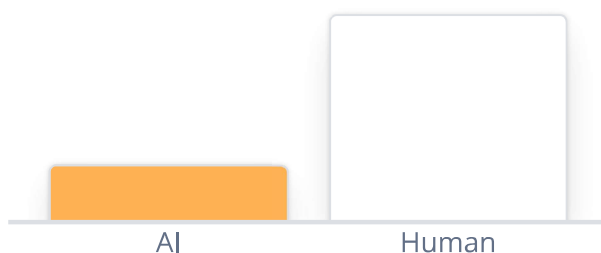




Results

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CHAPTER THREE

MATERIALS AND METHODS

In this part, the materials and experiments used in the preparation of the α -quaternary amino acids through the Kolbe electrochemical reaction are outlined.

3.1. Chemical Reagents and Equipment

3.1.1. Selection of Chemicals

The best chemicals were also taken to guarantee uniformity in the reactions and to reduce the existence of impurities that may disrupt the electrochemical reaction. Table 1 shows all the reagents and their specifications.

Primary Substrates:

Methyl 2-acetamidoacrylate, CAS: 35356-70-8, ThermoFisher, 98% purity.

In a few experiments, a co-substrate was introduced, which was propionic acid ($\text{CH}_3\text{CH}_2\text{COOH}$, CAS: 79-09-4, Fisher Scientific, 99.5% purity, Batch #PS7890).

Base System:

Potassium hydroxide (KOH, CAS: 1310-58-3, Merck, reagent grade, Batch number KOH9012) was included in the list of options since it is a base system to be optimized.

Supporting Electrolytes:

- Tetra-n-butylammonium tetrafluoroborate (TBABF₄, CAS 429-42-5, Acros Organics, 99-percent purity, Batch number TBABF123) was used as the main auxiliary electrolyte.

Solvents and Reagents:

- Diethyl ether (Et₂O, CAS: 60-29-7, Sigma-Aldrich, anhydrous, Batch #DEE901) to use as extractions and rinsing as an electrode.
- Methanol (CH₃OH, CAS: 67-56-1, Sigma-Aldrich, HPLC grade, Batch #MeOH234) to remove the electrolytes and extract as co-solvent.
- Acetone (C₃H₆O, CAS: 67-64-1, Sigma-Aldrich, reagent grade, Batch #ACE567) for electrode cleaning and extraction of electrolytes.
- Hexane (C₆H₁₄, CAS: 110-54-3, Sigma-Aldrich, HPLC grade, Batch #HEX456) to use in TLC analysis.
- Ethyl acetate (CH₃COOCH₂CH₃, CAS: 141-78-6, Fisher Scientific, HPLC grade, Batch #EtOAc789).
- Magnesium sulfate (MgSO₄, CAS: 7487-88-9, Fisher Scientific, anhydrous, Batch #MgSO4123) as drying agent.
- Silica gel (CAS: 112926-00-8, Merck, 60 Å, 230-400 mesh, Batch #SiGel123).

All chemicals were kept in the right conditions: KOH in closed containers with desiccant in order to avoid the absorption of moisture, volatile solvents in ambers with a nitrogen atmosphere, and TBABF₄ in the desiccator to keep them in dry air.

Table 1: Chemicals Used in α-Quaternary Amino Acid Synthesis

Chemical Name	CAS Number	Supplier	Purity	Batch/Lot
methyl 2-acetamidoacrylate	35356-70-8	ThermoFisher	98%	10170609
Propionic acid	79-09-4	Fisher Scientific	99.5%	PS7890
Potassium hydroxide	1310-58-3	Merck	Reagent grade	KOH9012
Sodium bicarbonate	144-55-8	Sigma-Aldrich	99.7%	NaHCO3789
TBABF ₄	429-42-5	Acros Organics	99%	TBABF123

Dichloromethane 75-09-2 VWR HPLC grade DCM5678
Diethyl ether 60-29-7 Sigma-Aldrich Anhydrous DEE901
Methanol 67-56-1 Sigma-Aldrich HPLC grade MeOH234
Acetone 67-64-1 Sigma-Aldrich Reagent grade ACE567
Hexane 110-54-3 Sigma-Aldrich HPLC grade HEX456
Ethyl acetate 141-78-6 Fisher Scientific HPLC grade EtOAc789
Magnesium sulfate 7487-88-9 Fisher Scientific Anhydrous MgSO4123
Silica gel 112926-00-8 Merck 60 Å, 230-400 mesh SiGel123

3.1.2. Electrochemical Equipment and Setup

Electrochemical System: The synthesis was conducted using an ElectraSyn 2.0 system (IKA, Model ES 2.0) equipped with precise current control capabilities. The electrochemical cell configuration consisted of:

- Working Electrode: Platinum electrode
- Reaction Vessel: 5 mL borosilicate glass vial with Teflon-lined cap
- Current Control: Constant current mode at 11.2 mA (current density: 2.1 F/cm²)
- Stirring System: Magnetic stirrer

Figure 3.1 : IKA Electrasyn 2.0 electrochemical synthesis system showing constant current operation at 11.2 mA with reaction vessel in position.

