

Microbial Baeyer-Villiger Oxidation – Stereopreference and Substrate Acceptance of Cyclohexanone Monooxygenase Mutants Prepared by Directed Evolution

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Supporting material

General procedure for multi-well plate screening:

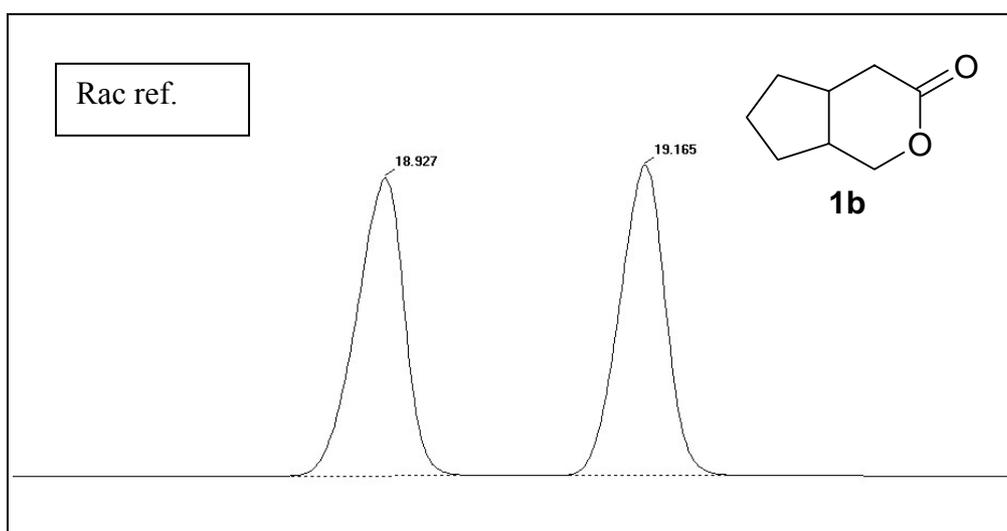
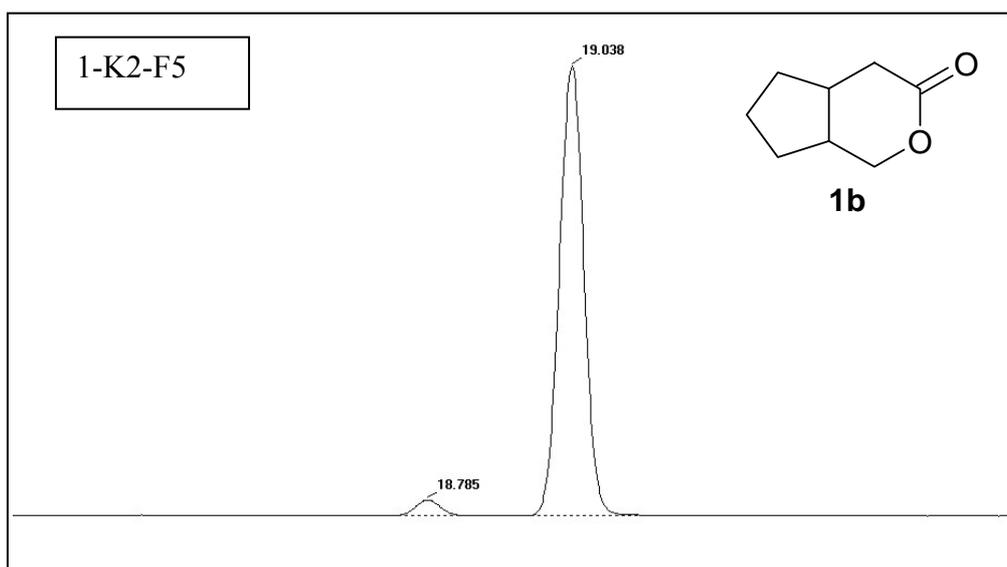
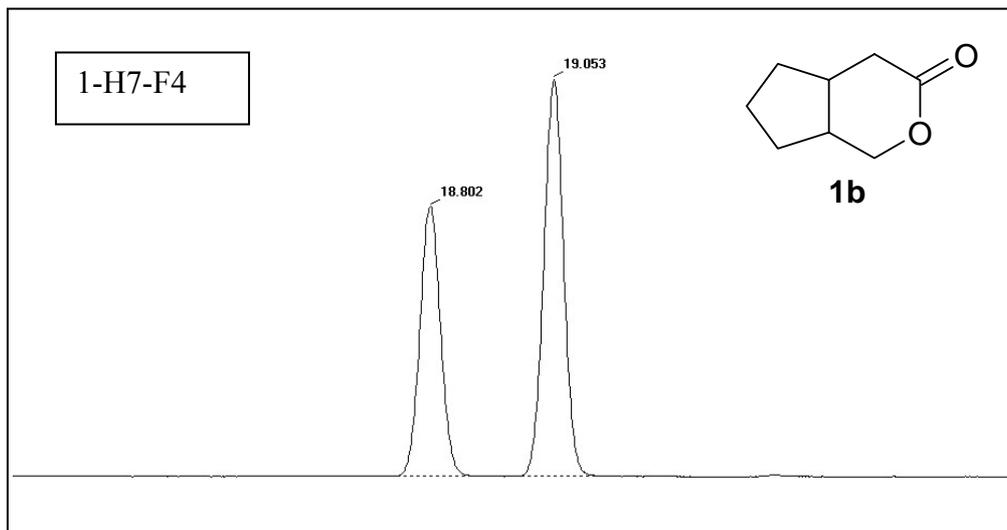
Fresh LB-amp medium (1-2mL) was inoculated with 1% of an overnight preculture of recombinant *E.coli* strains in 24 well dishes. The culture was incubated at 120 rpm at 37°C on an orbital shaker for 2 hours, and then IPTG was added to a final concentration of 0.025 mM. Standardized experiments were carried out with 0.5mg of substrate per mL of broth in 12- or 24-well dishes in the presence of 1 equiv. of β -cyclodextrin to facilitate biooxidation of slowly converted substrates. Transformations were analyzed after 24 hours of fermentation time at 24°C by extraction of the sample with EtOAc or CH₂Cl₂ supplemented by an internal standard *via* chiral gas chromatography.

