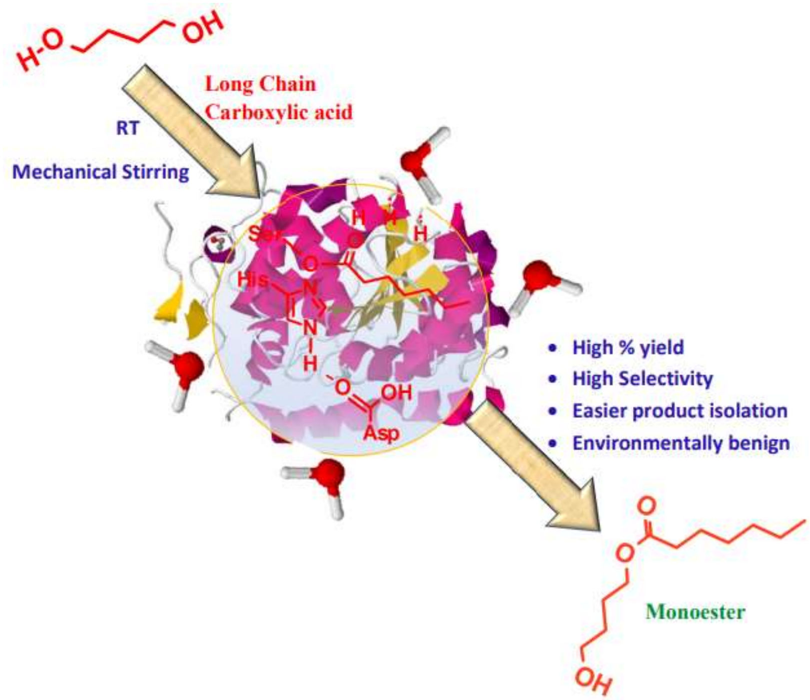
Chapter 2

Enzymatic Mono-esterification of Symmetric Diols: Restriction of Molecular Conformations Influences the Selectivity



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2.1 Introduction

Esterification is one of the most important reactions in organic synthesis. Organic esters find applications in diverse areas. For example, the fatty acid monoester and diester of 1,3-propanediol are useful in the production of soaps, detergents, shampoos, cosmetics, as well as several industrial applications. In these products, they find application as surfactant, emulsifier, dispersants, corrosion inhibitors, lubricants, demulsifiers, and so on.[1] However, the reaction is governed by equilibrium and as it proceeded in the forward direction, the reverse reaction i.e. hydrolysis of ester also compete, affect overall reaction yield. If water produced during the process is removed continuously using the simple apparatus like 'Dean and Stark', the reaction can be shifted to forward direction to almost completion. [2, 3] In most of the cases the reaction is carried out in excess of alcohol as a medium and catalysis by mineral acid is a routine practice. However, this simple procedure is not applicable for the monoesterification of symmetrical diol having both hydroxyl groups chemically equivalent.[4-8] The by-product diester (forms in high per cent yield) is unavoidable. Monoesters of diols are required as they find applications in diverse area like precursors in the multistep synthesis of bioactive natural products (such as amphidinolide B, spirastrellolide A, spiculoic acid A etc)[9 -12] and pheromones (for example, epoxide derivative of mono acylated diol is required during the synthesis of (+)-disparlure-the pheromone of gypsy moth, *Lymantria dispar* and its modified derivative- found as pheromone of ruby tiger moth, *Phragmatobia fuliginosa*).[13] Mono acylated diol is also useful for the synthesis of 2-piperidones present in many bioactive compounds such as cytisine, tacamonine, yaequinolone etc.[14] Hence development of strategy to suppress the production of diester is required.

The conventional procedure for the synthesis of monoester from symmetric diol is to treat the carboxylic acid with stoichiometric amount of diol in presence of mineral acid as catalyst; however, the desired selectivity for monoester formation cannot be achieved. It was demonstrated that the statistical distribution of unreacted diol, monoester and diester present in the mixture is in 1:2:1 ratio.[15, 16] In this procedure, also, the catalysis is of homogeneous type and the amount of mineral acid used for the purpose is comparable with that of reactants, not an environmental friendly practice and may degrade the acid sensitive reactants.

Application of protecting group chemistry is difficult due to almost similar chemical environment of both the hydroxyl groups in the diol molecule. Tetrahydropyranyl (THP) group is one of the most explored protecting groups for the purpose. For example: selective protection of one hydroxyl group as its tetrahydropyranyl ether in 1,*n*-symmetrical diol was achieved by iodine-catalyzed reaction of the diol with dihydropyranyl ether (DHP) under microwave irradiation. For this process microwave irradiation of 600 W for 150-170s is required to achieve high selectivity. On the other hand, it was observed that if reaction mixture was simply refluxed without microwave resulted into poor selectivity.[17] However, microwave irradiation is not a practical solution for large scale production. Eshghi et al achieved monoprotection of the diol with THP using P_2O_5/SiO_2 catalyst.[18]

In past, many methods were specifically developed for the selective mono-functionalization of 1,n-diol. These methods were innovative and highly contributed to the development of the field. But, these protocols use some specialty reagents or solvents which may difficult to handle. Solvent recovery during process is harmful to the environment on exposure or when applied for large scale production. Some of these are reported in Table 1 with their peculiarity and environmental concern.

Table 1. Summary of some selected protocols for mono-functionalization of 1,n-diol and issues concern with the environment.

Protocol	Strategy/Catalytic System developed	Remark	Ref.
Monosilylation of symmetrical 1,n-primary diols.	A biphasic process was developed for the selective protection of one of two chemically equivalent primary hydroxyl groups in 1,n-diols using t-butyldiphenyl silyl chloride (TBDPSCl) in diisopropyl ethyl amine (DIEA) and dimethyl formamide (DMF). DIEA had limited solubility in DMF and excess DIEA formed a light phase on top of the DMF phase. The unique selectivity under this biphasic condition was believed due to the constant concentration (16%) of base (DIEA) maintained in the reaction phase (DMF) during silylation.	**Strength.** Selectivity of monosilylation over disilylation is high upto 92 %. **Environment Concern** TBDPSCl is a hygroscopic, fuming and corrosive reagent. It has a high molecular weight as compared to diols, therefore it is used in large quantities. This reaction requires anhydrous atmosphere and all the reagents i.e. DIEA and DMF should be moisture free. After the reaction is completed the reaction mass is quenched in water due to which DMF is wasted and this is not environmental friendly. The pure product was obtained after column chromatography.	19

Selective mono protection of diols, diamines, and amino alcohols using cesium bases.	This protocol utilizes the three component coupling of diols, diamines, and amino alcohols using alkyl bromides and carbon disulfide in presence of a cesium base and tetrabutylammonium iodide (TBAI).	**Strength.** Process claims very high yield up to 93% in a short period ranging from 1.6 to 2.5 h. **Environmental Concern.** CS_2 is highly toxic with foul smell. DMF is used as a solvent, solvent recovery remains as a problem.	20
Selective Monoacylation of 1,n-diols.	Several 1,n-diols, ranging from 1,2-ethanediol to 1,16-hexadecanediol, were selectively monoacylated by transesterification in ester/octane solvent mixtures catalyzed by strongly acidic ion-exchange resins.	**Strength.** Good selectivity of mono esterification over diester, upto 92 %. **Environment Concern.** The reaction is carried out at high temperature of 100^oC and pH of dowex is strongly acidic. Some labile groups may not sustain at such extreme temperature and pH. Drying of the resin beads requires several days.	21

Catalytic monosilylation of 1,2-diols	The selective monosilylation of 1,2-diols catalyzed by dimethyltin dichloride was developed.	**Strength.** Very high selectivity of mono protected diols. The reaction is carried out for very short time that is about 1 h and at RT. **Environment Concern.** Me_2SnCl is toxic and corrosive. triethylsilyl chloride (used as 1.5 equiv) is hygroscopic and corrosive.	22
Monosilylation of primary and secondary diols.	Monosilylation of heptane diol was carried out using tert-butyl dimethyl silyl chloride (TBSCl) at RT in hexane/MeCN biphasic solvent system.	It was hypothesized that MeCN can solvate both diol and TBSCl while newly formed mono protected species would preferentially migrate to hexane phase due to difference in partition coefficient of diol and monoprotected product.	23
A practical and scalable process to selectively	Two chemically equivalent hydroxyl groups of water-soluble α,ω-diols were differentiated with dihydropyran (DHP) using a ~5-fold excess of diol in	**Strength**. Use of inexpensive DHP source and ease to remove excess water-soluble α,ω-diols and THP ether after deprotection render the process scale-friendly without need of column chromatographic separation. The application of present method was also illustrated in	24

monofunctionalize water-soluble α,ω-diols.	THF or CH_2Cl_2 in presence of a catalytic amount of p-toluenesulfonic acid (TsOH).	the preparation of heterobifunctional diols and well-defined extended to oligo(ethylene glycol). **Environment Concern.** Not suitable for acidic conditions.	
Highly selective silver (I) oxide mediated monoprotection of symmetrical diols.	Treatment of symmetrical diol with Ag_2O and alkyl halide produced the monoprotected derivative in good to excellent yield.	**Strength.** Very high isolated yields of the mono protected products (upto 93 %). **Environment Concern.** High amount of costly silver oxide is being used in the reaction (1.5 mol equivalent). High amount of diester is formed in some of the cases upto 16 %.	25
A remarkably simple process for monoprotecting diols	Lipase from pig pancreas (PPL) has been shown to catalyse selectively the hydrolysis of alkane-1,n-diol bis-acetates into the corresponding monoacetate.	**Strength.** The yields of the isolated products are high (79 -95 %). Use of Porcine pancreas lipase for the hydrolysis of the diester which is environmentally benign process.	26

Environment Concern. Initially bis acetates are prepared and then hydrolysis of one ester is carried out.

Preparation of diester with acetic anhydride requires anhydrous and moisture free reaction condition.

The hydrolysis of the diester is carried out at pH 6.9 which is maintained by addition of aq. NaOH solution periodically.

The pure product is obtained after column chromatography.

There are many reports regarding desymmetrization of prochiral diols,[27-29] however, few describe site selective monoesterification of diols having no asymmetric centre.[30-32] For example, Pathak et al reported a protocol for the monoesterification of symmetrical diols using resin bound triphenyl phosphonium iodide (TPP-I_2) in presence of 4-dimethyl amino pyridine (4-DMAP) in THF/DCM medium at room temperature.[33] They achieved the features like easy purification, low moisture sensitivity and good to excellent yield of the product. However, the difficulties using these solid phase synthesis protocol at large scale are (i) synthesis of resin bound TPP-I_2 complex requires anhydrous DCM as solvent and nitrogen atmosphere. TPP used for this purpose is itself environmentally hazardous (ii) storage at RT requires vacuum condition (iii) three molar equivalent of base 4-DMAP with respect to carboxylic acid and alcohol is necessary to drive the reaction to completion (iv) THF:DCM mixture in 1:3 mol ratio was used as reaction medium which are environmentally unfavourable for bulk production. Hence, the catalytic system which is mild, simple, highly selective, environmentally benign, easy to recover and recycle (heterogeneous in nature), scalable for large scale production, adaptable for wide range of working parameters such as temperature and pH, is the demand of the days.

Mimicking the natural processes for the purpose may be one of the solutions of this problem. Nature on billions of years of evolution process has developed highly specific catalytic system called enzyme having 100% selectivity for a given reaction under physiological conditions. Esterases (EC 3.1.1.1) and lipases (EC 3.1.1.3) are the enzymes find everywhere in natural processes sustaining life.[34] Commonly both enzymes hydrolyse the ester bond; however, esterases hydrolyse water soluble esters made from short chain carboxylic acids while lipases hydrolyse water insoluble triglycerides made from long chain aliphatic carboxylic acids.[35] This enzymatic system is so stubborn that it can easily survive in the organic media while retaining or enhancing (in most of the cases) its activity. It is observed that the enzymatic ‘pocket’ (responsible for the catalytic activity) gets open at lipid/water interface showing enhanced activity.[36-39] The function of these enzymes is not only limited to hydrolysis but they are also active towards thiols and amides.[40] Due to highly specific enantio and regio selectivity as well as substrate tolerance capacity they find wide range of industrial applications.[41-43]

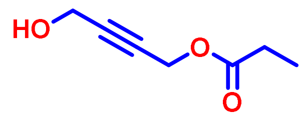
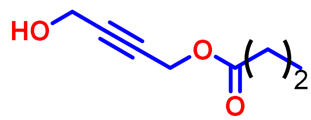
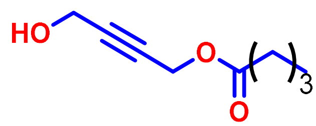
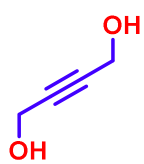
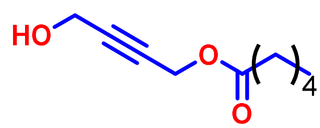
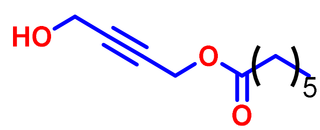
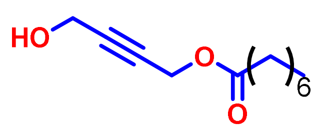
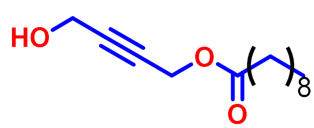
However, in almost all cases the lipases are used for variety of transesterification reaction in organic media with high regio and stereo selectivity. Here in present study, we have reported the use of *Candida Antarctica lipase B* (CALB) immobilized on polymethylmethacrylate matrix for direct monoesterification of different types of symmetrical diols in organic media with high selectivity and %yield. To study the effect of the molecular conformation on selectivity, we have selected the symmetrical diol molecules having double or triple bond in the center. By this way the molecular motion becomes restricted in a limited number of conformations. We obtained good to excellent monoesterification selectivity.

2.2 Results and discussion

It can be observed in the literature about working mechanism of transesterification involving lipase enzyme that Ser-His-Asp catalytic triad is responsible for the catalytic activity.[44, 45] Under suitable conditions the triad gets exposed allowing the substrate to enter in correct orientation. The reaction begins with nucleophilic attack of oxygen atom of serine residue on the carbonyl carbon of carboxylic acid with the formation of tetrahedral intermediate. The intermediate becomes very well stabilize by H-bonding with H-atoms of surrounding amino acids residue in the catalytic groove. Now, the alcohol or diol molecule takes part in the reaction drives the reaction towards the end.[46, 47] All steps are very well synchronized and concerted in nature that reflect in the selectivity of the product formed. Many factors affect the fate of reaction, such as (i) there should be a balance of lipophilicity between catalytic groove and substrate (ii) pH or pKa of the reaction medium (iii) solvent selected as reaction medium (iv) strength of the H-bonding between substrate-amino acid residues and between product-amino acid residues (iv) the by-product formed may initiate back reaction.

We had carried out esterification (a test reaction) of but-2-yne-1,4-diol with heptanoic acid in diethyl ether as a medium in absence of enzyme at room temperature for 24 h, but the reaction was not proceeded at all confirmed by Thin Layer Chromatography (TLC) analysis. To optimize the reaction parameters we fixed the diol (but-2-yne-1,4-diol) and carried out the reaction with carboxylic acid having different chain length in presence of CALB enzyme in organic reaction media (Table 2).

Table 2. Selective esterification of but-2-yne-1,4-diol with carboxylic acids having different chain length under optimized conditions.[a]

Entry	Alcohol	Acid	Product	Monoester[c]	Yield [b] %
1		Formic acid	No reaction	--	--
2		Acetic acid	No reaction	--	--
3		Propionic acid		92.0	64
4		Butyric acid		94.75	61
5		Pentanoic acid		92.75	62
6		Hexanoic acid		98.75	73
7		Heptanoic acid		91.25	71
8		Octanoic acid		97.5	62
9		Decanoic acid		88.0	45

[a] Carboxylic acid (1 mmol, 1 eq), diol (1 eq.), enzyme (50 mg), Diethyl ether (50 mL).

[b] isolated yield (mixture of monoester + diester only).

[c] % of monoester present in isolated yield.

The selectivity of monoester formation was studied by using ^{1}HNMR spectroscopy. For the purpose, the monoester and diester of diol was prepared and physically mixed in the predefined percent ratio (Table 3) and the intensity ratio of NMR signals of methylene protons next to acyl group at 4.3 and 4.7 ppm was calculated. The signal intensity ratio Vs % mono ester to diester was plotted as a straight line. From the slope of the straight line, the per cent ratio of monoester to diester present in the unknown sample can be calculated.

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Table 3. The proportion of monoester mixed with diester (of But-2-yne-1,4-diol and Heptanoic acid) and the signal (4.23:4.64) ratio.