3.2. Experimental Procedures

143mg of methyl 2 acetamidoacrylate, 100mg of tetrabutyl ammonium tetrafluoroborate, 45mg of potassium hydroxide, 4ml of acetonitrile and 60 micro litre of propionic acid were weighed and

measured respectively into the 5ml ElectraSyn vial with a magnetic stirrer bar. The vial along with the mixture were fixed to the platinum electrode and taken to JK laboratory for electrolysis. Constant current at 11.2mA was passed through the reaction with the following conditions 2.1 F/cm² and 10 F/cm².

After electrolysis, the product was transferred to a separating funnel, the platinum electrode was rinsed with 10ml diethyl ether to recover the adhered products. 50ml of distilled water was added, then extraction with 40ml of diethyl ether three times, the organic phase and aqueous phase were placed on two different beakers and labeled appropriately. The organic phase were added back into the separating funnel, and further extraction with 50ml of distilled water was used five times. Then magnesium sulphate was added to the organic phase to absorb humidity. The magnesium sulphate was filtered out. The resulting solvent was taken to the rotary evaporator, where it was dried in a round bottom flask and the weight was measured. Some of the solution were taken for NMR and GC-MS.

Table 2 showing various experimental conditions

exp	C6H9NO3	KOH	BU4NBF4	C3H6O2	CH3CN
	Potential	F/cm ²			
HO001	0.2mmol	0.8mmol	0.3mmol	0.8mmol	4ml 11.2mA 2.1
HO002	0.2mmol	0.8mmol	0.3mmol	0.8mmol	4ml 18mA 10
HO003	0.2mmol	0.8mmol	0.3mmol	0.8mmol	4ml 11.2mA 2.1
HO004	0.2mmol	0.8mmol	0.3mmol	0.8mmol	4ml 11.2mA 2.1
HO005	29mg	45mg	100mg	60microL	4ml 11.2m 2.1

Using methyl 2 acetamidoacrylate as starting material and the solution obtain after electrolysis as the product, both the starting material and product were analysed using Thin layer chromatography (TLC) plate. This was analyse to see if we were able to synthesize alpha quaternary amino acids. The TLC plates are shown below.

Similar experimental procedures were carried out on different days for the experiments.

Figure 3.2: TLC analysis showing baseline separation conditions (70:30 hexane:ethyl acetate, spots S and P indicating starting material and product)

Figure 3.3: TLC analysis under optimized conditions (H:E 90:10, hexane:ethyl acetate) showing improved product isolation

3.2.4. Analytical Methodology

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis:

- Instrument: Agilent 7890A GC coupled with 5975C MSD
- Column: HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm)
- Temperature Program: 50°C (2 min) → 10°C/min → 300°C (10 min)
- Injection: Split mode (10:1), 1 μL injection volume
- Carrier Gas: Helium at 1.0 mL/min constant flow
- Sample Preparation: Crude products dissolved in dichloromethane (1 mg/mL)

Nuclear Magnetic Resonance (NMR) Spectroscopy:

- ¹H NMR: Bruker Avance 400 MHz spectrometer
- ¹³C NMR: 100 MHz with proton decoupling
- Solvent: CDCl₃ with TMS internal standard (δ 0.00 ppm)
- Sample Concentration: 10–20 mg in 0.6 mL CDCl₃

3.2.5. Safety and Quality Control

Safety Protocols:

- All manipulations conducted in well-ventilated fume hood
- Personal protective equipment: nitrile gloves, safety glasses, lab coat
- Electrical safety procedures for electrochemical equipment
- Proper disposal of electrolyte waste according to institutional guidelines

Sample Nomenclature:

Systematic sample labeling system employed:

- HO001, HO002, HO003
- HO002_b: Post-workup analysis of HO002
- HO004-end: End-point analysis of optimized conditions
- HO004-90min: Extended reaction time analysis
- HO005: Comprehensive analysis

This methodology ensures reproducible synthesis conditions while providing systematic optimization data for α-quaternary amino acid formation via electrochemical Kolbe reaction.