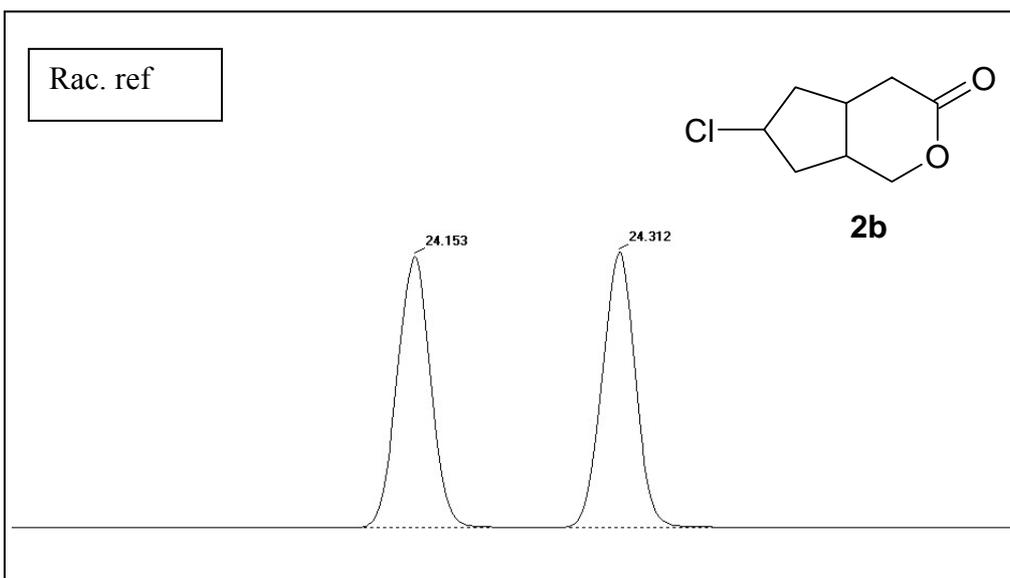
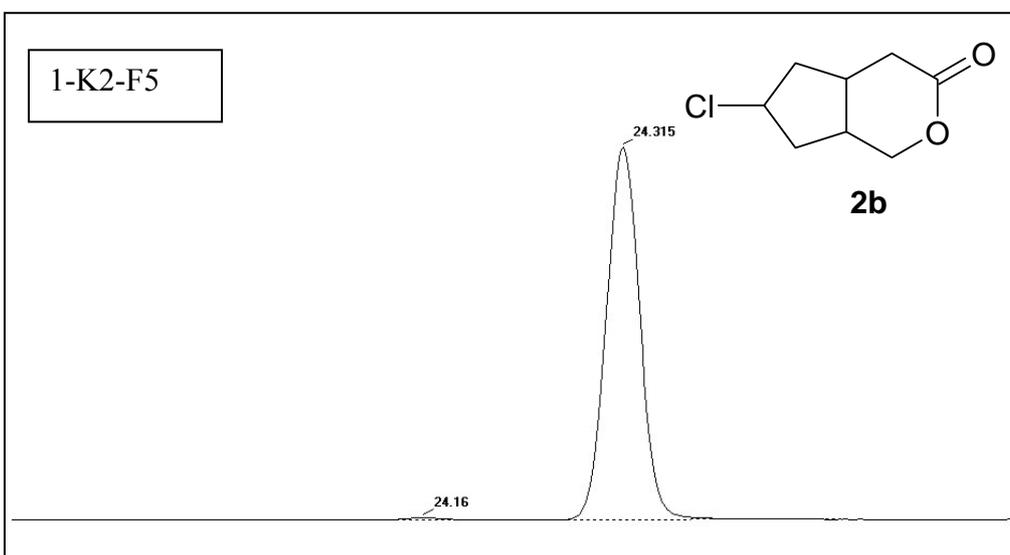
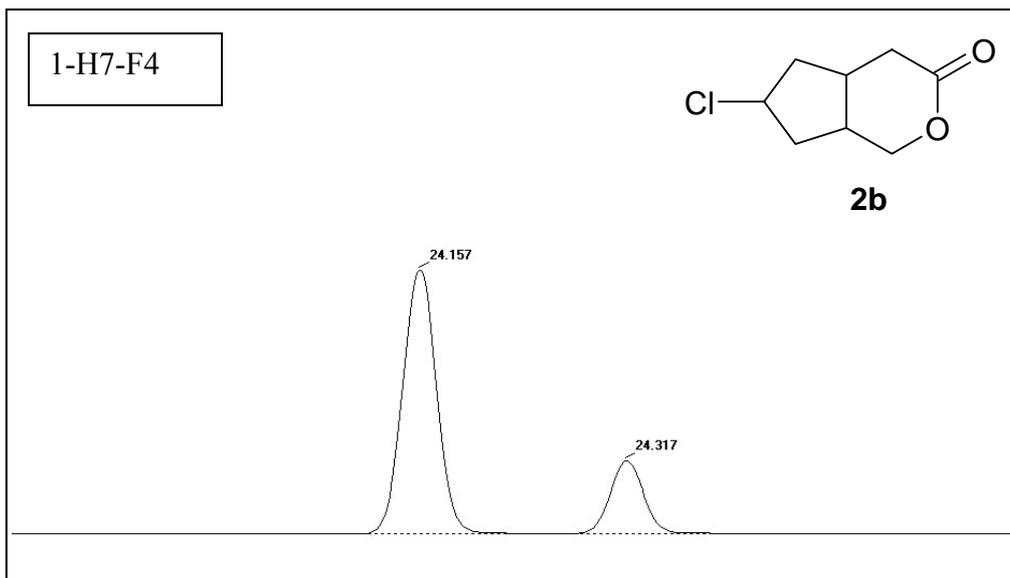
GC-analysis was performed on a ThermoFinnigan Trace GC 2000 with a BGB 173 (30m \times 0.25mm ID, 0.25 μ m film) or BGB 175 (30m \times 0.25mm ID, 0.25 μ m film) column (carrier gas: He) and retention time units are minutes.

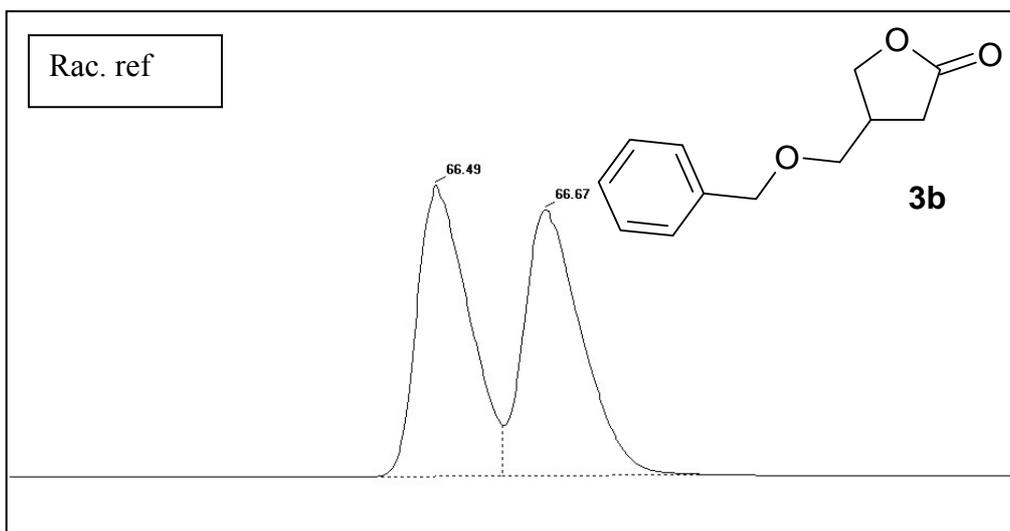
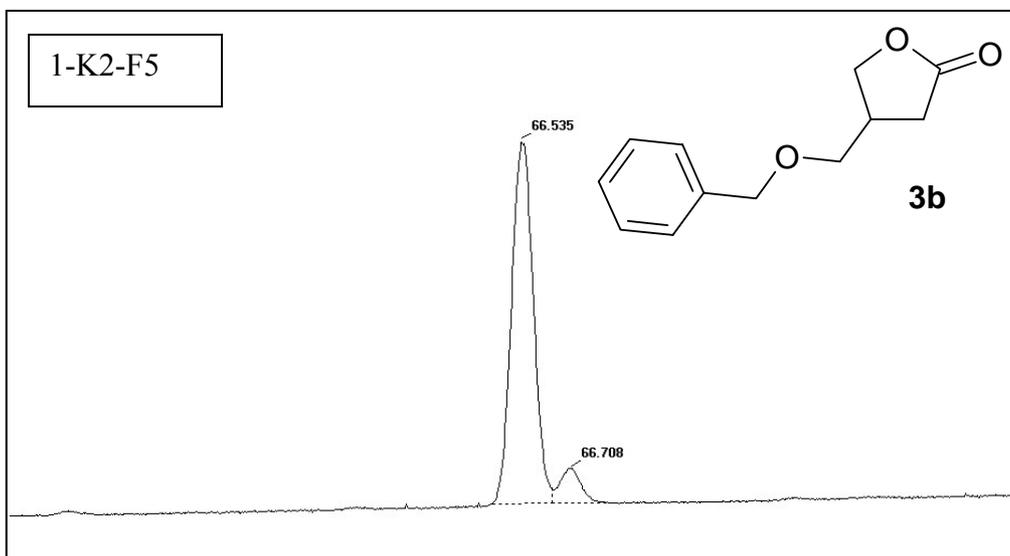
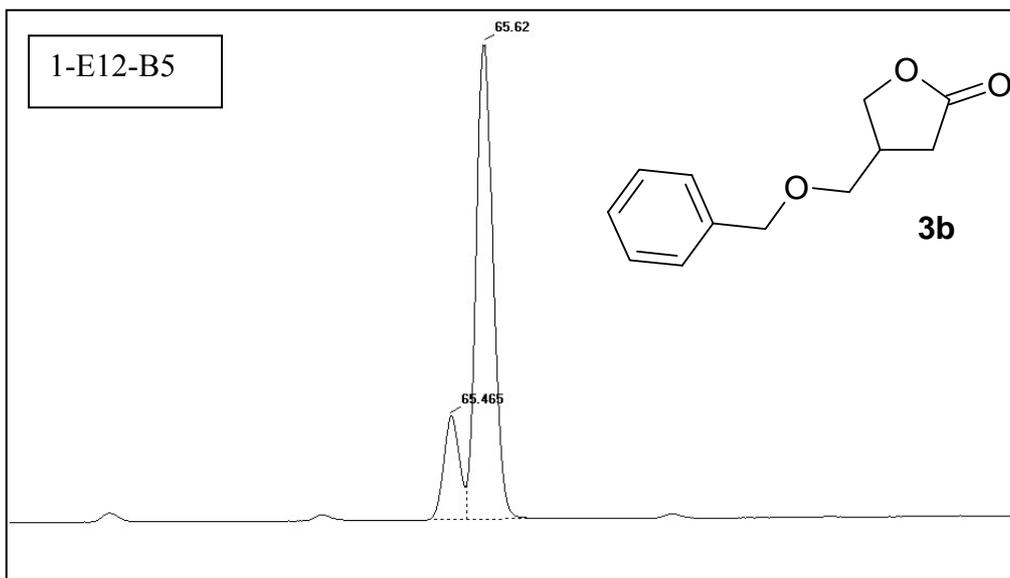
short GC-method (runtime-33min): 80°C-2min; \rightarrow 160°C-5°C/min; \rightarrow 220°C-10°/min; 220°C-7min