	Monoester spiked with % of diester	The ratio of signal at 4.23 and 4.64
1	0	0.89
2	25	0.65
3	50	0.42
4	75	0.20

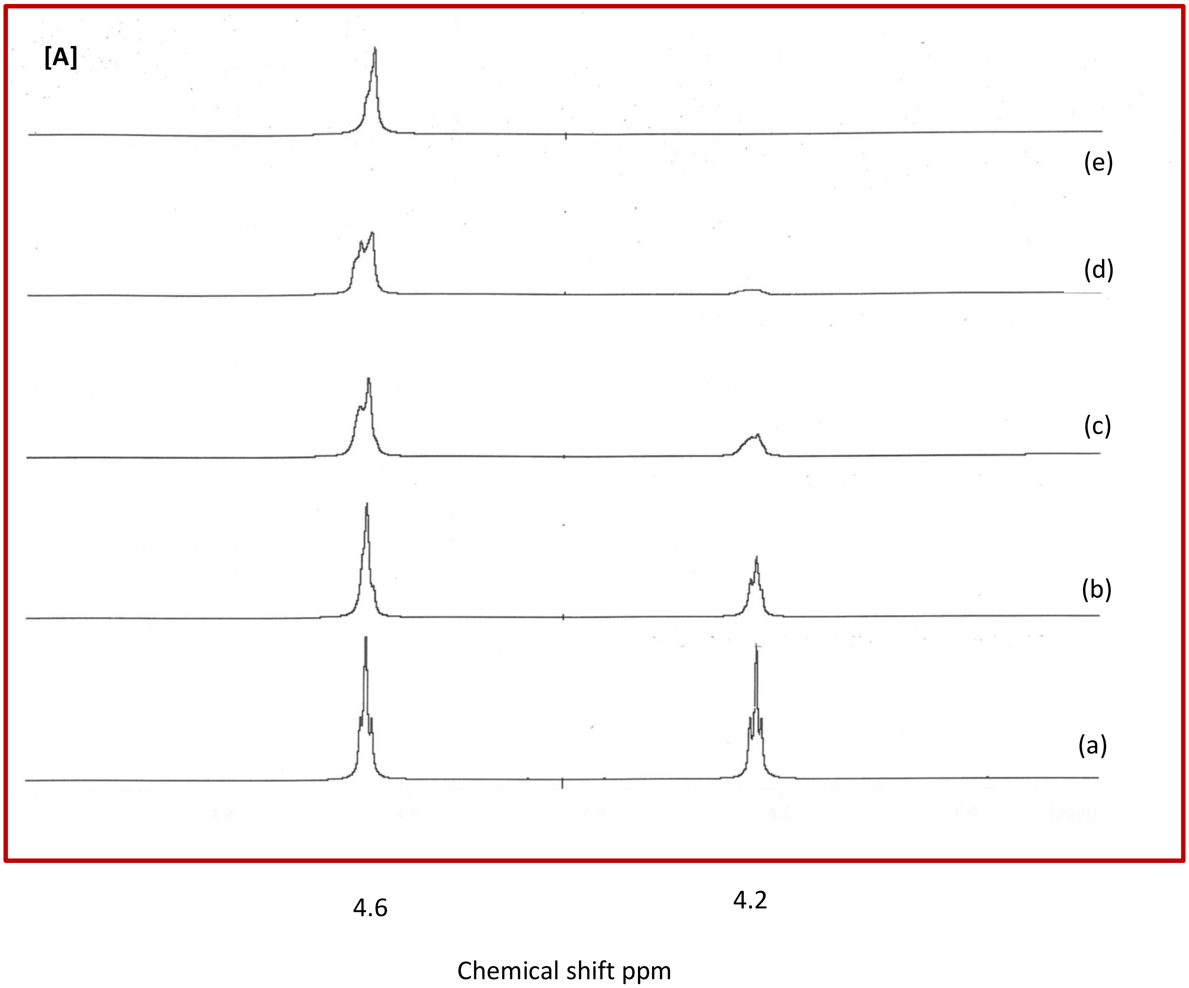


Fig. 1. Variation in ^{1}HNMR signal intensity with changing the proportion of monoester and diester (of But-2-yne-1,4-diol and Heptanoic acid) in a mixture. (a) 100 % Monoester (b) 25 % Diester + 75 % Monoester (c) 50 % Diester + 50% Monoester (d) 75 % Diester + 25% Monoester (e) 100 % Diester.

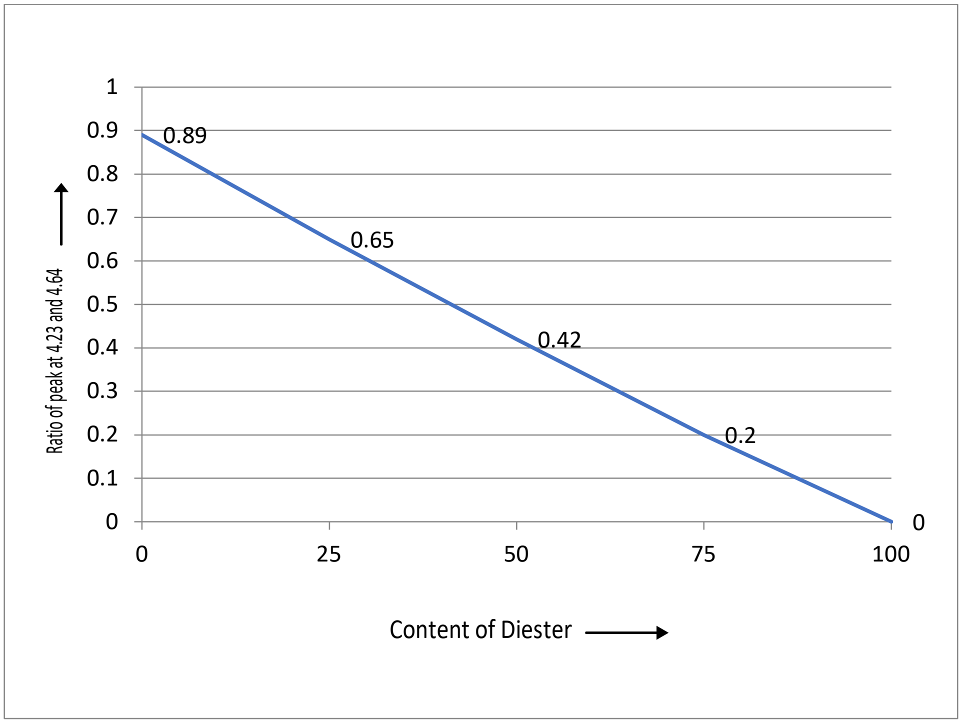


Fig. 2. Calibration curve to calculate the monoester to diester ratio in reaction mixture.

Calculation of slope

Slope = y2-y1/x2-x1

= 0.65 – 0.42 / 25 - 50

= - 0.0092

Using the slope the content of mono ester and diester can be calculated for any unknown sample.

Content of diester when the ratio of peak at 4.23 and 4.64 is about 0.65

X = y-c / m

= 0.65 – 0.89 / - 0.0092

= 26 %

The % yield of each reaction with the monoester formed is tabulated (Table 2). We observed an interesting result from this set of reactions as: the reaction was carried out for formic acid to decanoic acid as a substrate. The reaction does not take place at all for formic acid (pKa

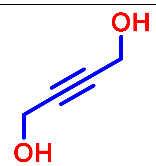
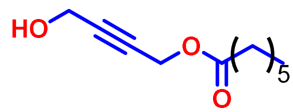
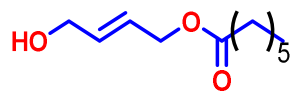
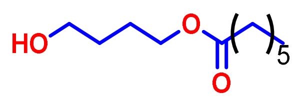
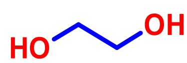
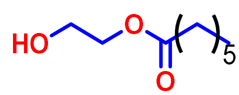
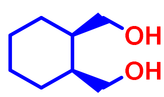
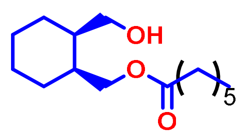
3.75) and acetic acid (pKa 4.76) may be due to the denaturation of enzyme. From propionic acid onwards up to octanoic acid the product formed conveniently. While for nonaic acid and decanoic acid the reaction proceeded well but during isolation of the product a heavy emulsion was observed made isolation of the product difficult. When we correlated these data with the pKa of each carboxylic acid, we found that the enzyme works efficiently in the range of 4.8 to 5.0 pKa values for straight chain carboxylic acid (Table 4). This could be one of the reasons that enzyme shows its activity in unbuffered reaction media.

Table 4. pKa values of carboxylic acids with different chain length.

Acid	**pKa value**
Benzoic acid	4.2
Formic acid	3.75
Acetic acid	4.76
Propionic acid	4.86
Butyric acid	4.83
Pentanoic acid	4.84
Hexanoic acid	4.85
Heptanoic acid	4.89
Octanoic acid	4.89
Nonanoic acid	4.96

From this study we observed that hexanoic and heptanoic acid are the perfect match for the reaction with high selectivity and yield for monoesterification. Therefore, for mono esterification of different diols we designed next set of experiments in which heptanoic acid was selected as a substrate and type of diol varied (Table 5). In the earlier discussion we proposed that the conformation of diol directly affects the fate of reaction. Hence, we selected symmetrical diols with different levels of unsaturation at the centre to arrest the molecular conformation and stabilize the same through H-bonding in the catalytic pocket (Scheme I).

Table 5. Selective esterification of heptanoic acid with diols having different levels of unsaturation and chain length under optimized conditions.[a]

Entry	Acid	Alcohol	Product	Monoester[c]	Yield %[b]
1				91.25	71
2				93.0	66
3	Heptanoic acid			90.25	68
4				99.75	64
5				98.00	69

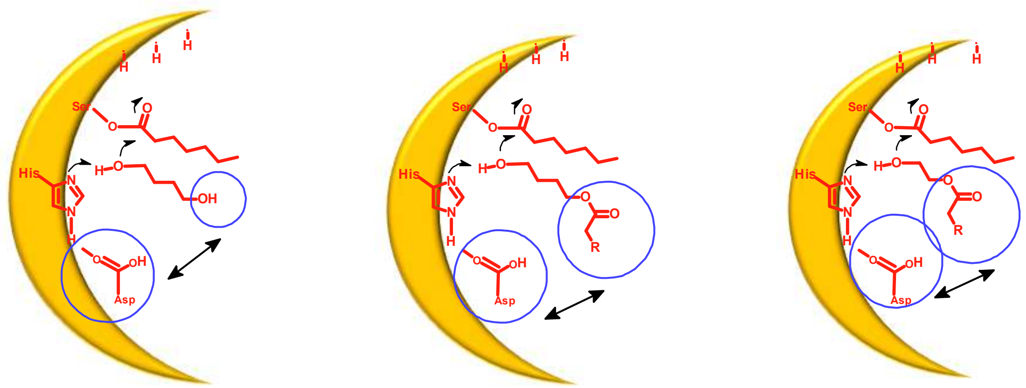
[a] Carboxylic acid (1 mmol, 1 eq), diol (1 eq.), enzyme (50 mg), Diethyl ether (50 mL).

[b] isolated yield (mixture of monoester + diester only).

[c] % of monoester present in isolated yield.

Based on this fact, we propose the mechanism for this reaction. It can be observed from scheme SI that second esterification is very difficult once the monoester is formed. It is obvious that monoester molecule cannot be oriented properly and stabilized in the catalytic groove compared to diol molecule.

It was observed from previous studies that the catalytic Ser-His-Asp triad remains inside the surface loop of the lipase and possesses hydrophobic environment. In aqueous medium it is in the ‘close’ conformation and not available to the substrate, however, it can be ‘open’ by forcing the enzyme to change its conformation.[48] Molecular docking studies demonstrated that two mobile α-helices surrounding the active site (α5 and α10) play an important role to change the conformation of the enzyme.[49, 50] It was proved that at lipid/water interface the microenvironment around the enzyme drives it to conformational change and makes it open for a given substrate. During reaction, the micro-environment around the enzyme changes in every moment with the generation of water molecules as by- product. This situation creates environment like lipid/water interface in organic media facilitates the enzyme to retain its changed confirmation resulting into high yield.[51] Here, one question arises: what about the hydrolysis of the product (monoester) by the water molecules present in the vicinity? Well, this fact cannot be denied. During the experiment we observed the intensity of the product’s spot on TLC plate every 4 h, the TLC analysis showed that at the beginning (upto 8 h) the rate of product formation was very fast and became slow and then stable after 8 h up to 24 h. However, the amount of product never decreases in 24 h. Looking at the last few steps of the mechanism, it can be said that the hydrophobic environment of the reaction site try to keep water molecules away from the electrophilic carbonyl center of attack. In this situation the water molecules prefer to make stronger H-bonding with His residue pushing the product out of the pocket rather than initiating the hydrolysis of the product formed. However, % yield values show that some hydrolysis of the product may be occurred. The same reaction was carried out using heptanoic acid as substrate but now ethane-1,2- diol was selected instead, to study the effect of the size and structure of diol on selectivity. To our surprise, we found 99% selectivity for monoester in overall 64% yield. Proposed scheme I explains the performance for high selectivity for monoester product. These diol molecules can very well accommodate and stabilize in the catalytic pocket by H-bonding with Asp oxygen atom (Scheme Ia). The same is the case with cyclic aliphatic diol, (Entry 5). The monoester accommodated in the catalytic groove cannot be stabilized due to steric hindrance with Asp residue (Scheme Ib) and steric hindrance is the most effective in case of monoester of ethane-1,2-diol (Scheme Ic) explains high selectivity.



(a) No hinderance (b) hinderance (c) Maximum hinderance

Scheme I. Proposed steric hindrance between monoesters and Asp residue in the catalytic groove explains high selectivity for monoester of ethane-1,2-diol.

2.3 Experimental

2.3.1 Materials

Cis-1,2-cyclohexanedimethanol was purchased from Sigma-Aldrich, trans-2-butene-1,4-diol was purchased from TCI chemicals and decanoic acid was purchased from Loba Chemie Pvt. Ltd. All solvents, carboxylic acids and diols other than above were purchased from Spectrochem Pvt. Ltd. India. All chemicals were used without further purification. *Candida Antarctica Lipase B*, Novozym® 435FG, (Novozymes, Denmark) was supplied by the manufacturer in an immobilized form embedded in a solid inert methacrylate granulate carrier. The declared activity was more than 5000PLU/g.

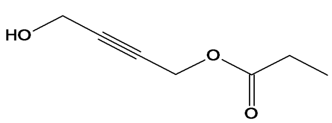
2.3.2 General Procedure for monoesterification of symmetrical diols

1,4-dihydroxy-2-butyne (400 mg, 4.64 mmol) was dissolved in diethyl ether (30 mL) at room temperature. Then, octanoic acid (669 mg, 4.64 mmol) was introduced and the reaction mixture was stirred for 5 min. Now, CALB (20 mg) was added and the solution was stirred with mechanical stirrer at 100 rpm for 24 h. After 24 h the reaction mixture was filtered to separate the enzyme. 30 mL water was added to the filtrate followed by addition of solid sodium bicarbonate in small portions till effervescence ceased. Aqueous layer was separated and discarded. The ether layer was initially washed with water (15 mL) and was distilled to give clear oil (500 mg, 2.35 mmol). The separated enzyme was recycled for three consecutive cycles without losing activity.

2.3.3 Characterization

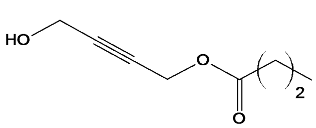
The FTIR (Perkin-Elmer, Spectrum Two) spectra of the synthesized products were obtained in the range of 400 to 4000 cm^{-1}. The ^{1}H NMR , ^{13}C NMR and Attached Proton Test (APT, 1D ^{13}C NMR) of the products were recorded on a Bruker Avance 300 MHz spectrometer using TMS as an internal standard in $CDCl_3$. HR-MS was recorded on XEVO-G2-S QTOF in Methanol as solvent.

4-hydroxybut-2-yn-1-yl propanoate



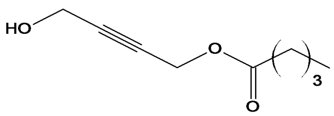
The compound was a colorless oil: Rf = 0.32 (Silica TLC, 33% EtOAc/petroleum ether); 1 H NMR (300 MHz, CDCl3) δ 1.15 (t, 3H), 2.38 (q, 2H), 2.97 (brs, 1H), 4.30 (s, 2H), 4.72 (s, 2H), 13C NMR-APT (75 MHz, CDCl3) δ 8.87, 27.32, 50.6, 52.2, 79.4, 85.1, 174.0; IR (neat, cm-1) 3387, 2983, 2883, 1731, 1141, 1172, 1001, 559, 499; HRMS (ESI) Calcd. for $C_7H_{11}O_3$ $(M+H)^+$: 143.0708; Found: 143.0708.

4-hydroxybut-2-yn-1-yl butanoate



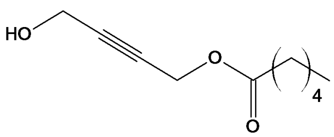
The compound was a colorless oil: Rf = 0.30 (Silica TLC, 33% EtOAc/petroleum ether); 1 H NMR (300 MHz, CDCl3) δ 0.96 (t, 3H), 1.67 (q, 2H), 2.33 (t, 2H), 2.56(brs, 1H), 4.3 (s, 2H), 4.7 (s, 2H), 13C NMR-APT (75 MHz, CDCl3) δ 13.5, 18.2, 35.87, 50.8, 52.1, 79.6, 85.0, 173.2; IR (neat, cm-1) 3385, 2967, 2938, 2877, 1728, 1168, 1140, 1019, 972, 555, 492; HRMS (ESI) Calcd. for $C_8H_{13}O_3$ $(M+H)^+$: 157.0865; Found: 157.0865.

4-hydroxybut-2-yn-1-yl pentanoate



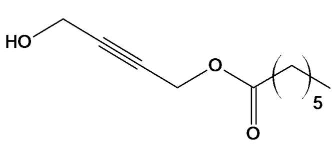
The compound was a colorless oil: Rf = 0.32 (Silica TLC, 33% EtOAc/petroleum ether); 1 H NMR (300 MHz, CDCl3) δ 0.92 (t, 3H), 1.34 (q, 2H), 1.62 (q, 2H), 2.35 (t, 2H), 2.80 (brs, 1H), 4.30 (s, 2H), 4.71 (s, 2H), 13C NMR-APT (75 MHz, CDCl3) δ 13.62, 22.15, 26.81, 33.72, 50.73, 52.16, 79.54, 85.08, 173.37; IR (neat, cm-1) 3425, 2960, 2935, 2873, 1739, 1159, 1107, 1022, 965, 553, 501; HRMS (ESI) Calcd. for $C_9H_{15}O_3$ $(M+H)^+$: 171.1021; Found: 171.1021.

4-hydroxybut-2-yn-1-yl hexanoate



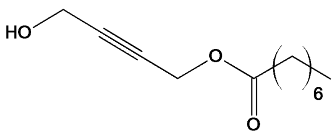
The compound was a colorless oil: Rf = 0.29 (Silica TLC, 33% EtOAc/petroleum ether); 1 H NMR (300 MHz, CDCl3) δ 0.89 (t, 3H), 1.30 (m, 4H), 1.64 (m, 2H), 2.33 (m, 2H), 3.32 (brs, 1H), 4.30 (s, 2H), 4.71 (s, 2H), 13C NMR-APT (75 MHz, CDCl3) δ 13.85, 22.25, 24.46, 31.20, 33.94, 50.82, 52.15, 79.68, 85.00, 173.38; IR (neat, cm-1) 3418, 2957, 2932, 2872, 1740, 1156, 1098, 1021, 556, 479; HRMS (ESI) Calcd. for $C_{10}H_{17}O_3$ $(M+H)^+$: 185.1178; Found: 185.1175;

4-hydroxybut-2-yn-1-yl heptanoate



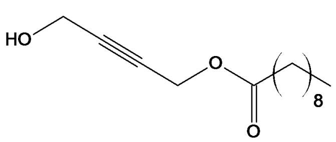
The compound was a colorless oil: Rf = 0.34 (Silica TLC, 33% EtOAc/petroleum ether); 1 H NMR (300 MHz, CDCl3) δ 0.89 (t, 3H), 1.30 (m, 8H), 1.63 (m, 2H), 2.34 (m, 2H), 4.30 (s, 2H), 4.71 (s, 2H), 13C NMR-APT (75 MHz, CDCl3) δ 13.99, 22.45, 24.76, 28.73, 31.39, 34.04, 50.95, 52.10, 79.82, 84.93, 173.27; IR (neat, cm-1) 3425, 2956, 2930, 2859, 1740, 1417, 1379, 1155, 1020, 727; HRMS (ESI) Calcd. for $C_{11}H_{19}O_3$ $(M+H)^+$: 199.1334; Found: 199.1334.

