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RESEARCH PRODUCTS

Instructions for Use

Ethyl 2-hydroxy-2-methyl-3-oxobutanoate (methyl-^{13}C, 99%)

CLM-9935

Catalog Numbers	Required Reagents	Required Equipment
CLM-9935-0.25 CLM-9935-1	0.06 M K_2HPO_4 (in D_2O) 10 N NaOH 6 N HCl	Small stir bar Magnetic stirrer Micro-pipette Micro pH electrode and pH meter Clamp

Suggested Hydrolysis Procedure for CLM-9935-0.25:

1. Add 1.02 mL of 0.06 M K_2HPO_4 (in D_2O) to the vial. Tightly close the vial. Remove label to allow visualization of the vial's contents.
2. Mix contents in vial by vortexing for a minimum of 5 seconds. Ensure the entire inner surface of the vial and cap get thoroughly wetted.
3. Add small stir bar to vial. Clamp in place over magnetic stirrer to ensure contents to do not spill. Carefully insert calibrated pH electrode. Additional K_2HPO_4 (up to 1 mL) may be added to ensure adequate coverage of the electrode.
4. While stirring, carefully add NaOH in 10-20 µL amounts until pH reaches 11-12. DO NOT exceed pH 13. The pH will decrease as the hydrolysis reaction proceeds. Add additional NaOH once the pH drops below ~9.5.
5. Once the pH stabilizes, wait an additional 10 minutes before removing an aliquot to measure the ^{1}H-NMR spectrum.
   a. The extent of the hydrolysis reaction is monitored by inspection of the triplet and/or quartet from the ethyl group. At a pH of ~11, the methylene proton from the ethyl group resonates at ~4.1 ppm while in the ester form and at ~3.5 ppm after hydrolysis.
   b. The extent of deuteration of the 4-CH_3 group is also monitored by ^{1}H-NMR. Deuteration is complete when the integral for this methyl resonance (~1.5 ppm) is <1% of the integral for the 2-$^{13}CH_3$ methyl group (~2.2 ppm).
   c. Proceed to the next step only if the hydrolysis reaction is complete and the deuteration level is acceptable. If the hydrolysis reaction is incomplete, or if the deuteration is incomplete, transfer contents from NMR tube to the original vial and add additional NaOH and wait until the pH stabilizes.
6. Once verified by ^{1}H-NMR that the hydrolysis reaction is complete and the deuteration level is acceptable, add contents of NMR tube back to vial. While stirring, carefully add 6 N HCl to bring pH to 7.4.
7. Transfer contents to 1 L of suitable labeled minimal media 1 hr before induction.

Suggested Hydrolysis Procedure for CLM-9935-1:

1. Add 4.08 mL of 0.06 M K_2HPO_4 (in H_2O) to the vial. Tightly close the vial.
2. Follow steps 2-6 stated above for CLM-9935-0.25.
3. Divide solution into four equal aliquots, then freeze at -80°C or use fresh. Each aliquot contains enough precursor to prepare 1 L of suitable labeled minimal media.

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