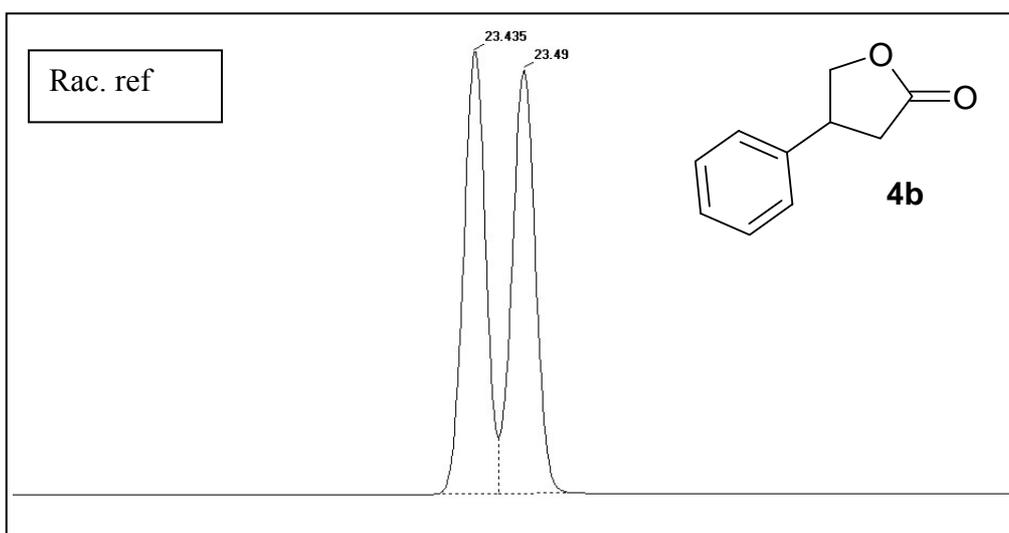
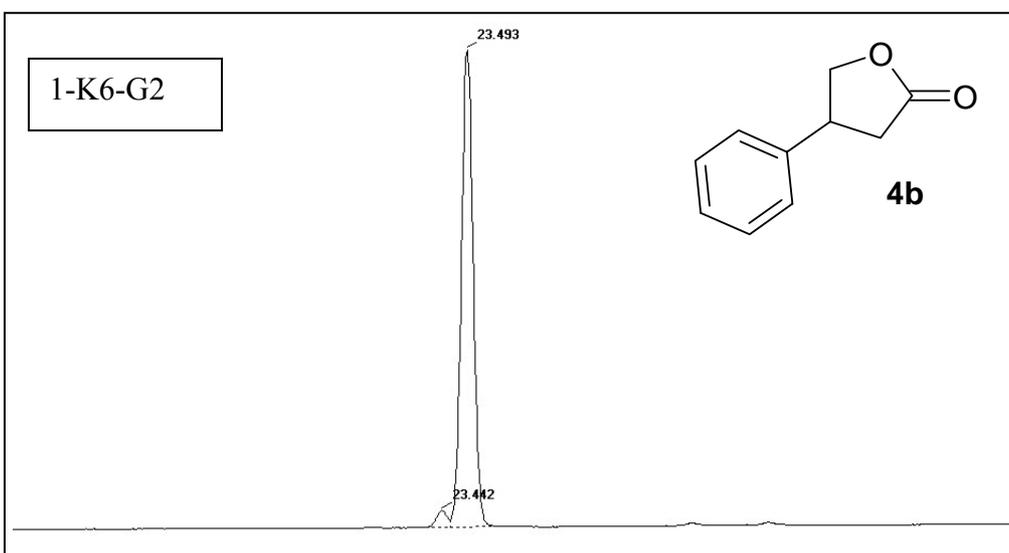
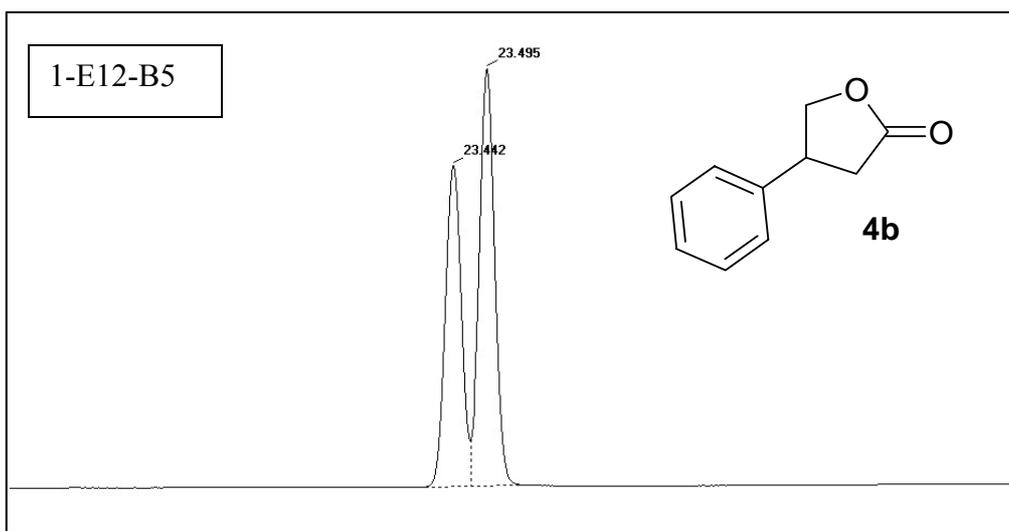
long GC-method (runtime-80min): 80°C-2min; \rightarrow 220°C-2°C/min; 220°C-8min