4-hydroxybut-2-yn-1-yl octanoate



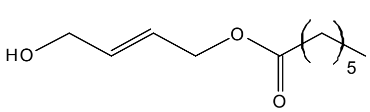
The compound was a colorless oil: Rf = 0.31 (Silica TLC, 33% EtOAc/petroleum ether); 1 H NMR (300 MHz, CDCl3) δ 0.89 (t, 3H), 1.28 (m, 8H), 1.62(m, 2H), 2.34 (m, 2H), 4.30 (s, 2H), 4.71 (s, 2H), 6.01 (brs, 1H); 13C NMR-APT (75 MHz, CDCl3) δ 14.02, 22.56, 24.79, 28.86, 29.01, 31.61, 33.98, 50.83, 52.13, 79.76, 84.92, 173.35; IR (neat, cm-1) 3425, 2927, 2857, 1741, 1455, 1379, 1224, 1150, 1022, 972, 724; HRMS (ESI) Calcd. for $C_{12}H_{21}O_3$ $(M+H)^+$: 213.1491; Found: 213.1491.

4-hydroxybut-2-yn-1-yl decanoate



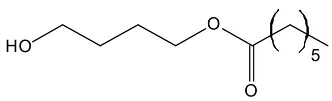
The compound was a colorless oil: Rf = 0.31 (Silica TLC, 33% EtOAc/petroleum ether); 1 H NMR (300 MHz, CDCl3) δ 0.88 (t, 3H), 1.26 (m, 12H), 1.63(m, 2H), 2.34 (m, 2H), 4.30 (s, 2H), 4.71 (s, 2H), 6.01 (brs, 1H); 13C NMR-APT (75 MHz, CDCl3) δ 14.07, 22.64, 24.79, 29.21, 29.23, 29.38, 29.68, 31.84, 34.10, 50.87, 52.11, 80.76, 84.93, 173.29; IR (neat, cm-1) 2924, 2854, 1743, 1711, 1378, 1149, 1024, 722; HRMS (ESI) Calcd. for $C_{14}H_{25}O_3$ $(M+H)^+$: 241.1804; Found: 241.1804.

(2E)-4-hydroxybut-2-en-1-yl heptanoate



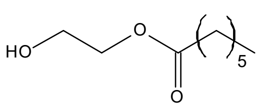
The compound was a colorless oil: Rf = 0.29 (Silica TLC, 33% EtOAc/petroleum ether); 1 H NMR (300 MHz, CDCl3) δ 0.89 (t, 3H), 1.30 (m, 6H), 1.61 (m, 2H), 2.30 (t, 2H), 3.15 (brs, 1H), 4.25(d, 2H), 4.68 (d, 2H), 5.61 (m, 1H), 5.83 (m, 1H); 13C NMR-APT (75 MHz, CDCl3) δ 13.95, 22.41, 24.82, 28.8, 31.3, 34.16, 58.19, 59.9, 125.42, 133.31, 174.01 ; IR (neat, cm-1) 3443, 2929, 2859, 1734, 1458, 1164, 1102, 1026, 726, HRMS (ESI) Calcd. for $C_{11}H_{21}O_3$ $(M+H)^+$: 201.1491; Found: 201.1491.

4-hydroxybutyl heptanoate



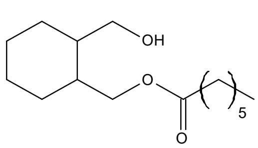
The compound was a colorless oil: Rf = 0.33 (Silica TLC, 33% EtOAc/petroleum ether); 1 H NMR (300 MHz, CDCl3) δ 0.88 (t, 3H), 1.30 (m, 6H), 1.67 (m, 6H), 2.30 (t, 2H), 3.68(d, 2H), 4.09 (d, 2H); 13C NMR-APT (75 MHz, CDCl3) δ 14.0, 22.4, 24.93, 25.32, 28.74, 28.80, 31.4, 34.36, 62.29, 64.06, 174.08; IR (neat, cm-1) 3455, 2956, 2930, 2859, 1734, 1460, 1234, 1167, 1102, 1040, 727; HRMS (ESI) Calcd. for $C_{11}H_{23}O_3$ $(M+H)^+$: 203.1647; Found: 203.1647.

2-hydroxyethyl heptanoate



The compound was a colorless oil: Rf = 0.31 (Silica TLC, 33% EtOAc/petroleum ether); 1 H NMR (300 MHz, CDCl3) δ 0.90 (t, 3H), 1.30 (m, 6H), 1.67 (m, 2H), 2.35 (t, 2H), 2.65 (brs, 1H) 3.81(m, 2H), 4.20 (m, 2H); 13C NMR-APT (75 MHz, CDCl3) δ 13.95, 22.4, 24.8, 28.7, 31.4, 34.16, 61.04, 65.8, 174.3, (neat, cm-1) 3455, 2930, 1734, 1234, 1167, 727; HRMS (ESI) Calcd. for $C_9H_{19}O_3$ $(M+H)^+$: 175.1334; Found: 175.1334.

[2-(hydroxymethyl)cyclohexyl]methyl heptanoate



The compound was a colorless oil: Rf = 0.35 (Silica TLC, 33% EtOAc/petroleum ether); 1 H NMR (300 MHz, CDCl3) δ 0.81 (t, 3H), 1.18-1.56 m(16H), 1.8 (m, 1H), 2.02(m, 1H), 2.23(t, 2H), 3.43-

3.57 (m, 2H), 3.96(m, 1H), 4.08(m, 1H) 13C NMR-APT (75 MHz, CDCl3) δ 13.97, 22.45, 22.85, 24.07, 24.91, 25.64, 27.03, 28.78, 31.41, 34.41, 35.58, 40.50, 63.67, 64.40, 174.13; IR (neat, cm-1) 3444, 2925, 2857, 1734, 1395, 1168, 1101, 726; HRMS (ESI) Calcd. for $C_{15}H_{29}O_3$ $(M+H)^+$: 257.2117; Found: 257.2117.

2.3.4. Spectral data of the synthesized compounds:

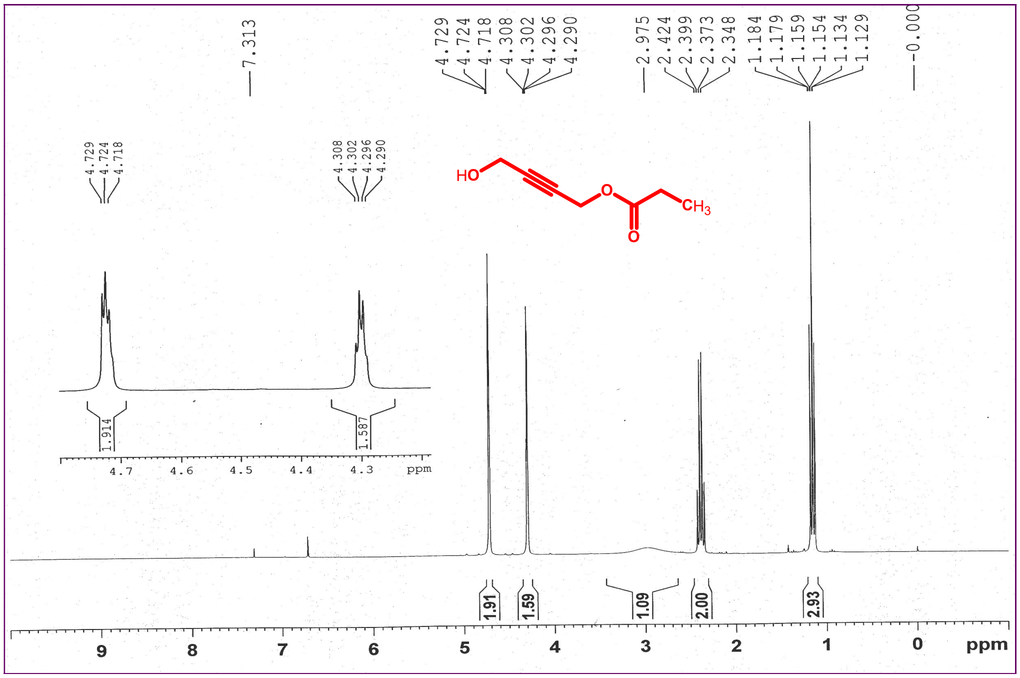


Figure 3. 1HNMR of 4-hydroxybut-2-yn-1-yl propanoate

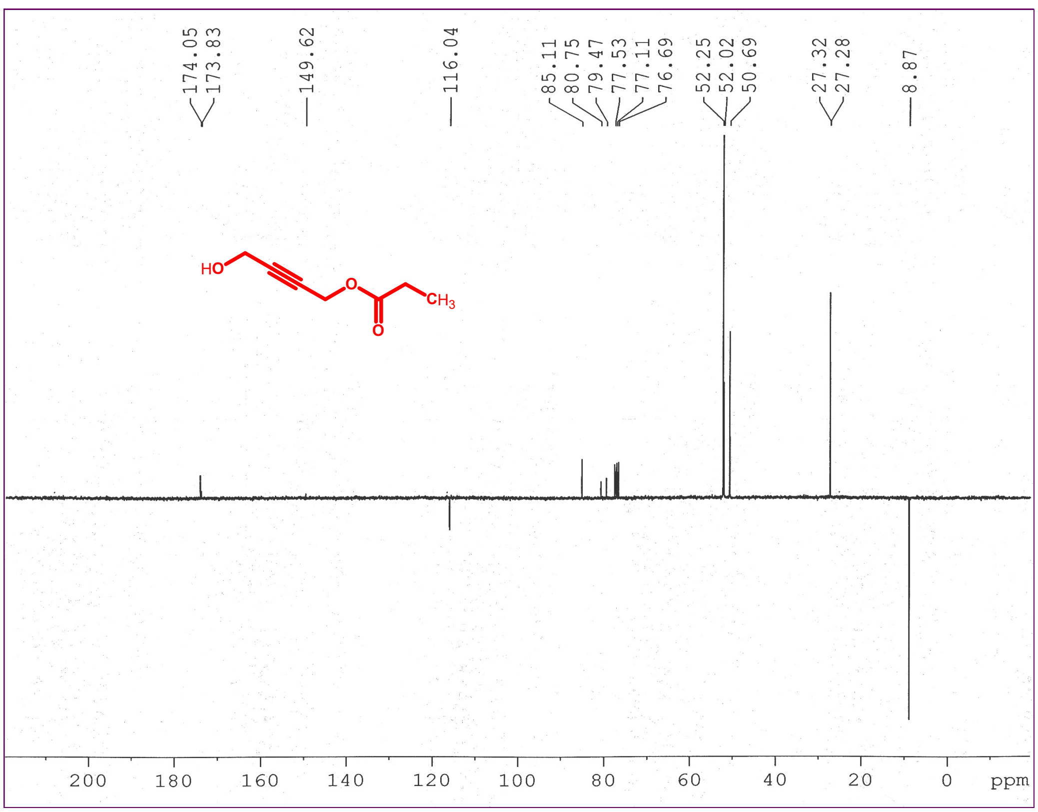


Figure 4. APT of 4-hydroxybut-2-yn-1-yl propanoate

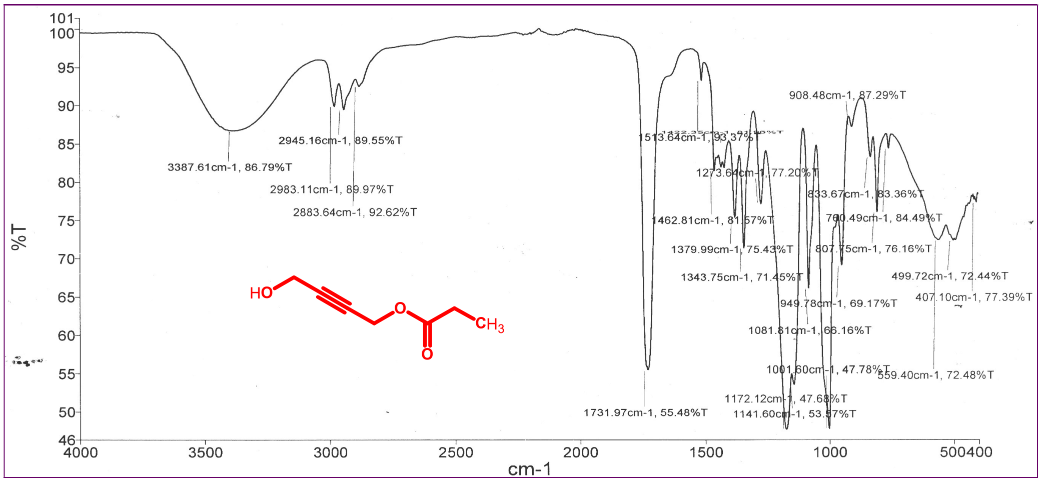


Figure 5. FTIR of 4-hydroxybut-2-yn-1-yl propanoate.

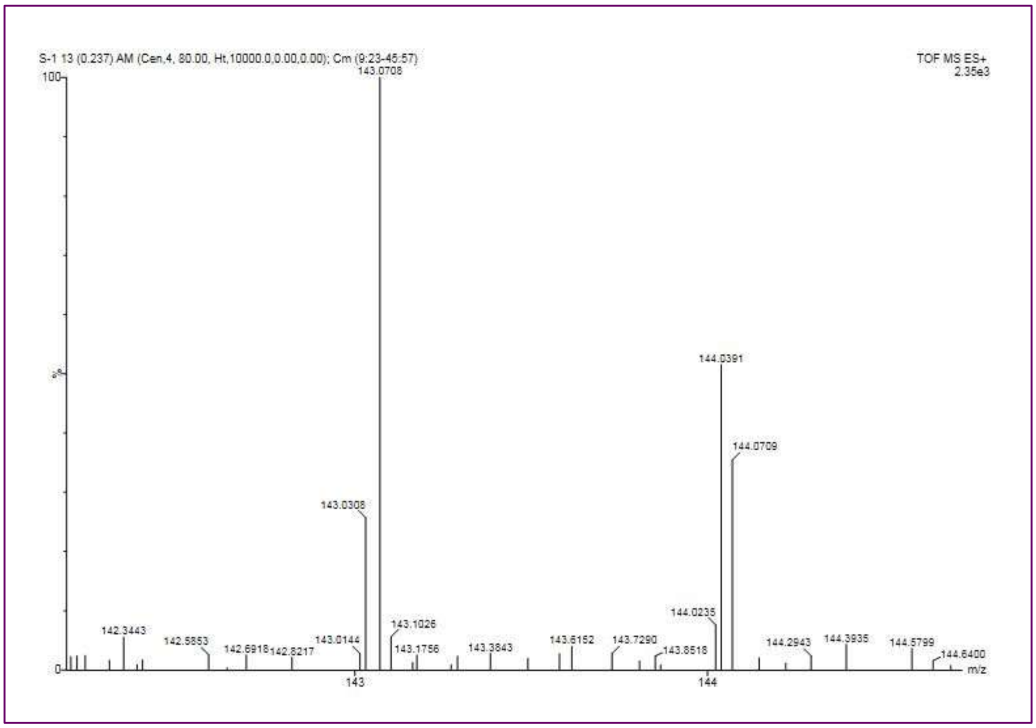


Figure 6. HR-MS spectra of 4-hydroxybut-2-yn-1-yl propanoate.

