparatus and are uncorrected.

4.1.4 Polarimetry

Specific rotations were measured using a 10 or a 50 mm cell, a Jasco DIP-370 digital polarimeter, and the values quoted were an average of 5-10 measurements. They are reported by the following convention: optical rotation [$10^{-1} \cdot \text{deg} \cdot \text{cm}^3 \cdot \text{g}^{-1}$] (concentration, solvent). Uncertainties in the values quoted are $\pm 5\%$.

4.1.5 Mass Spectrometry

Mass spectra were obtained on a VG Quatro mass spectrometer (low resolution), and on a VG Autospec mass spectrometer (high resolution). HRMS (exact masses) were obtained *in lieu* of element analysis, and ^1H and ^{13}C NMR were used as criteria for purity.

4.1.6 Microwave Reactions

These were conducted using a Milestone ETHOS SEL microwave reactor. All reactions were conducted in a 250 mL Teflon tube with a 100 bar pressure cap, and strict control of the internal temperature.