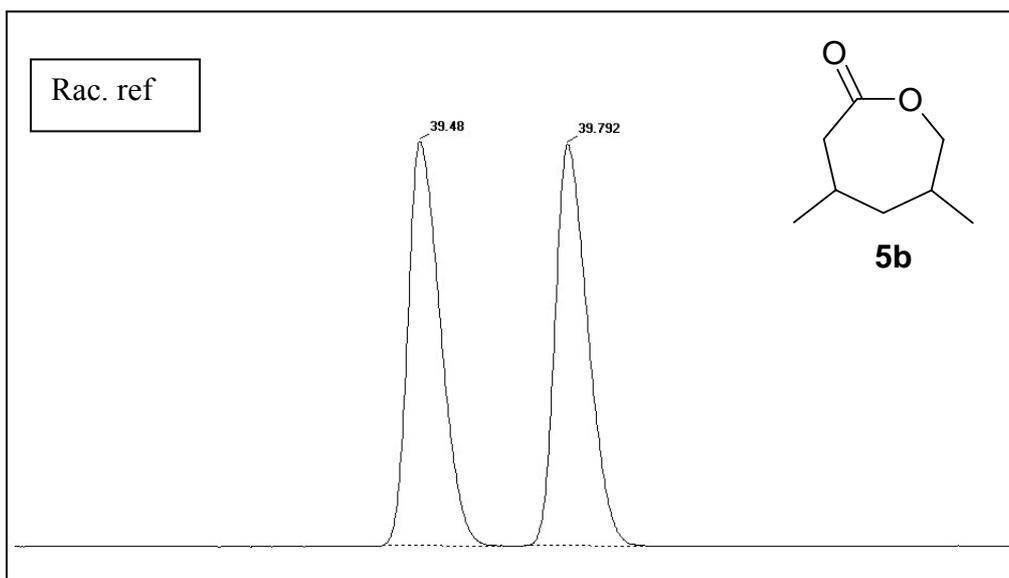
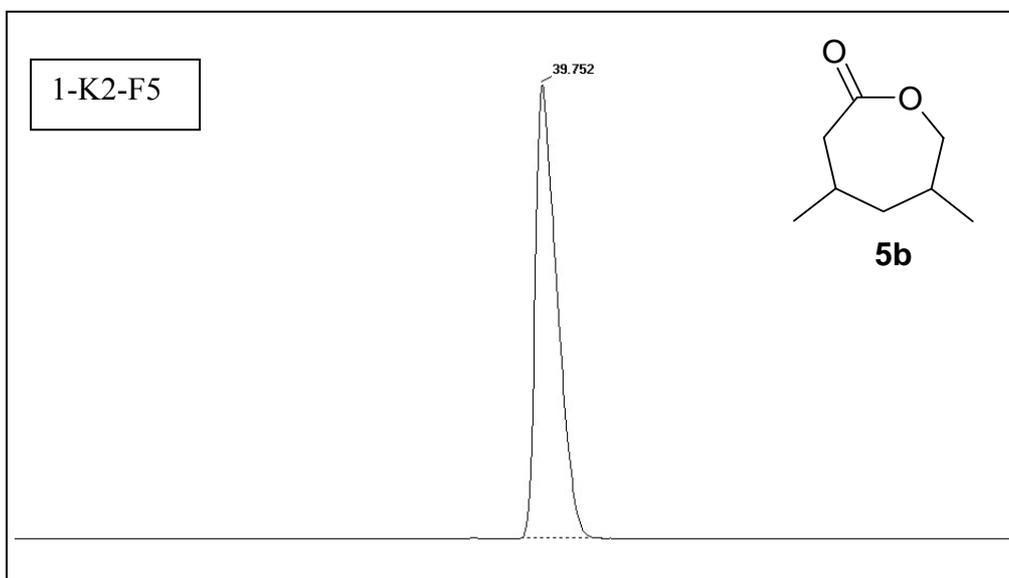
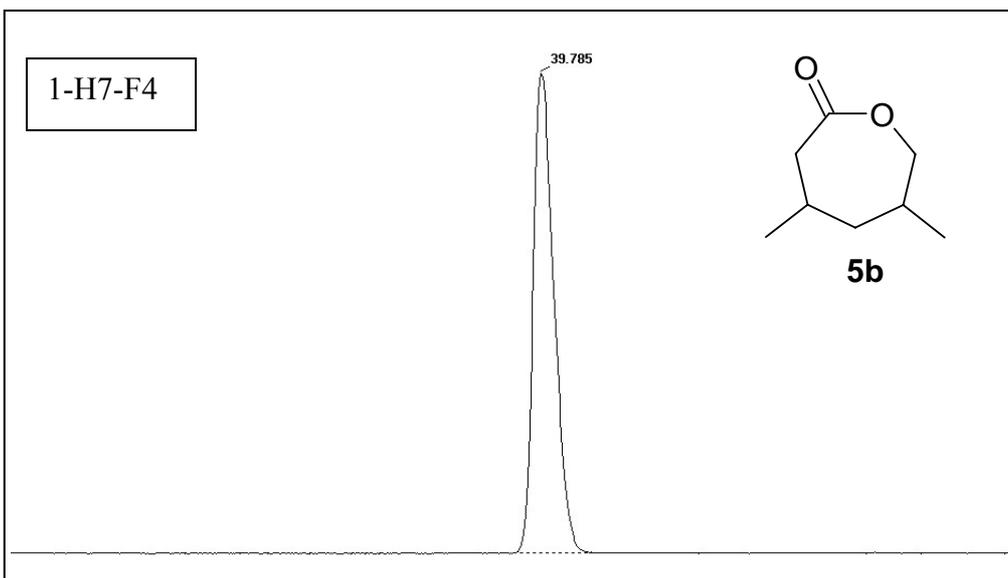
Selected GC analyses of biooxidation products:

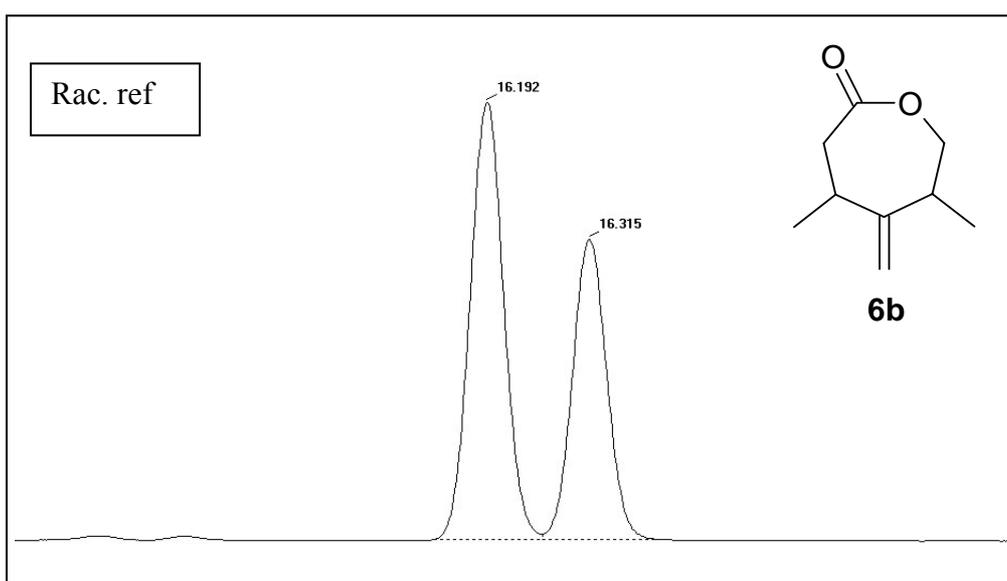
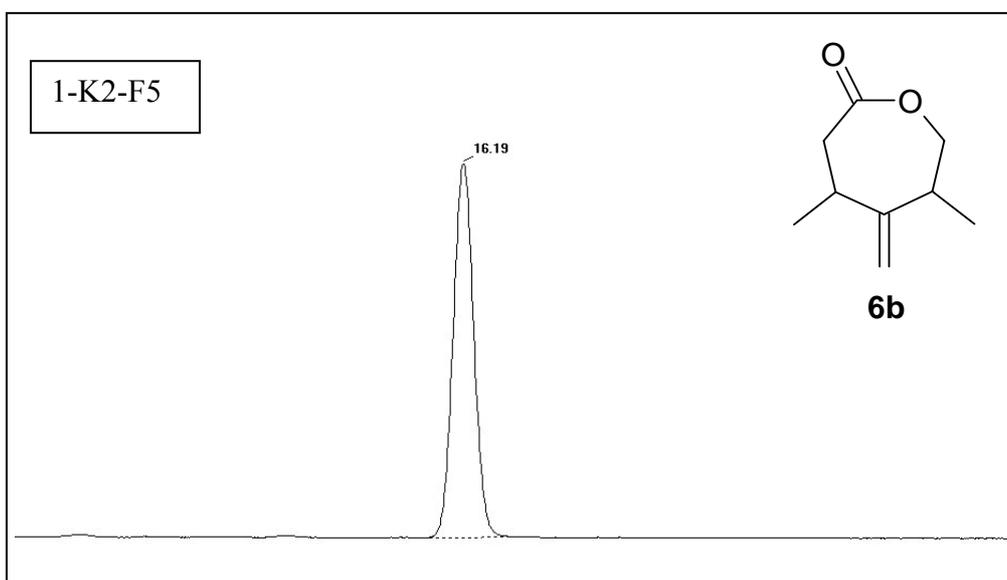
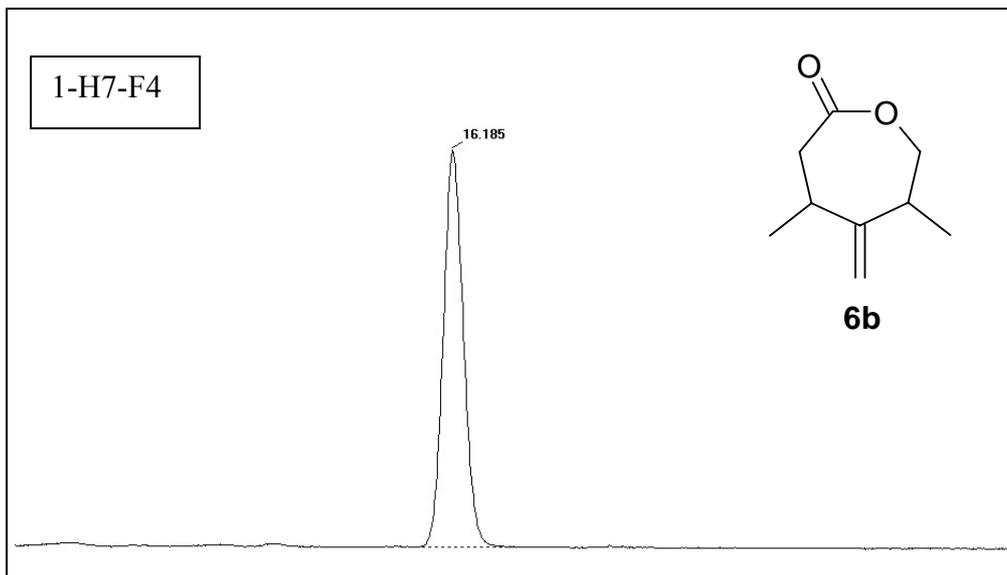