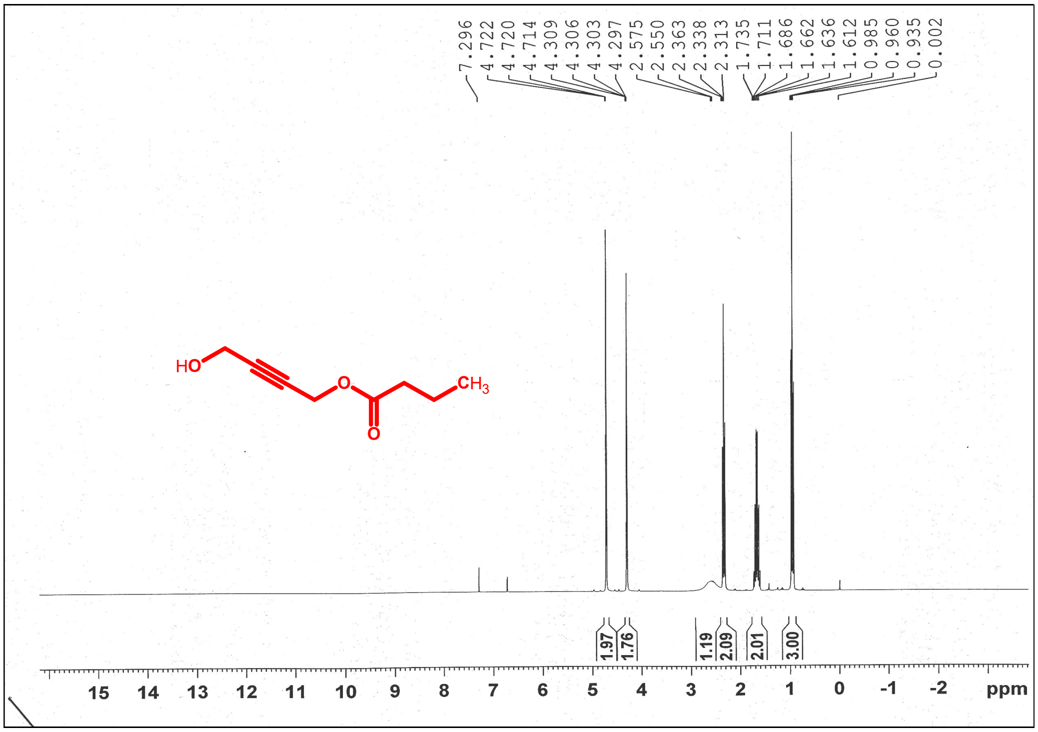


Figure 7. 1HNMR of 4-hydroxybut-2-yn-1-yl butanoate

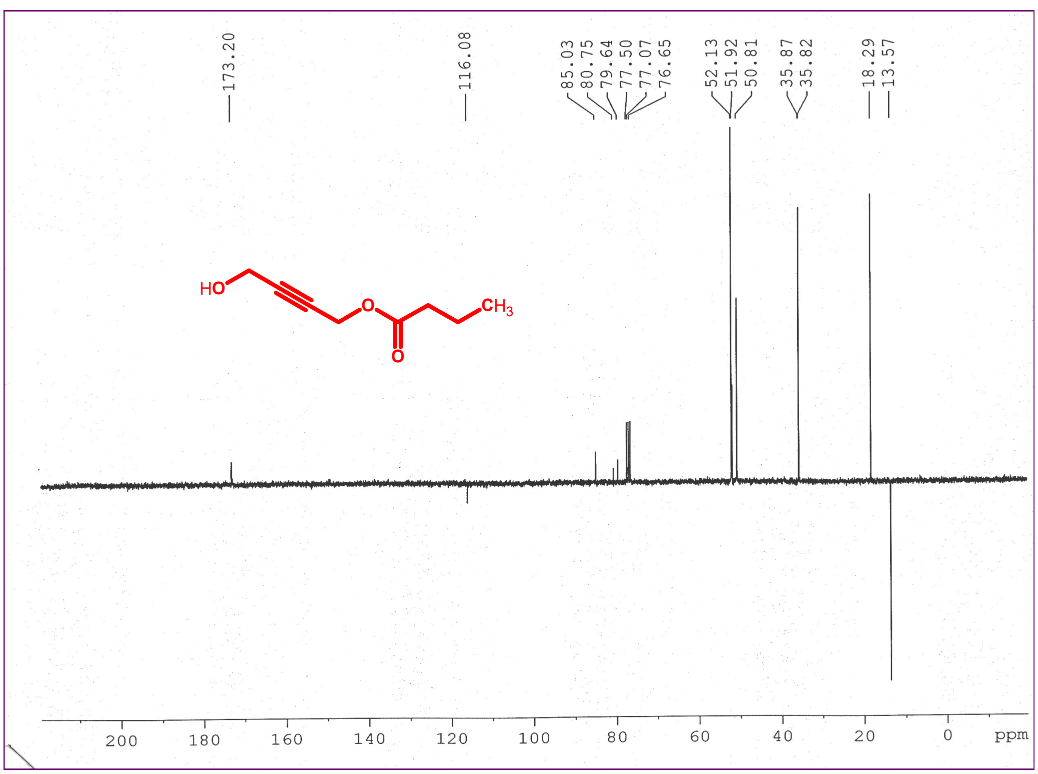


Figure 8. APT of 4-hydroxybut-2-yn-1-yl butanoate.

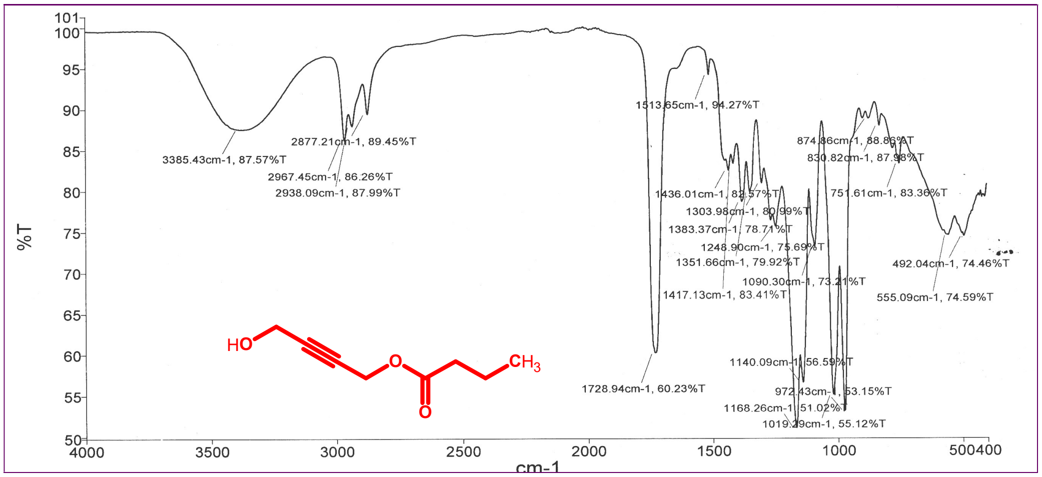


Figure 9. FTIR of 4-hydroxybut-2-yn-1-yl butanoate.

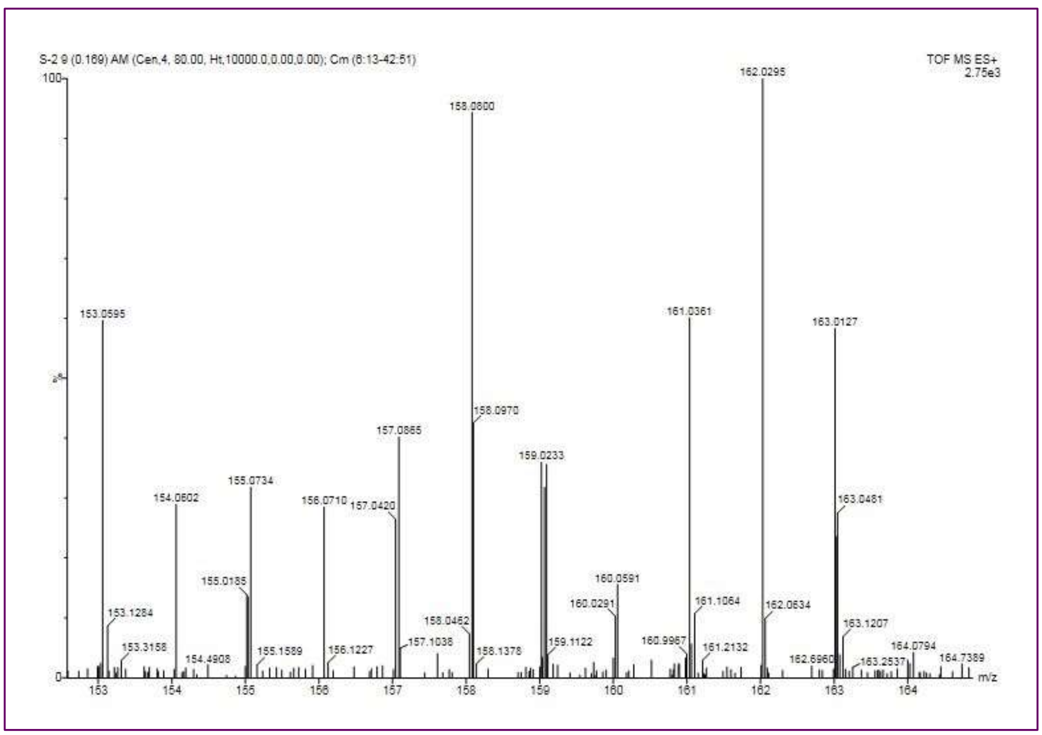


Figure 10. HR-MS spectra of 4-hydroxybut-2-yn-1-yl butanoate.

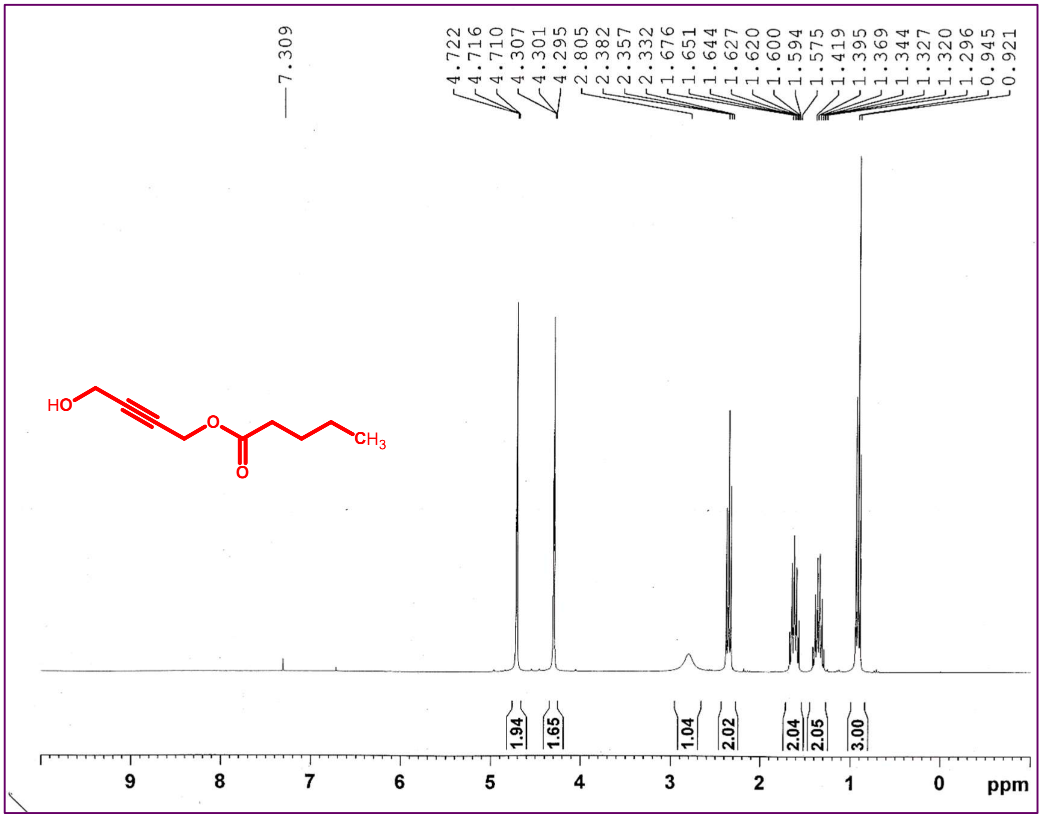


Figure 11. HNMR of 4-hydroxybut-2-yn-1-yl pentanoate.

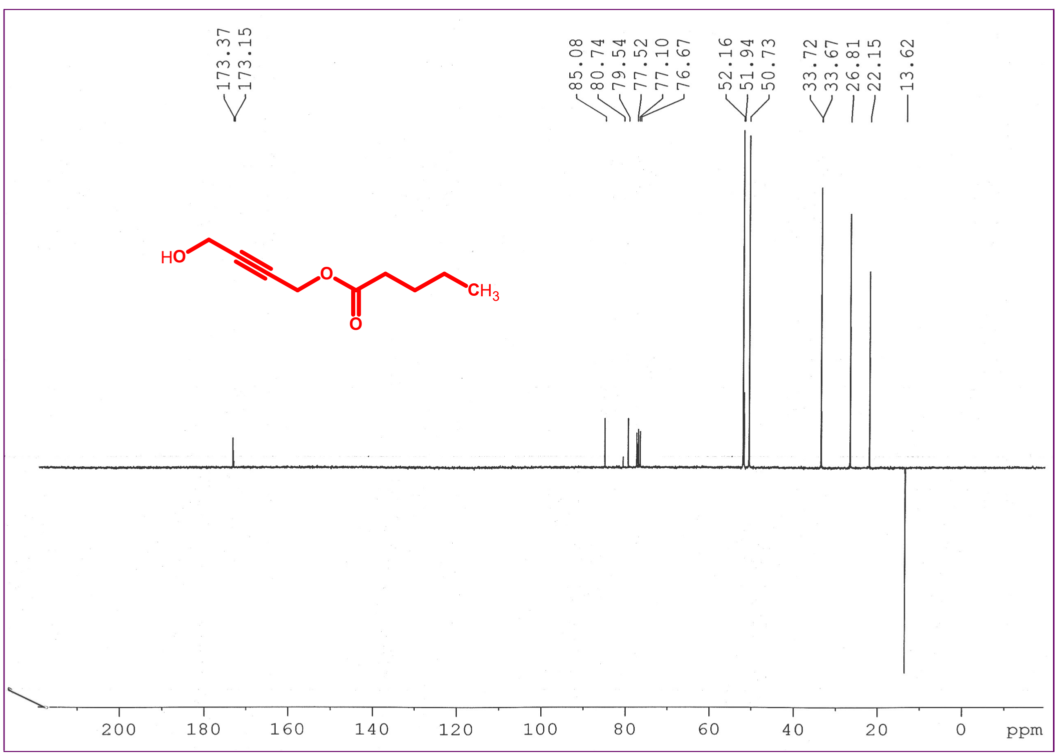


Figure 12. APT of 4-hydroxybut-2-yn-1-yl pentanoate.

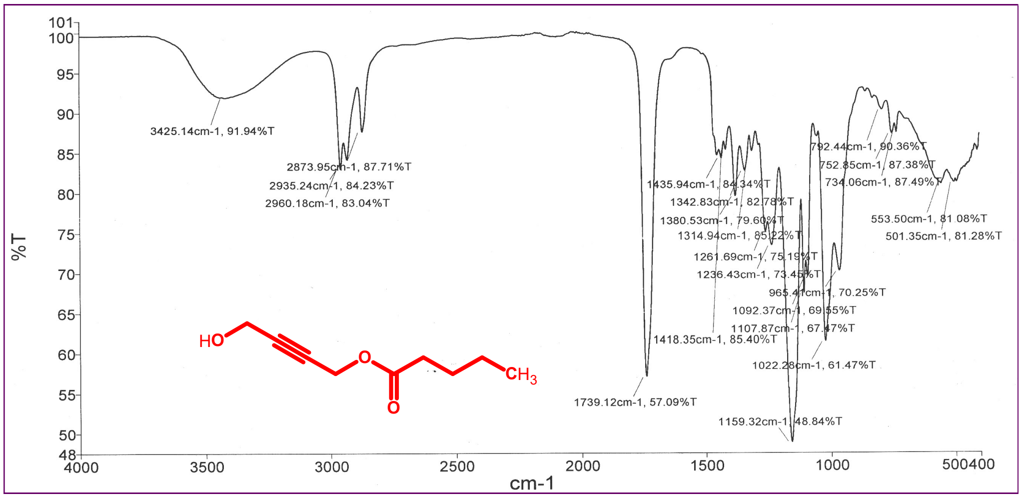


Figure 13. IR of 4-hydroxybut-2-yn-1-yl pentanoate.

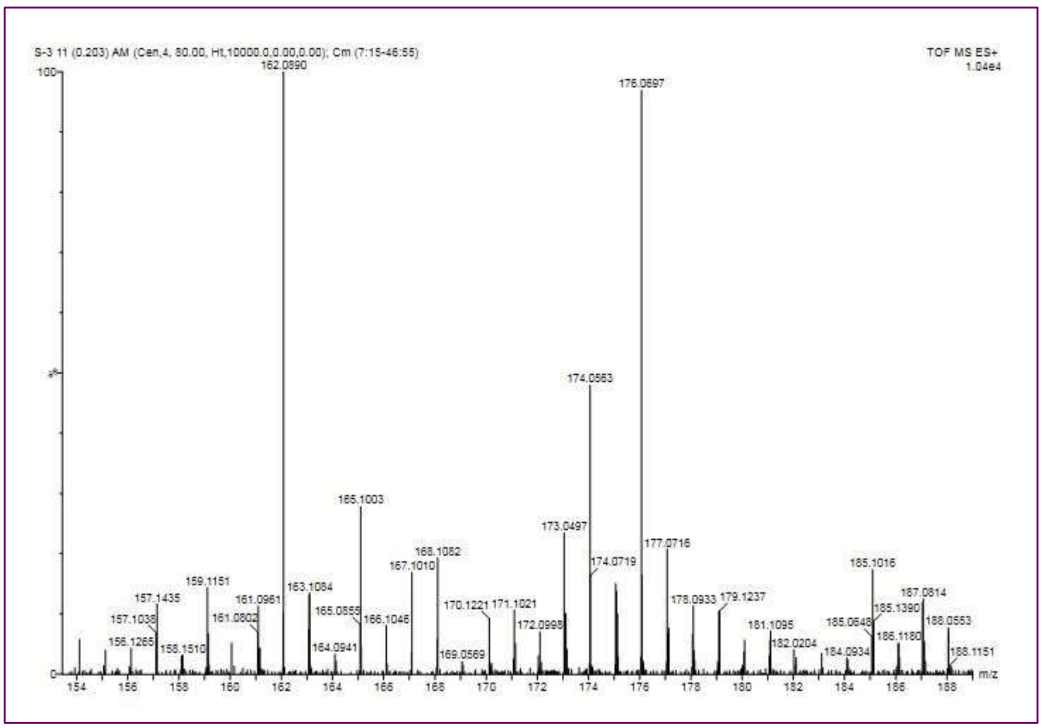


Figure 14. HR-MS spectra of 4-hydroxybut-2-yn-1-yl pentanoate.