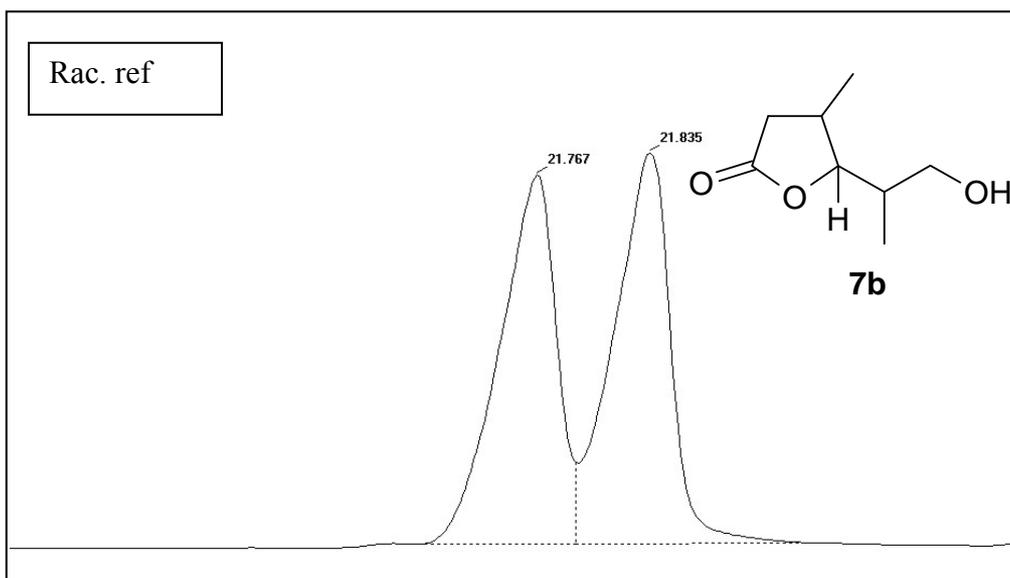
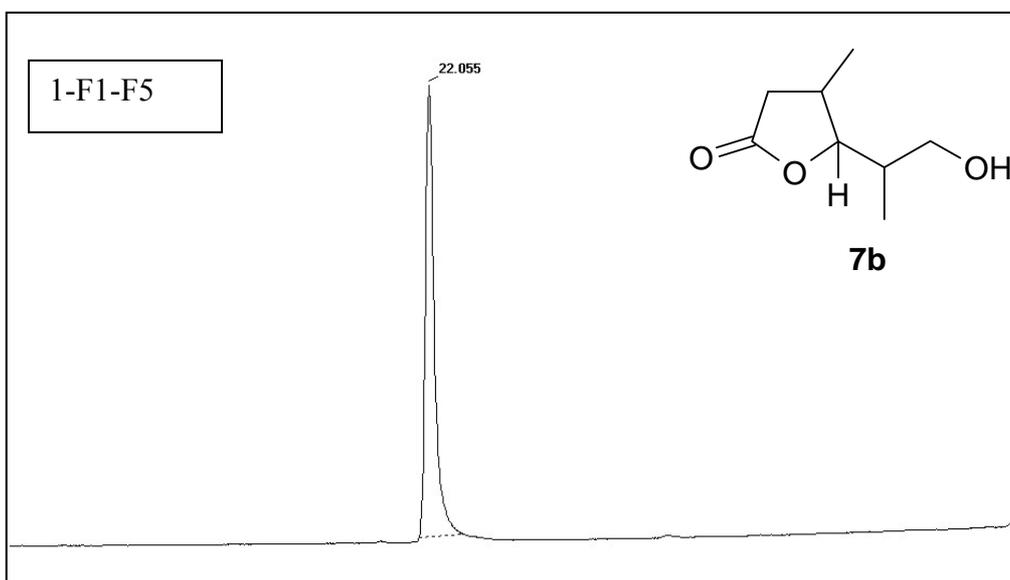
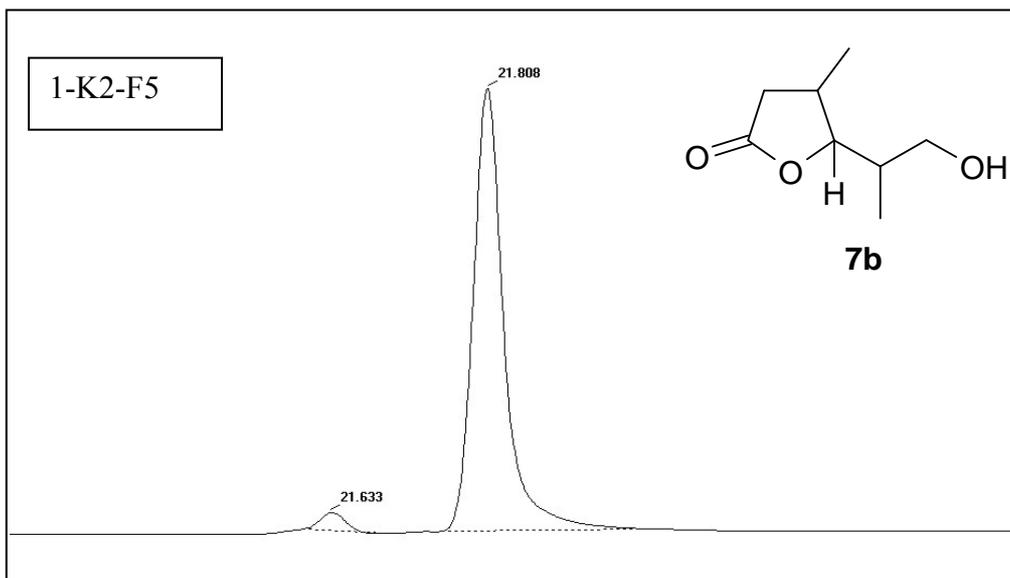


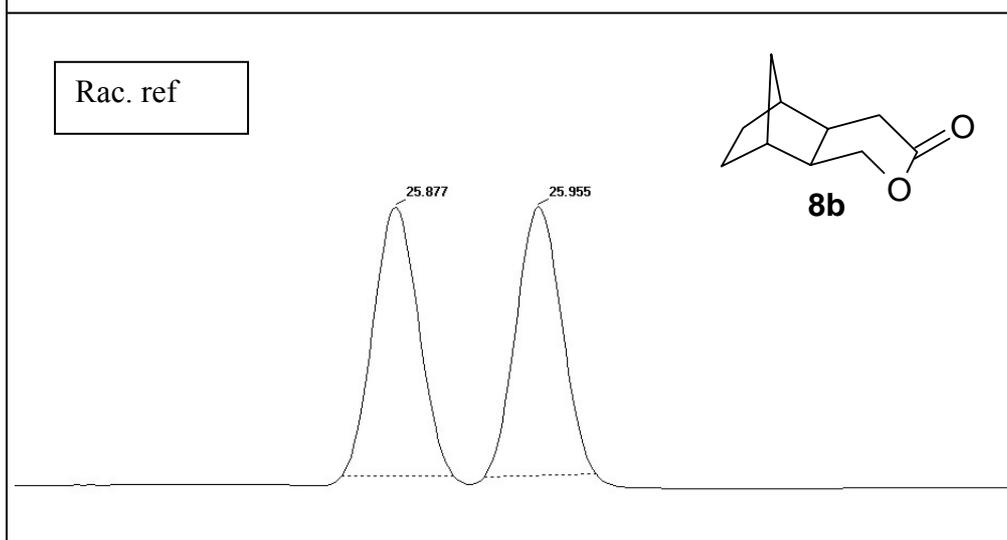
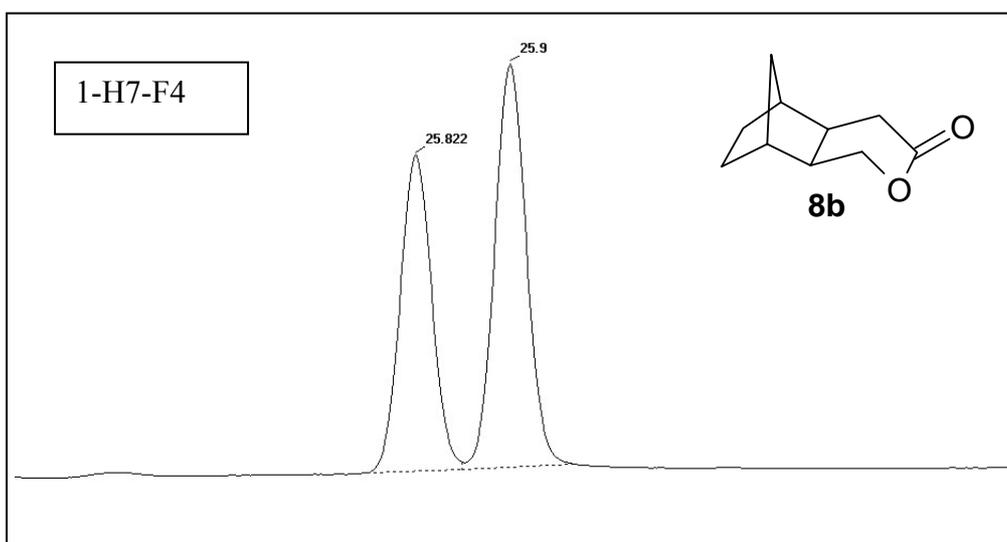
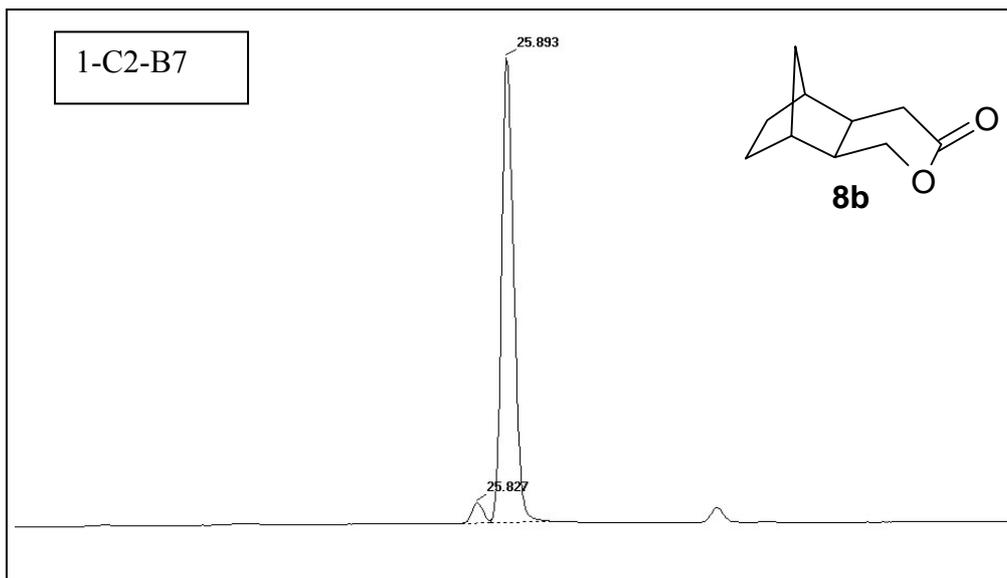


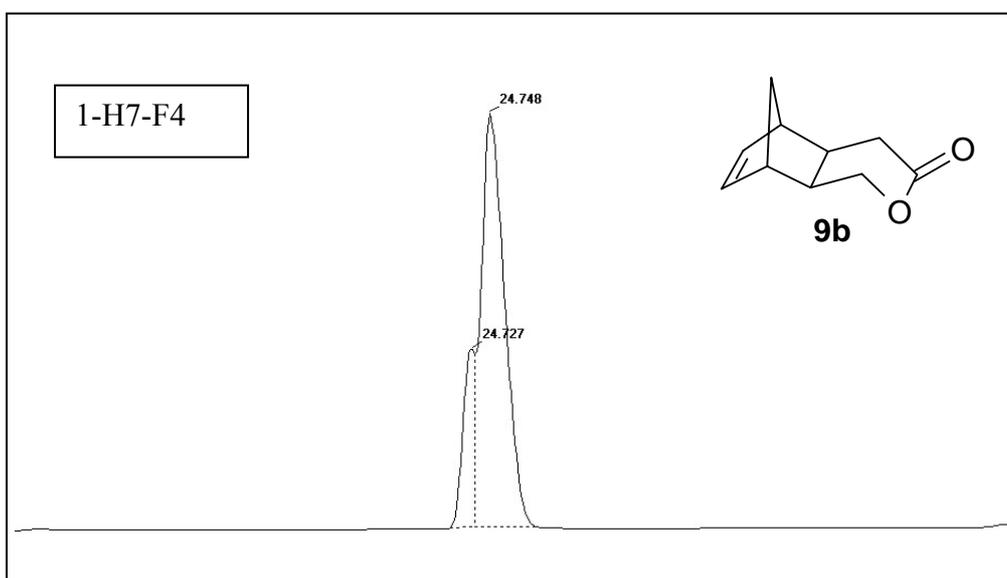
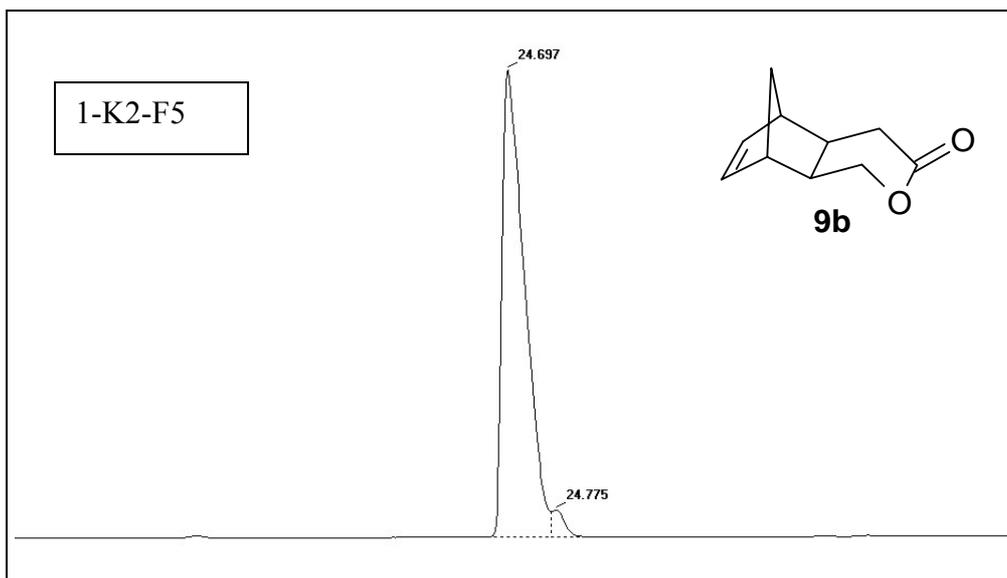