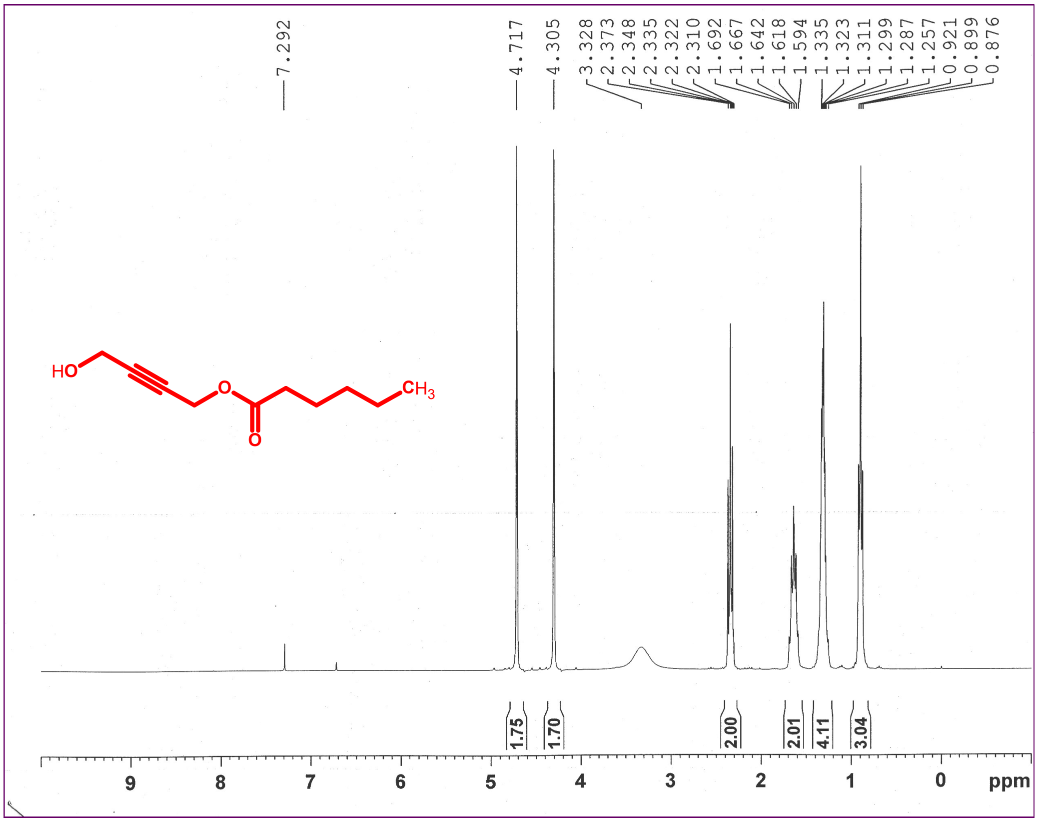


Figure 15. HNMR of 4-hydroxybut-2-yn-1-yl hexanoate.

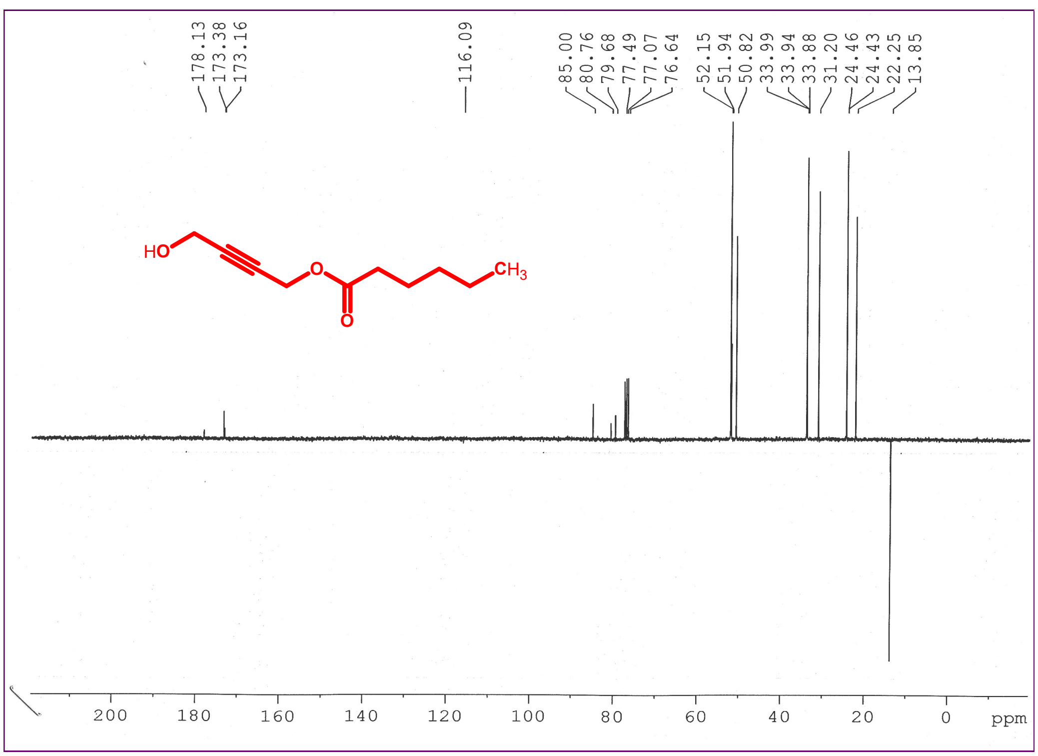


Figure 16. APT of 4-hydroxybut-2-yn-1-yl hexanoate.

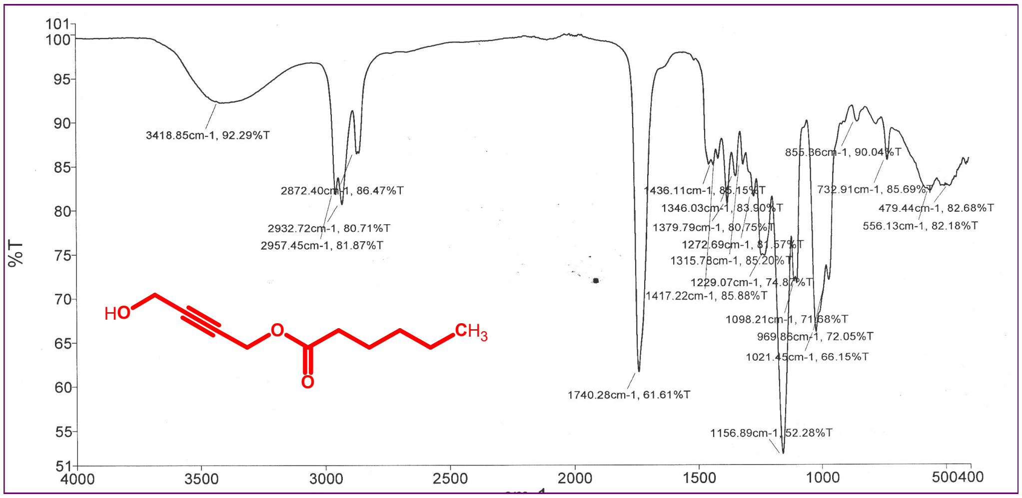


Figure 17. FTIR of 4-hydroxybut-2-yn-1-yl hexanoate.

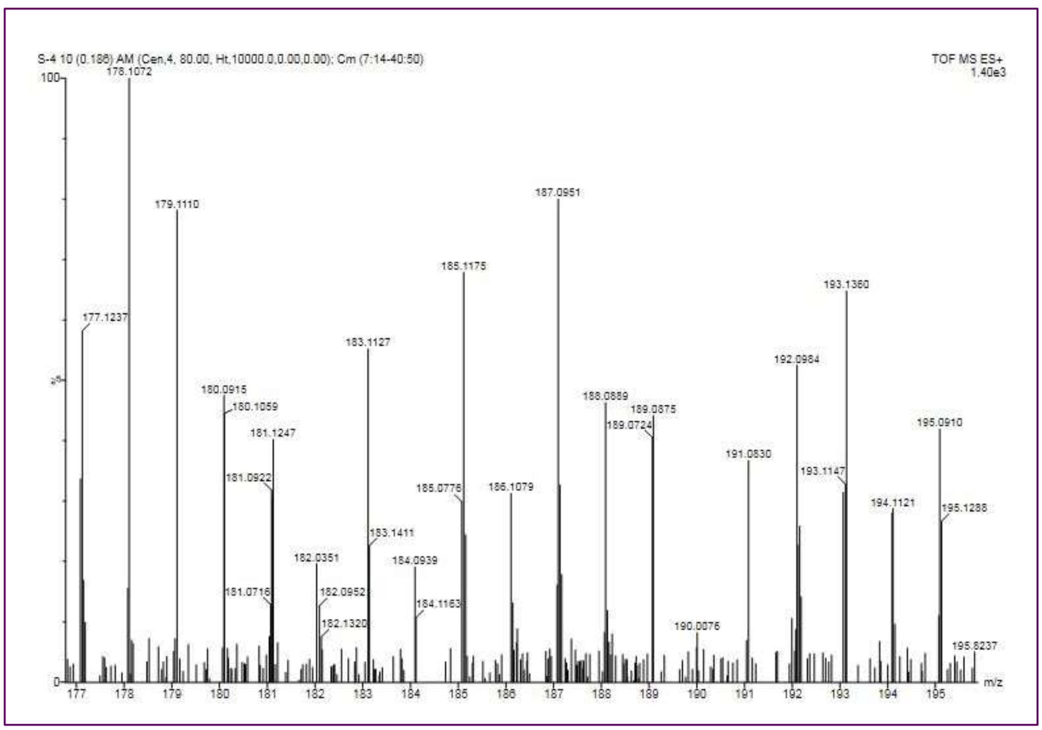


Figure 18. HR-MS spectra of 4-hydroxybut-2-yn-1-yl hexanoate.

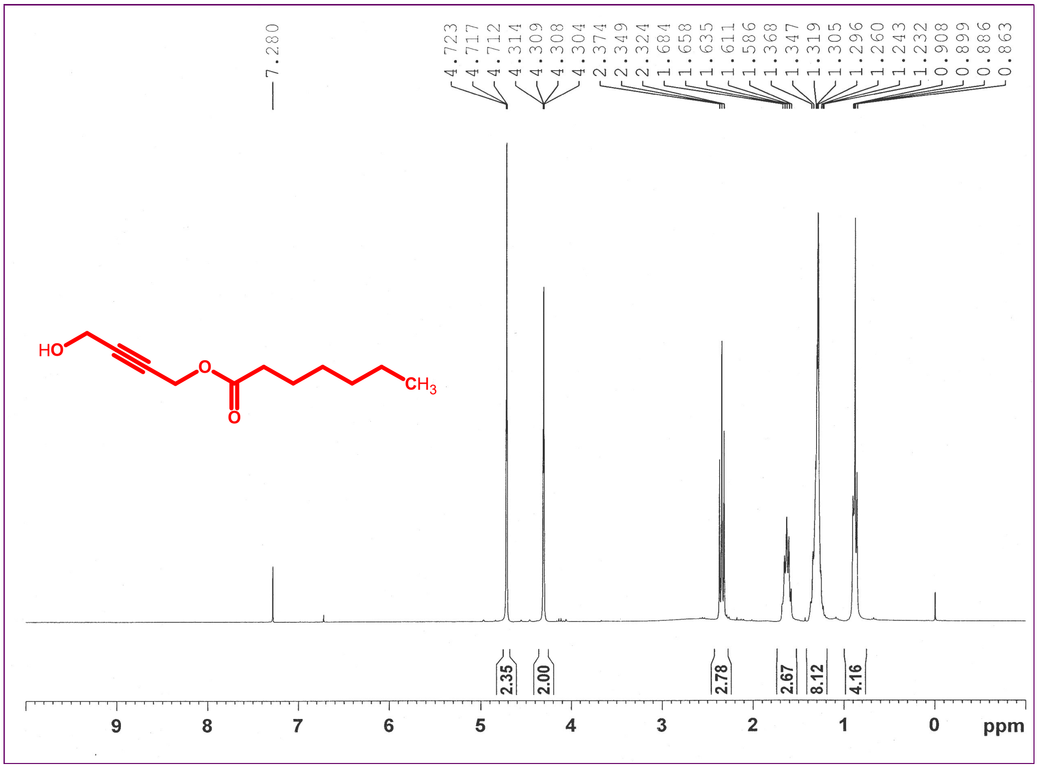


Figure 19. HNMR of 4-hydroxybut-2-yn-1-yl heptanoate.

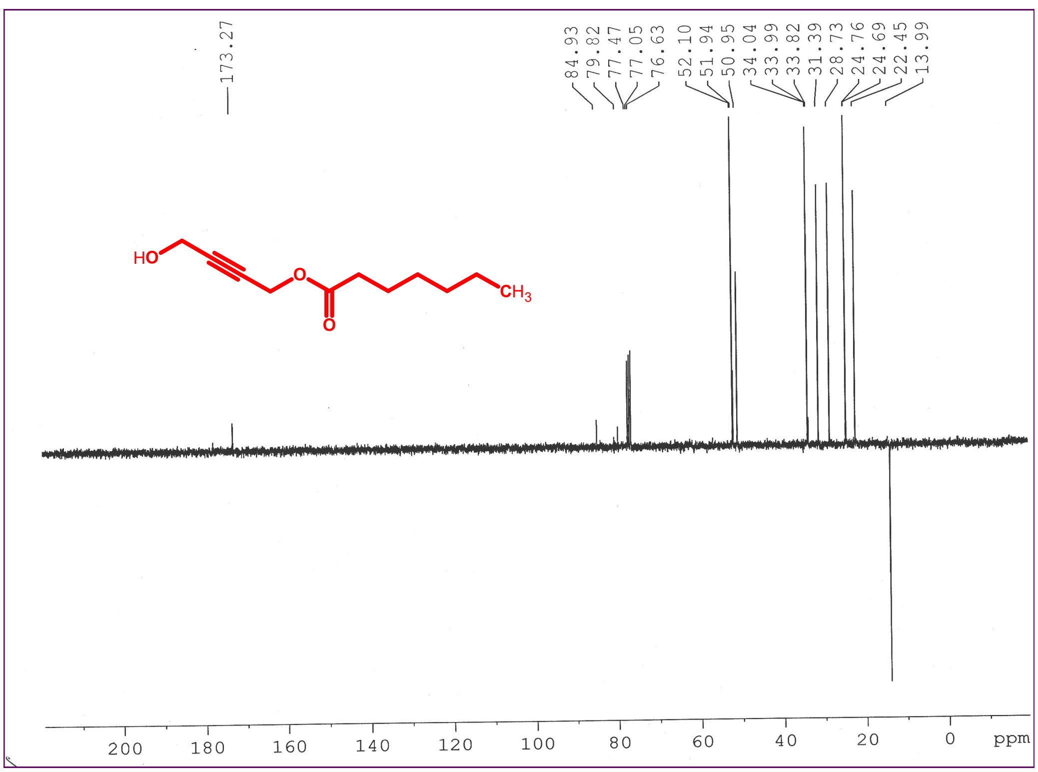


Figure 20. APT of 4-hydroxybut-2-yn-1-yl heptanoate.

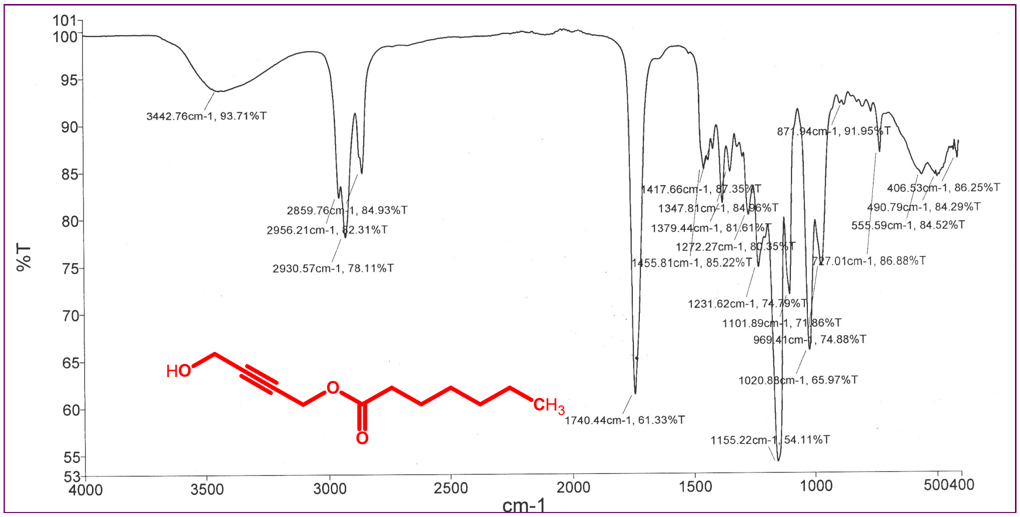


Figure 21. FTIR of 4-hydroxybut-2-yn-1-yl heptanoate.

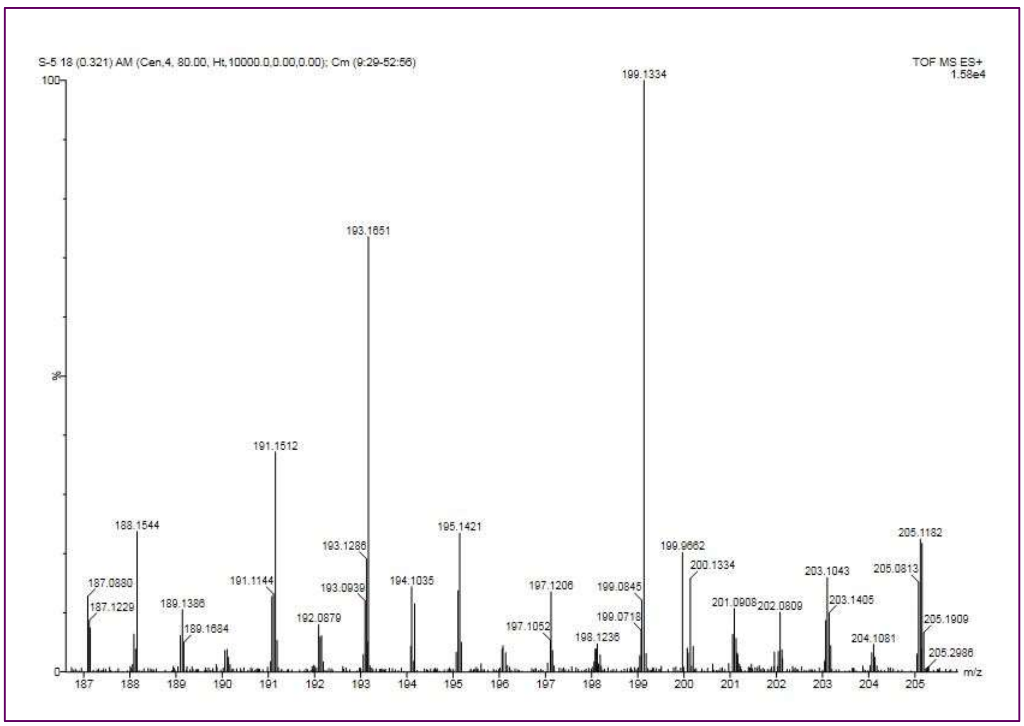


Figure 22. HR-MS spectra of 4-hydroxybut-2-yn-1-yl heptanoate.

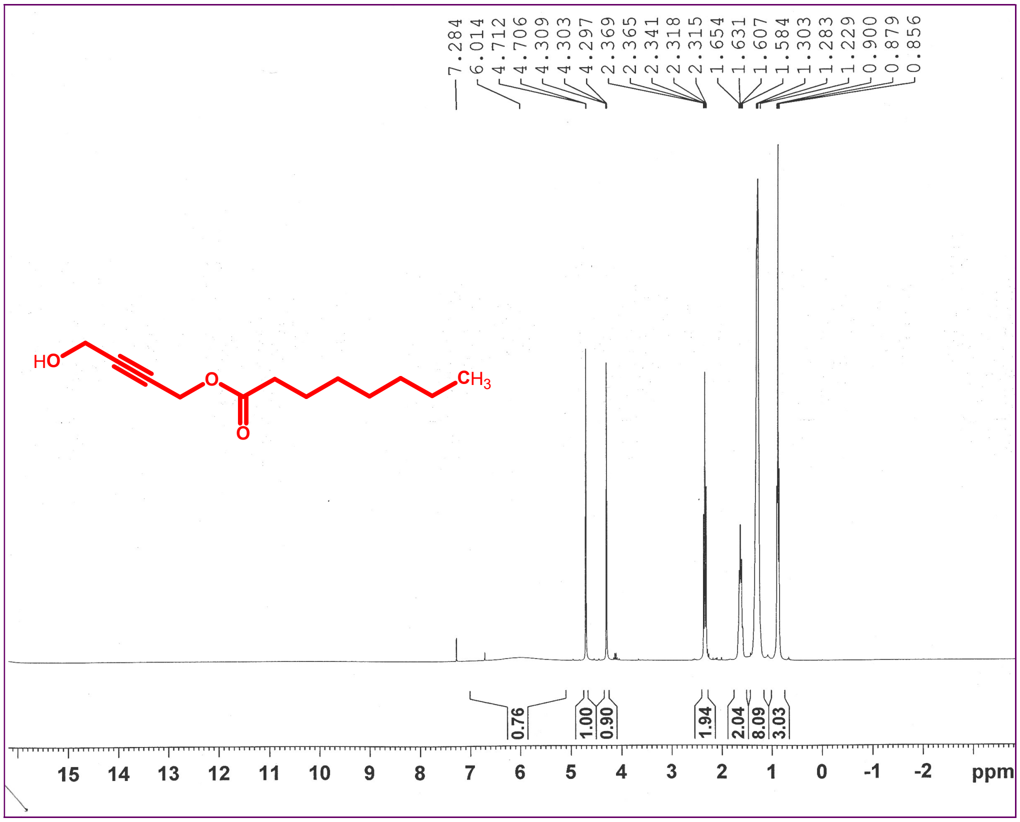


Figure 23. HNMR of 4-hydroxybut-2-yn-1-yl octanoate.

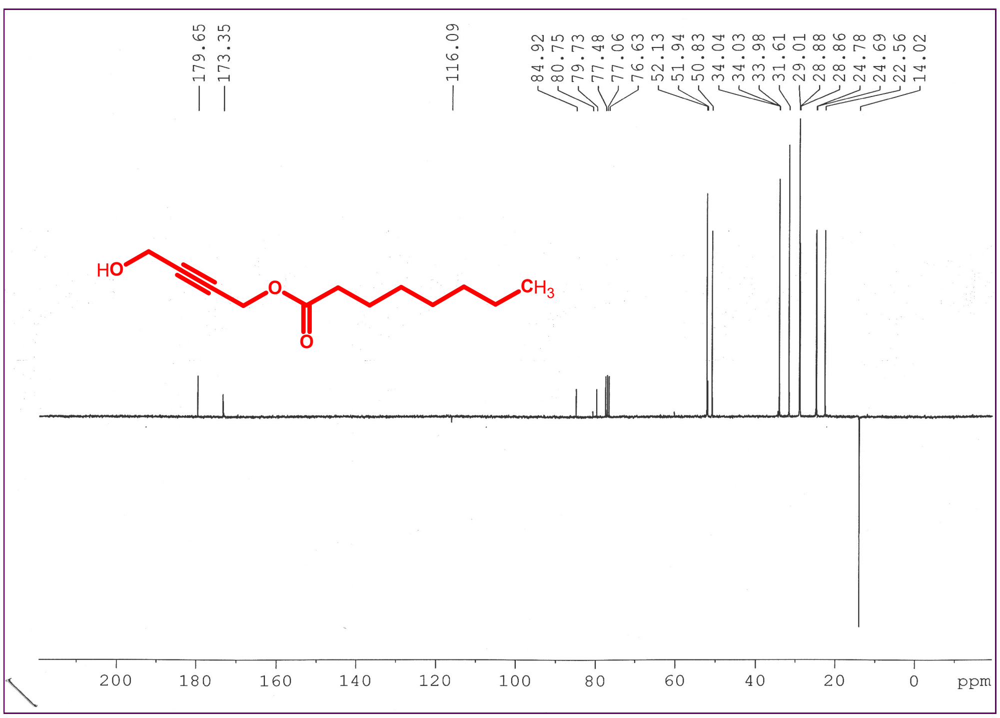


Figure 24. APT of 4-hydroxybut-2-yn-1-yl octanoate.

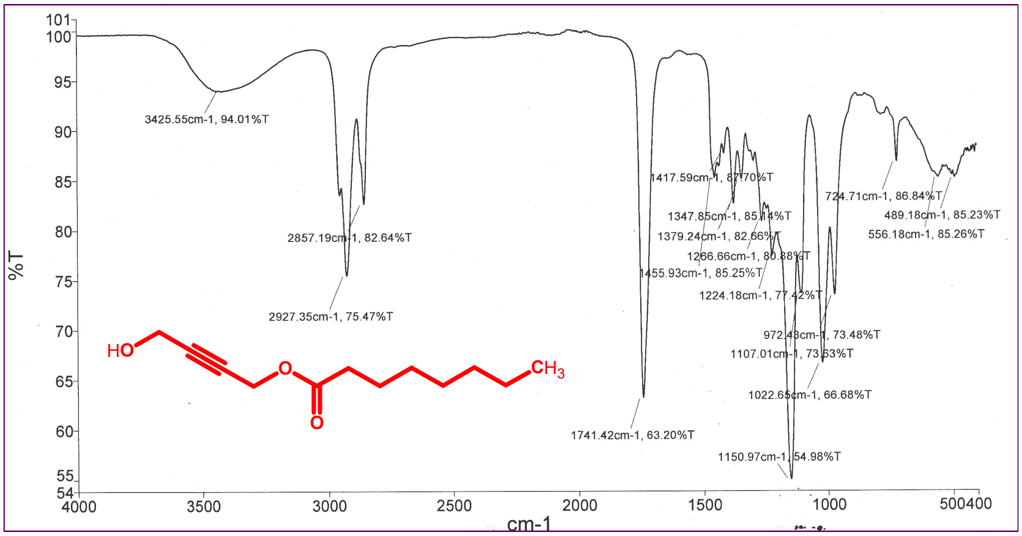


Figure 25. FTIR of 4-hydroxybut-2-yn-1-yl octanoate.

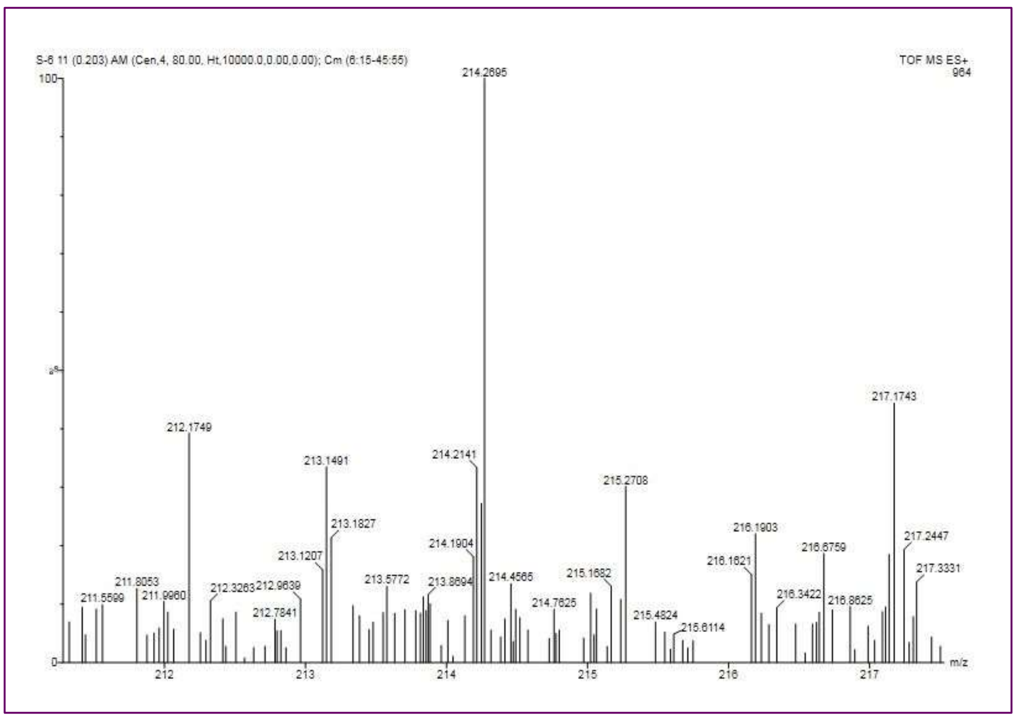


Figure 26. HR-MS spectra of 4-hydroxybut-2-yn-1-yl octanoate.

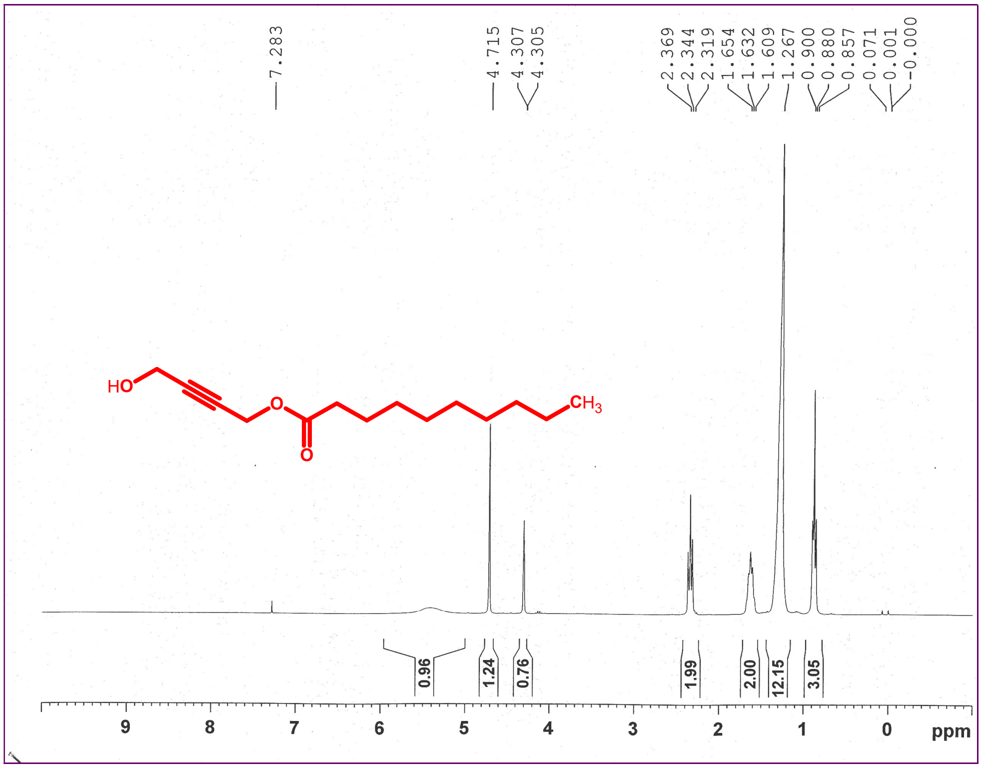


Figure 27. HNMR of 4-hydroxybut-2-yn-1-yl decanoate.

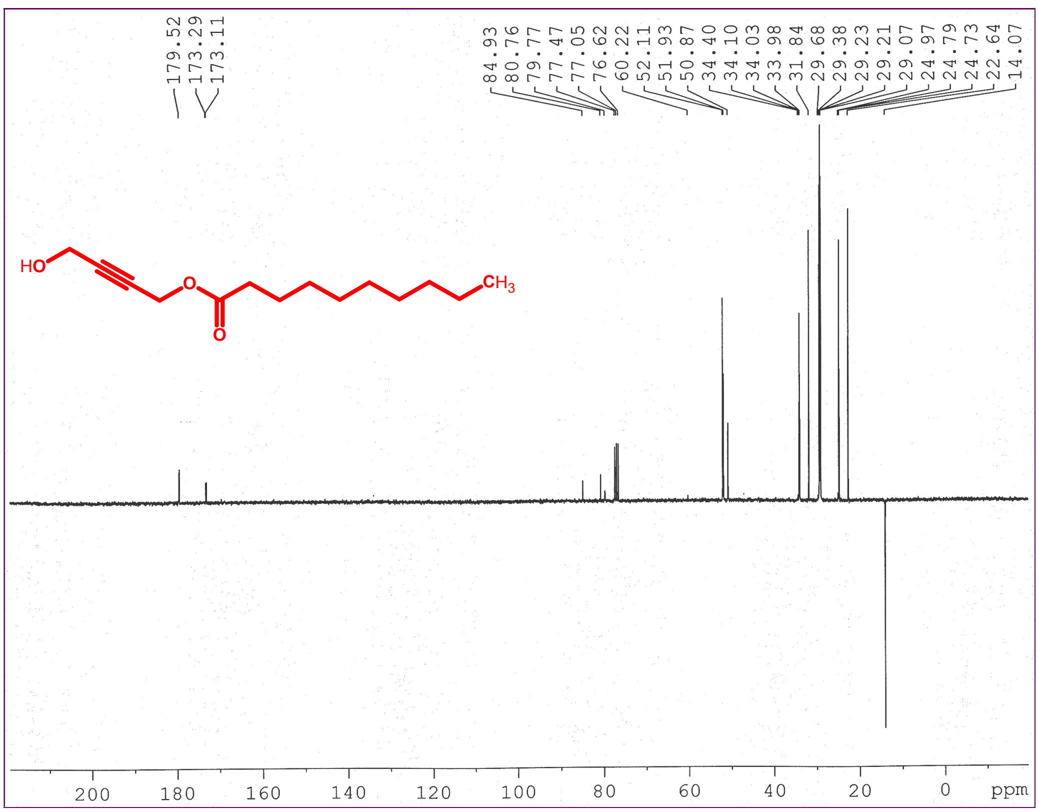


Figure 28. APT of 4-hydroxybut-2-yn-1-yl decanoate.

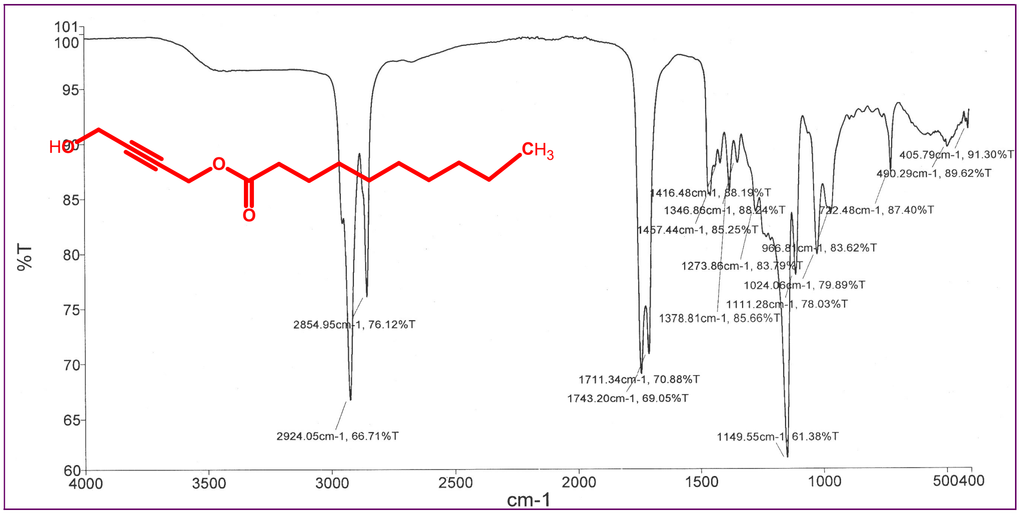


Figure 29. FTIR of 4-hydroxybut-2-yn-1-yl decanoate.

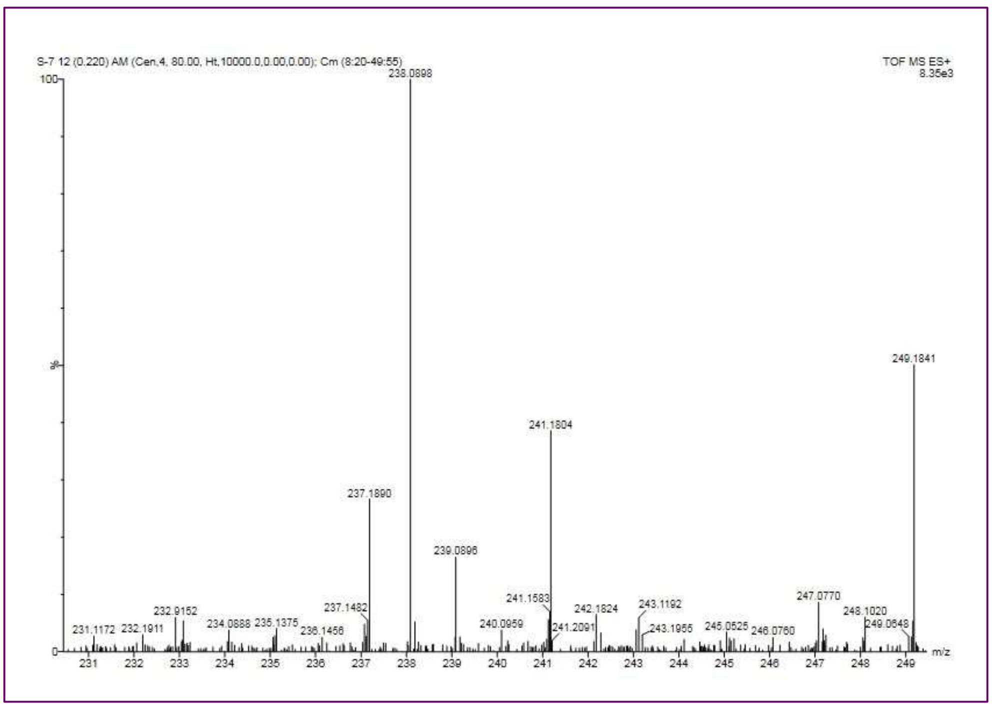


Figure 30. HR-MS spectra of 4-hydroxybut-2-yn-1-yl decanoate.