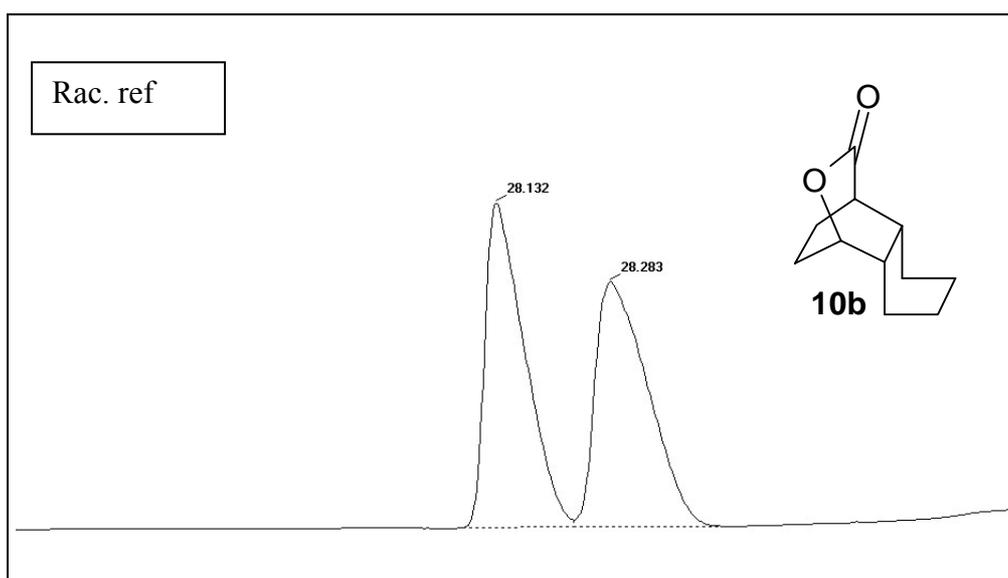
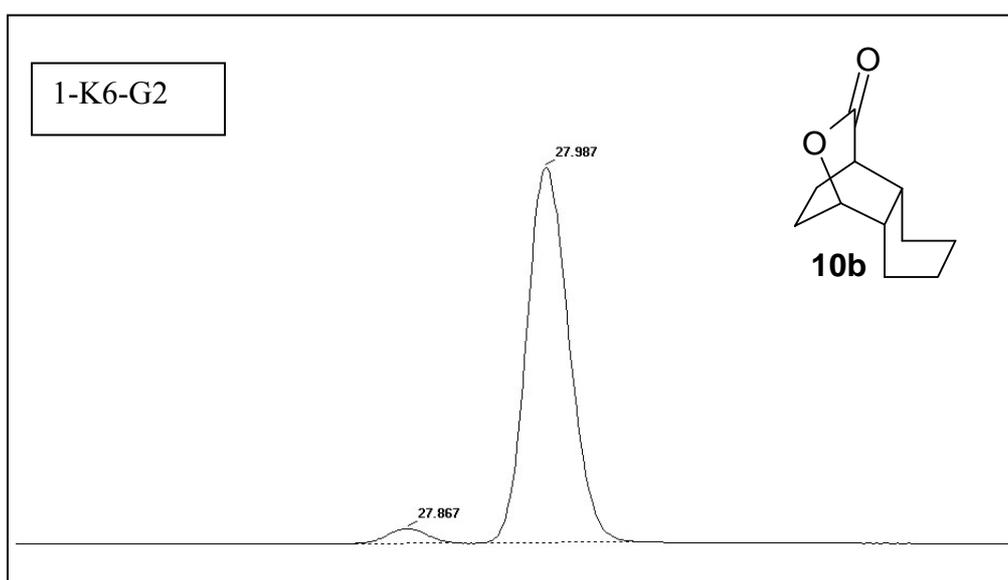
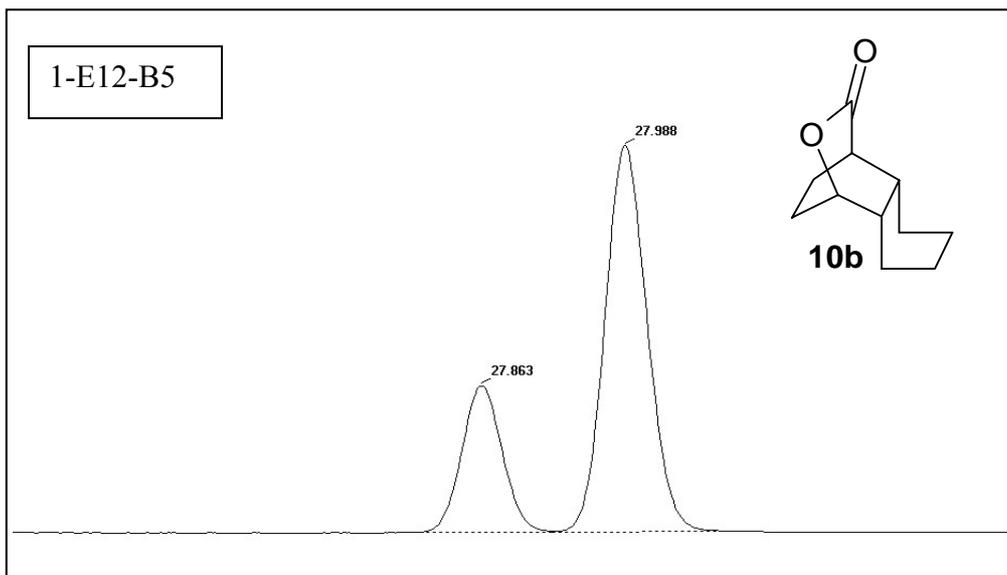


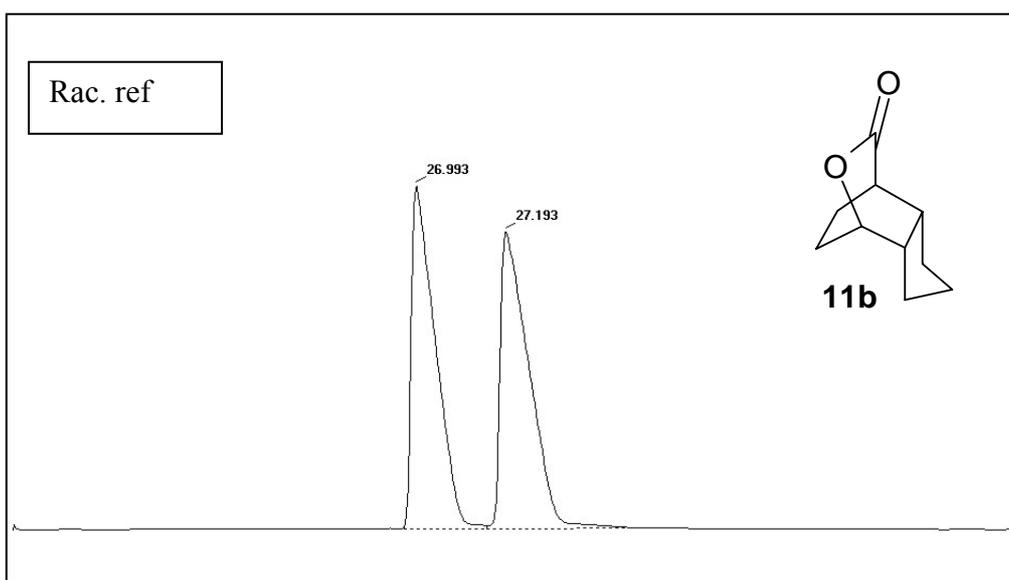
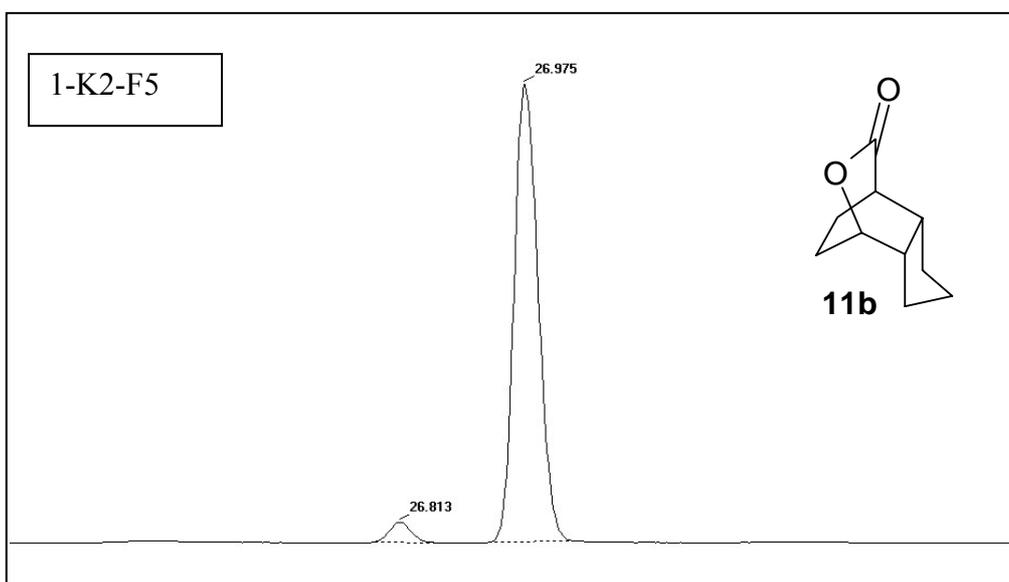
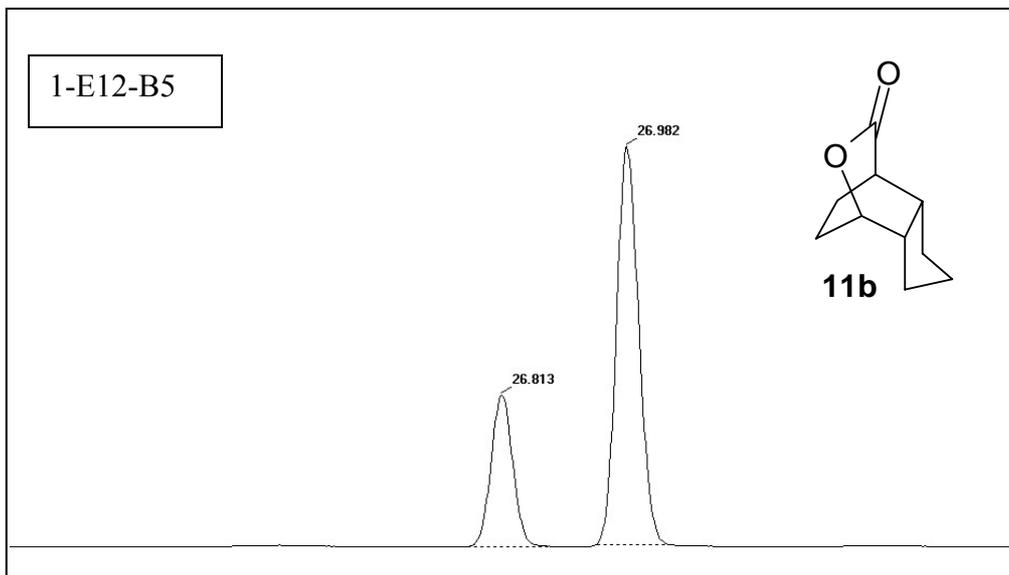