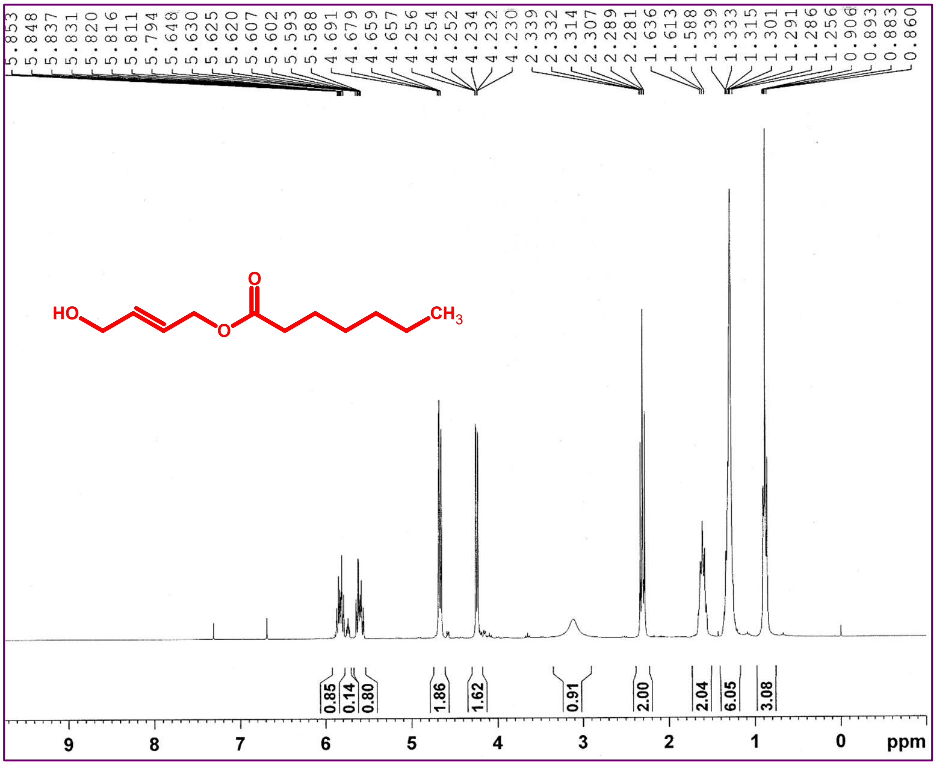


Figure 31. HNMR of (2*E*)-4-hydroxybut-2-en-1-yl heptanoate.

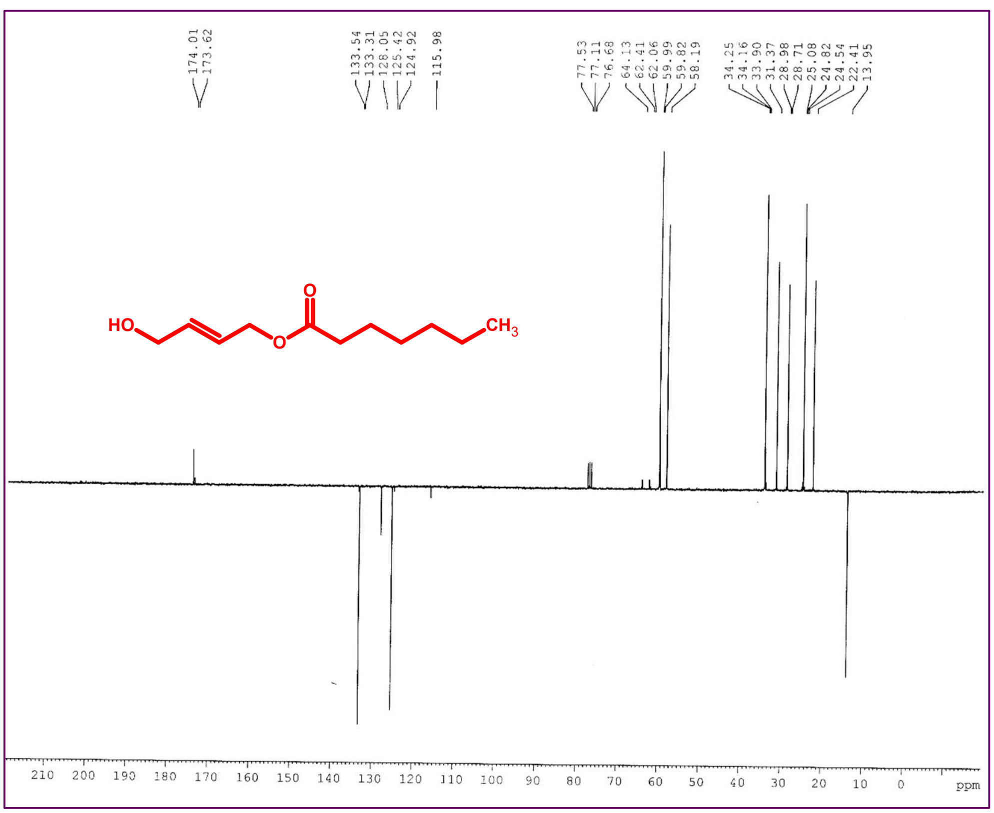


Figure 32. APT of (2*E*)-4-hydroxybut-2-en-1-yl heptanoate.

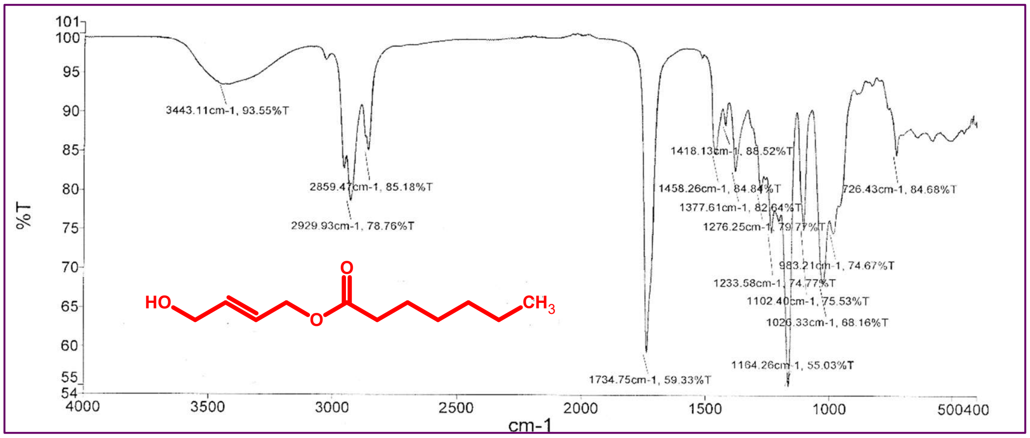


Figure 33. FTIR of (2*E*)-4-hydroxybut-2-en-1-yl heptanoate.

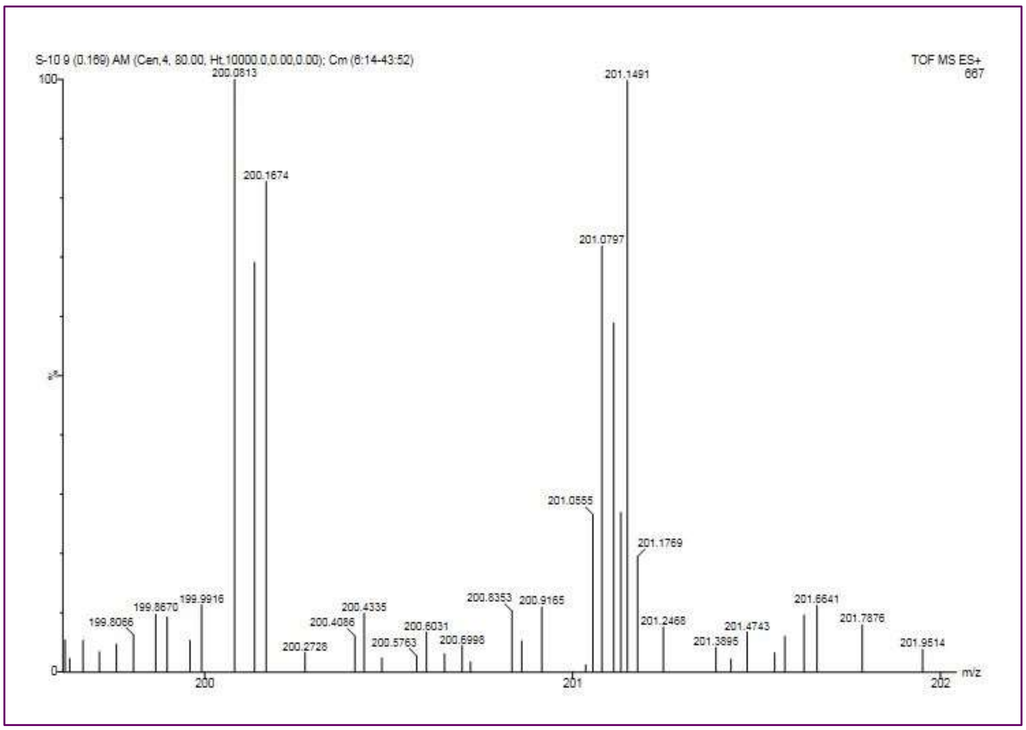


Figure 34. HR-MS spectra of (2*E*)-4-hydroxybut-2-en-1-yl heptanoate.

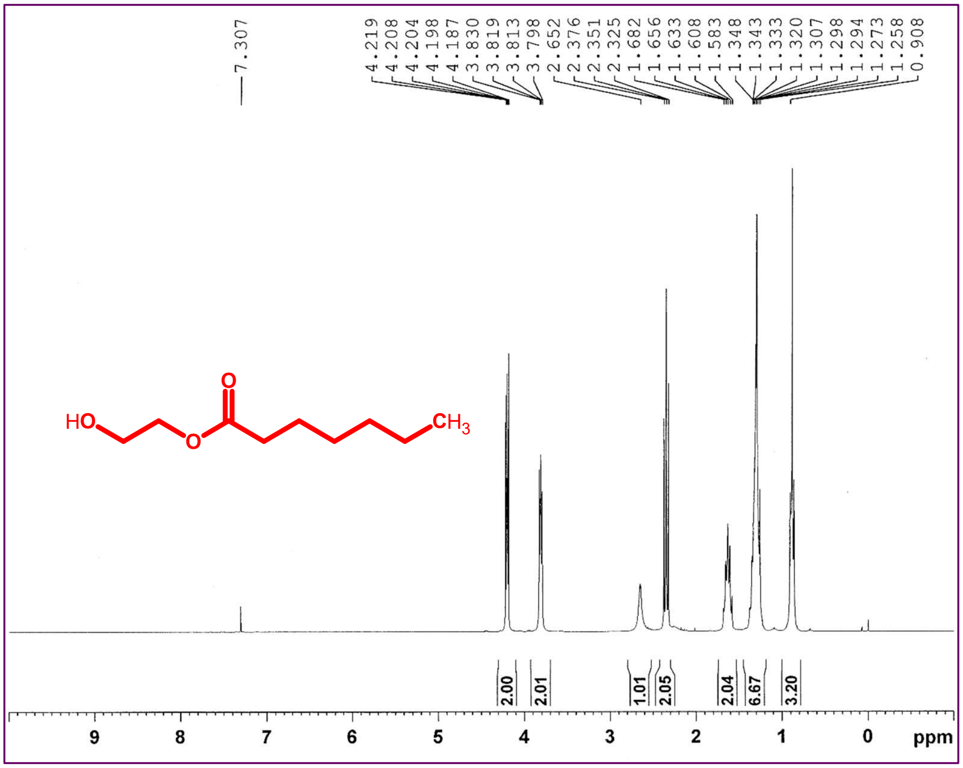


Figure 35. HNMR of 2-hydroxyethyl heptanoate.

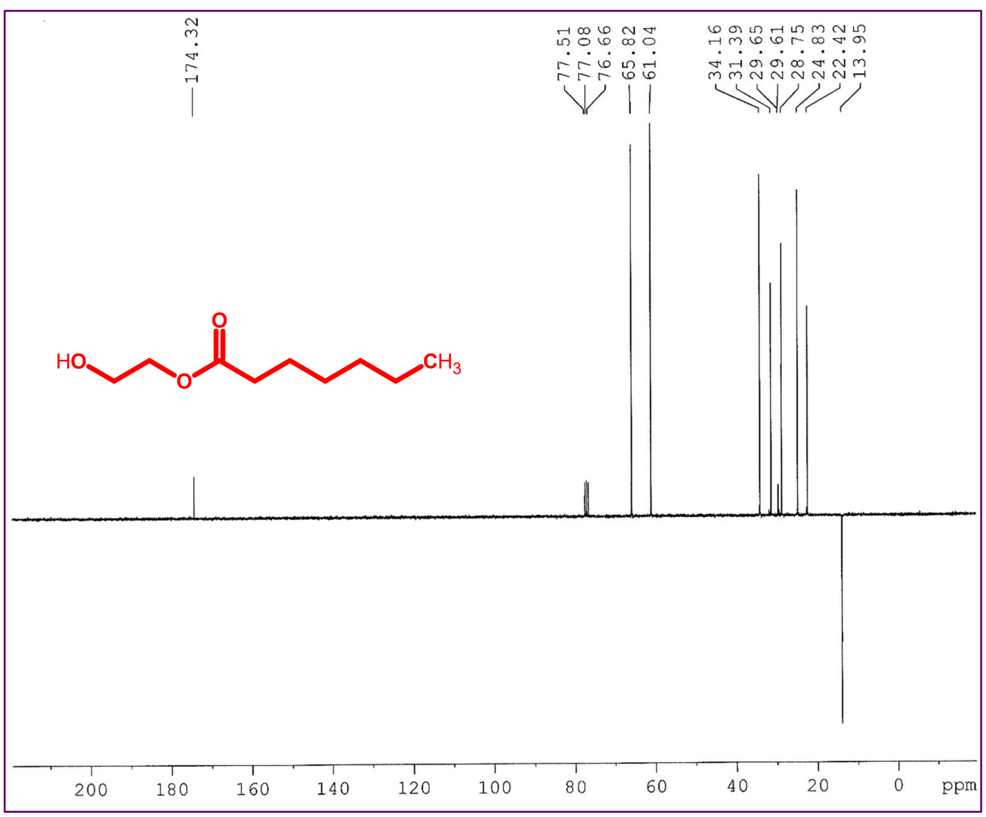


Figure 36. APT of 2-hydroxyethyl heptanoate.

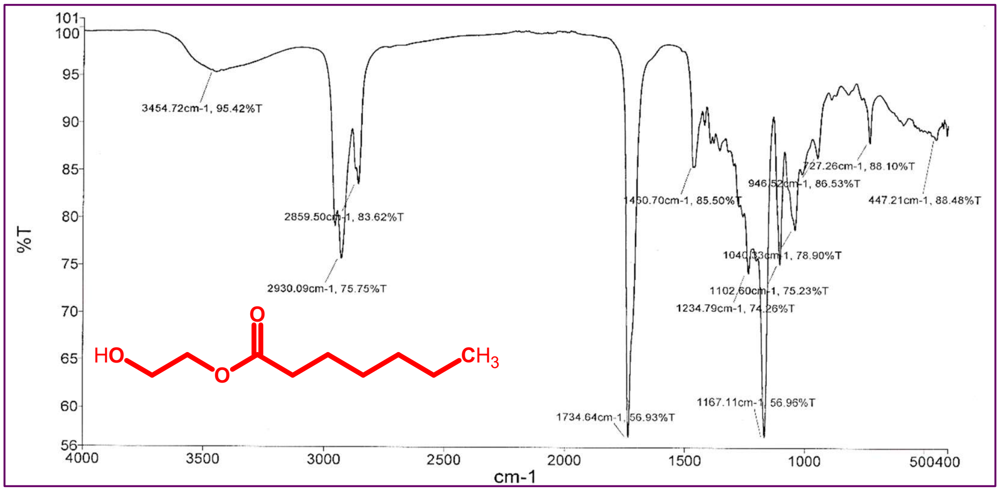


Figure 37. FTIR of 2-hydroxyethyl heptanoate.

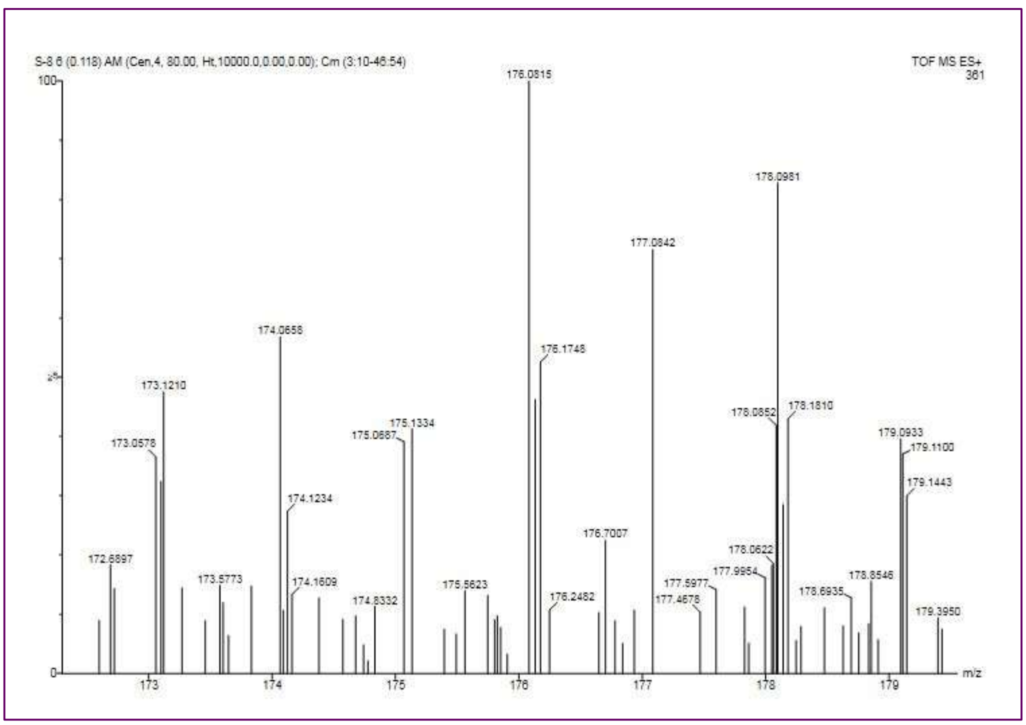


Figure 38. HR-MS spectra of 2-hydroxyethyl heptanoate.

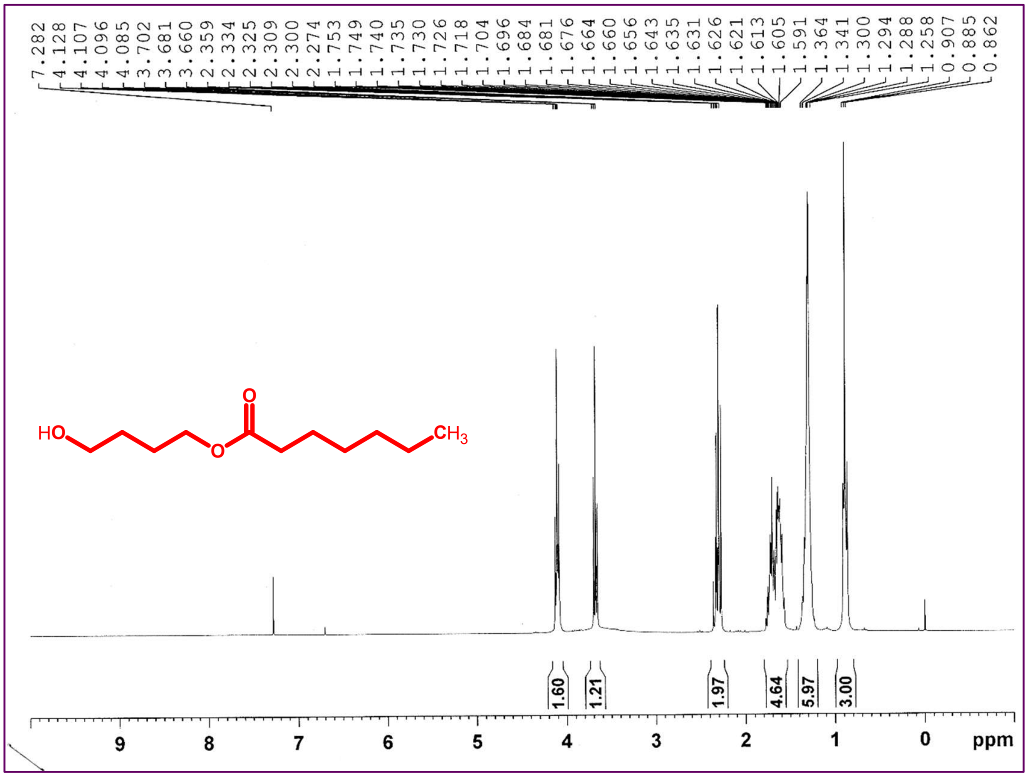


Figure 39. HNMR of 4-hydroxybutyl heptanoate.

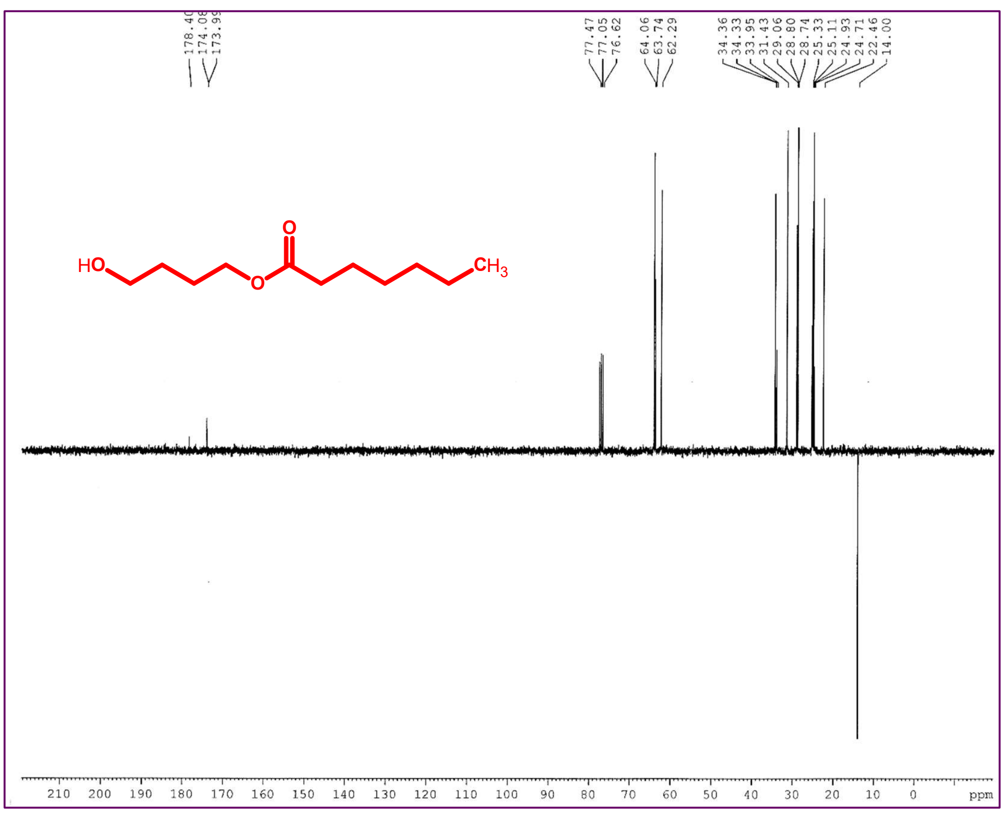


Figure 40. APT of 4-hydroxybutyl heptanoate.

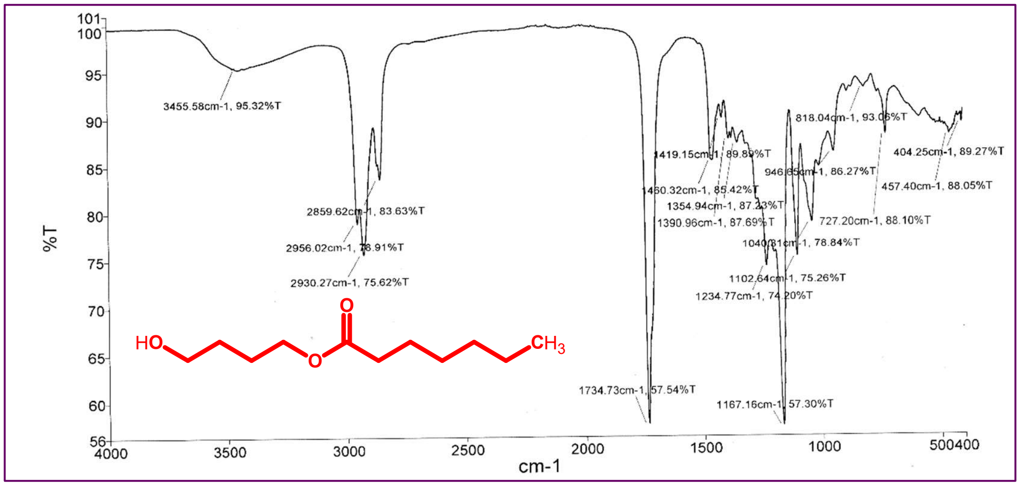


Figure 41: IR of 4-hydroxybutyl heptanoate

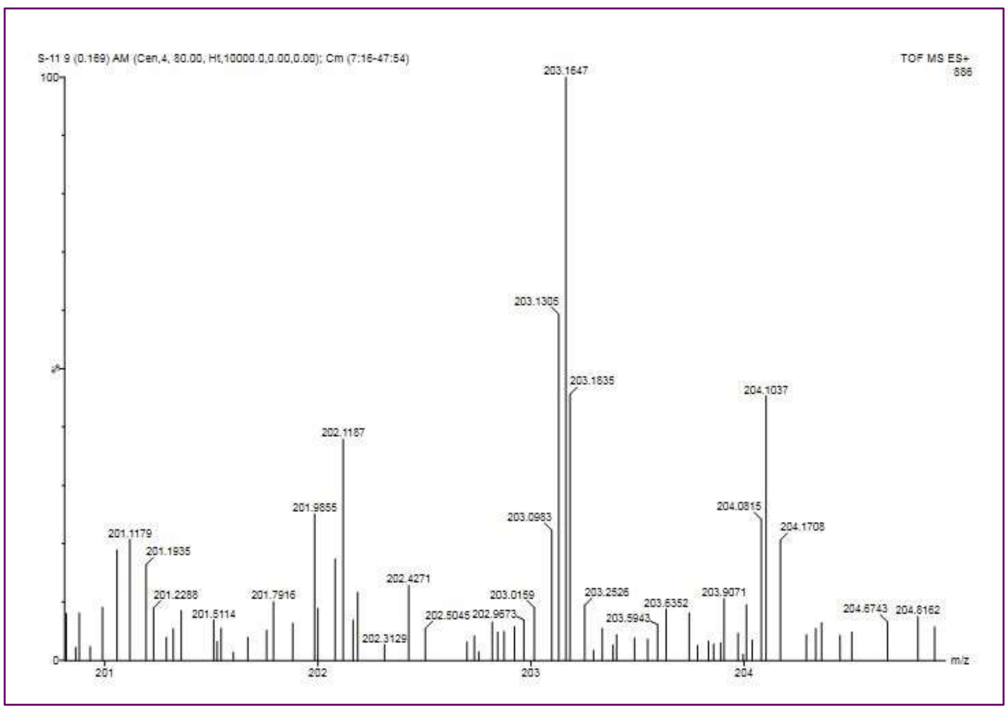


Figure 42: HR-MS spectra of 4-hydroxybutyl heptanoate

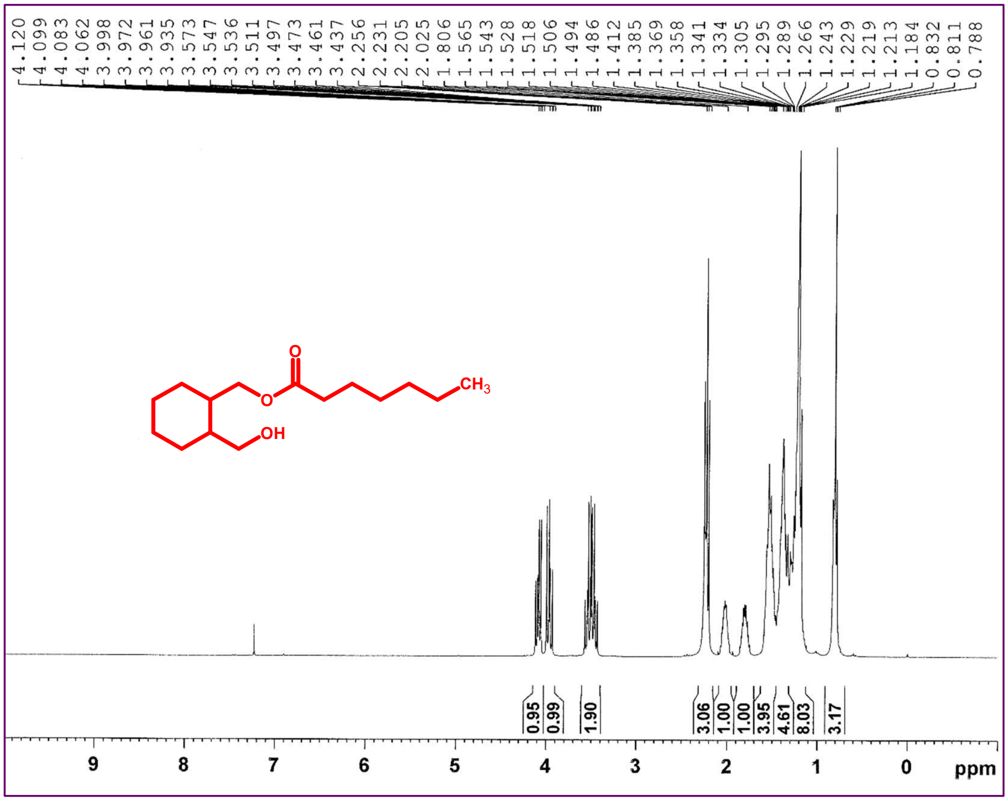


Figure 43: HNMR of [2-(hydroxymethyl)cyclohexyl]methyl heptanoate

Figure 44: APT of [2-(hydroxymethyl)cyclohexyl]methyl heptanoate

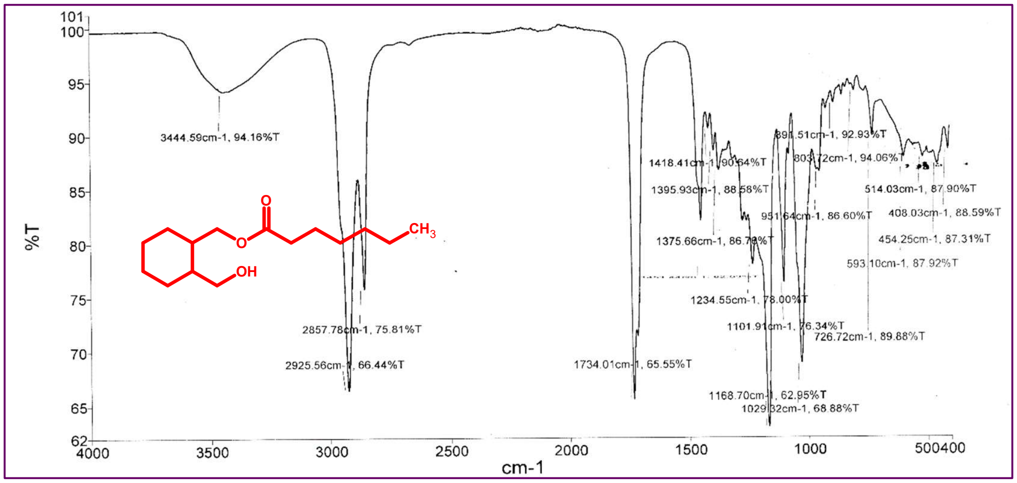


Figure 45: IR of [2-(hydroxymethyl)cyclohexyl]methyl heptanoate

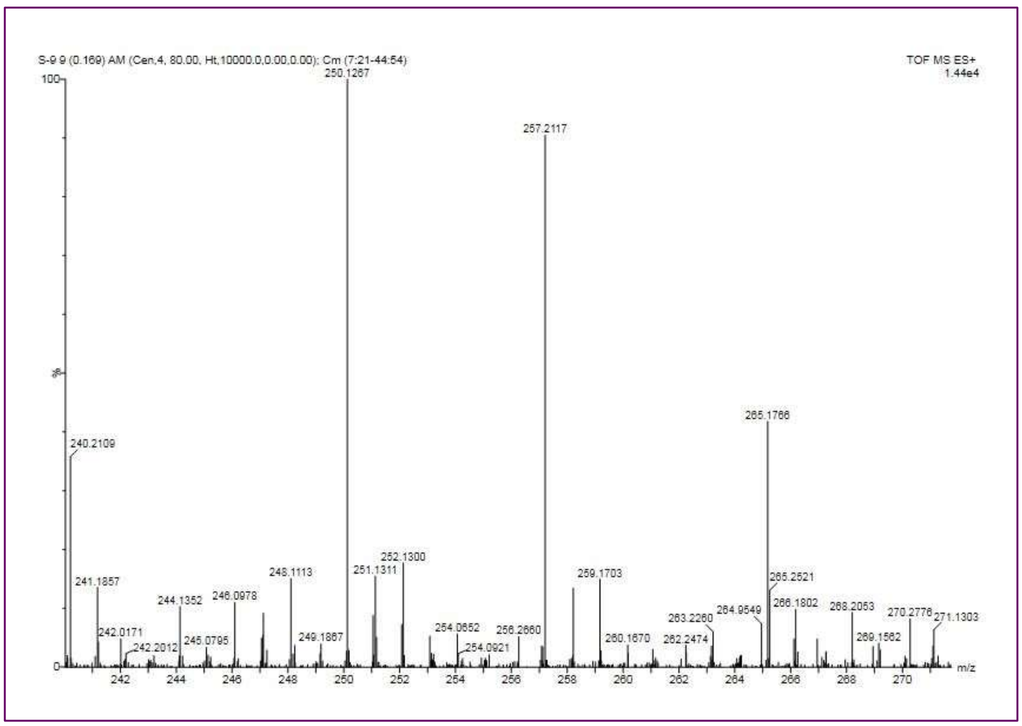


Figure 46: HR-MS spectra of [2-(hydroxymethyl)cyclohexyl]methyl heptanoate

2.4 Conclusion

Through this study we demonstrated that lipase category of enzymes like CALB can be used for esterification reaction too, besides transesterification for the production of monoester derivatives with higher percent selectivity. The by-product water formed during the reaction can tune the microenvironment around the catalytic lid of the enzyme mimicking the lipid/ water interfacial environment. This results in high % yield of the reaction. The liphophilicity of the enzymatic pocket as well as preference for stronger H-bonding should be the reason for preferential selectivity for a specific product. High selectivity can be achieved if molecular conformation of the diol can be locked. This study may provide a direction to future research in the field of molecular dynamic and docking to further support the observations. This process is suitable for the design of continuous flow reactor. By making few modifications and optimizing some parameters (eg. thickness of the catalytic bed of immobilized enzyme, rate of diffusion, removal of by-product water at certain stage etc), diol and carboxylic acid can be set as inputs while the product monoester becomes continuous output from the reactor.

2.5 References

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