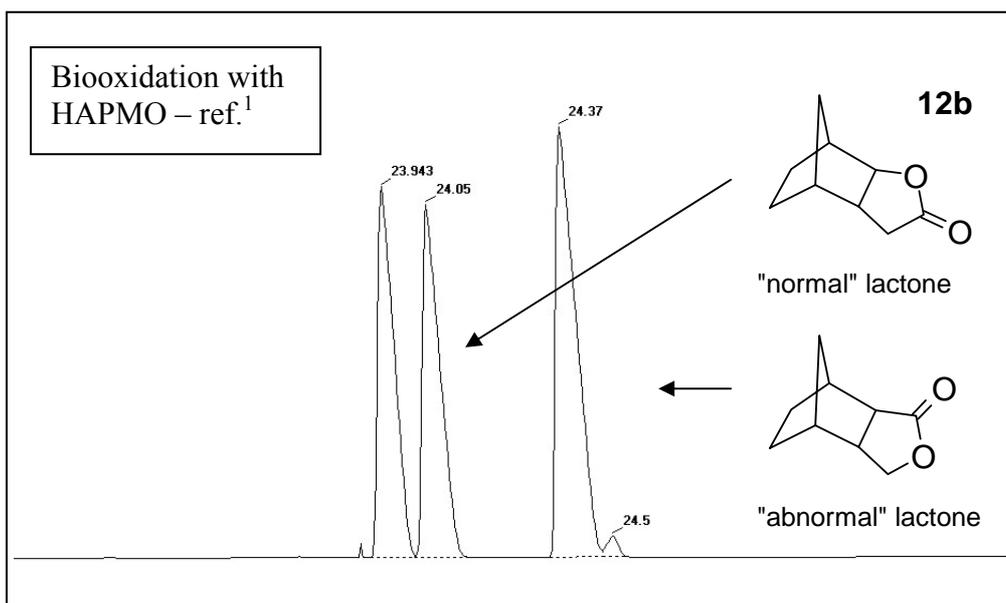
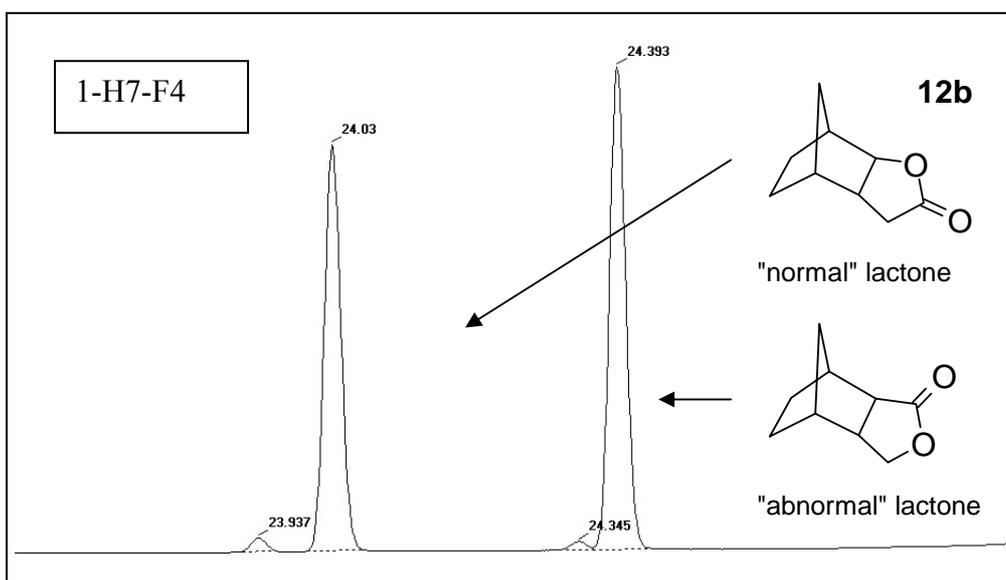
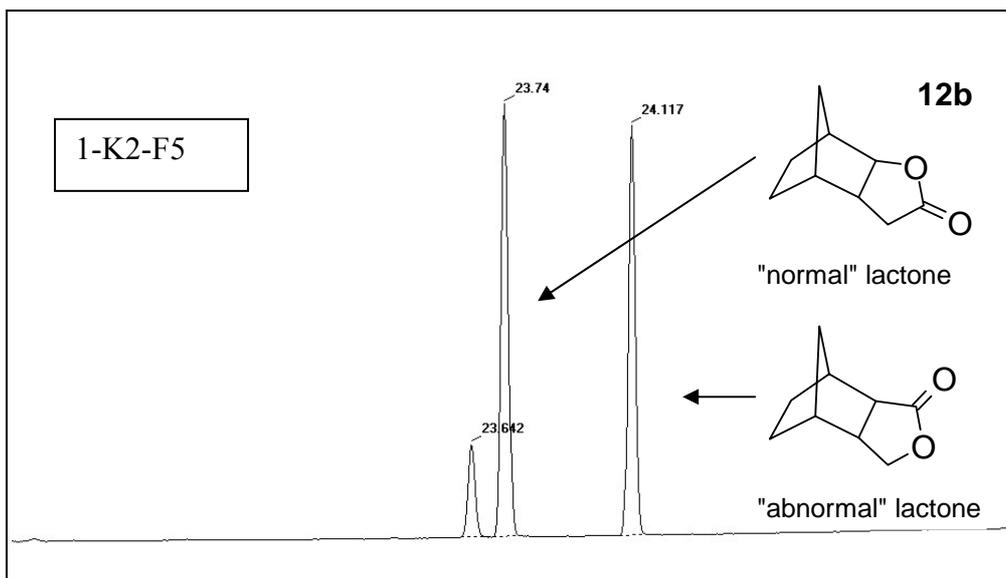












¹ Mihovilovic, M.D.; Kapitan, P.; Rydz, J.; Rudroff, F.; Ogink, F.H.; Fraaije, M.W. *J. Mol. Catal. B: Enzym.* **2005**, *32*, 135.