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Candida Antarctica Lipase-Catalyzed Synthesis and Characterization of Novel Acrylic Teroligomers

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Abstract:-The synthesis of three novel low molecular weight acrylic terpolymers, containing at random sequences of ethyl acrylate, acrylic acid and N-(2-hydroxyethyl)acrylamide, catalyzed by Candida antarctica lipase was successfully conducted in organic media. For the first time, these products have been enzymatically synthesized using ethyl acrylate as the only monomer starting material and taking advantage of a triple activity displayed by the lipase. In the presence of ethanolamine, the enzyme not only catalyzes the chain polymerization of ethyl acrylate but also the aminolysis and hydrolysis of the pendant ester groups affording the terpolymers. The products were characterized by ^{1}H and ^{13}C NMR and UV-MALDI-TOF-MS.

Keywords:-Lipase-catalyzed, acrylic terpolymers, ethyl acrylate.

I. INTRODUCTION

Low molecular weight acrylic terpolymers acrylate are a high performance polyelectrolytes with multi-functional properties including emulsion [1], dispersion [2], scale inhibition [3], crystal growth distortion [4], and plasticizing [5].

The family of terpolymers is generally obtained by reversible addition-fragmentation chain transfer (RAFT) polymerization [6] or chemical modification of a polyacrylamide chain [7]. Although RAFT allows for preparation of polymer with controlled molecular weight and polidispersity [8] the process is performed at high temperature and using toxic catalysts, so that a friendly synthetic alternative for the environment, involving mild reaction conditions and biodegradable catalysts would be useful.

In the last years, enzymatic polymerization has been used as a substitute for the toxic catalytic systems [9] and this new methodology allowed to obtain new materials, which otherwise are difficult to prepare [10]. By using lipase catalysis, functional aliphatic polyesters have been synthesized by various polymerization modes: ester interchange reactions [11], ring-opening polymerization of lactones [12] and direct esterification of hydroxyacids [13] or diacid/diol combinations [14].

In our laboratory we have performed the polymerization of glycerol with ethyl adipate in dry dioxane using lipase from *Candida antarctica* B as catalyst. A low molecular weight poly(1,3-glyceryladipate) was formed in

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high regioselectivity and low polidispersity [15].

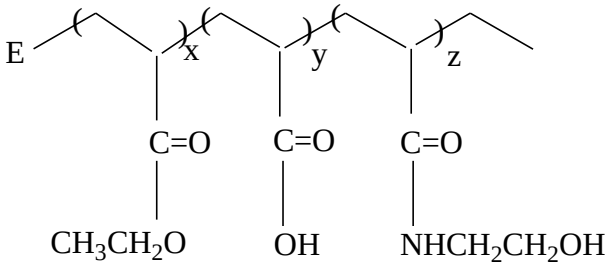
In addition, we reported the first lipase-catalyzed synthesis of acrylic polymers. By using ethyl acrylate as the only monomer starting material and variable amounts of ethanolamine, in the presence of *Candida antarctica* B lipase, low molecular weight copolymers containing at random sequences of poly(ethyl acrylate) and poly(N-(2-hydroxyehtyl)acrylamide) were obtained [16].

Recently, we reported the enzymatic synthesis of a linear polyamidoamine oligomer obtained by polymerization of ethyl acrylate and *N*-methyl-1,3-diaminopropane. The activity showed by the lipase in the polymerization reaction was highly selective and allowed us to obtain a product with biomedical applications [17].

In continuation with our work on enzymatic synthesis of polymers, we carried out new polymerization experiments on this occasion using ethyl acrylate and small amounts of ethanolamine.

Using ethanolamine: ethyl acrylate ratios between 0.05 and 0.25 we obtained three terpolymers containing at random sequences of poly(ethyl acrylate), poly(acrylic acid) and poly(*N*-2-hydroxyethyl)acrylamide (Figure 1).

Figure 1. General formula of the products 1-3 prepared through lipase-catalyzed oligomerization of ethyl acrylate. The sequence of x, y and z is at random.



Ea: T= $HO(CH_2)_2NH$- **Eb**: T'= CH_3CH_2O-

thanolamine/Ethyl acrylate	0.05	0.10	0.25
Product	1	2	3

In this paper, we describe the lipase-catalyzed synthesis and characterization of these polymerization. The composition, average molecular weight and polydispersity of the terpolymers 1-3 were determined by spectroscopic methods (^1H and ^{13}C NMR) and matrix-assisted ultraviolet laser-desorption/ionization time-of-flight mass spectrometry (UV-MALDI-TOF-MS).

ISSN No:-2456 –2165

II. RESULTS AND DISCUSSION

A. Optimization of reaction conditions

Selection of enzyme and effect of enzyme: substrate ratio

We evaluated the activity of four commercial lipases *Candida rugosa* lipase (CRL), *Candida antarctica* lipase B (CAL B), Lipozyme: lipase from *Rhizomucor miehei* (LIP) and porcine pancreatic lipase (PPL)) in the polymerization reaction of ethyl acrylate with ethanolamine at a ratio ethanolamine:ethyl acrylate: 0.25, 25ºC and an enzyme substrate ratio of 1. The tested lipases showed variable activity, CAL B giving the most satisfactory results in terms of monomer conversion at 72 h of reaction. This was determined from the ratio of peak intensities due to polymer and monomer from the ^{1}H NMR spectrum of the crude reaction mixture of each sample. The disappearance of signals of vinyl protons of ethyl acrylate was considered as the end of the reaction.

The optimum enzyme:substrate ratio E/S was studied between 0.1 and 2 , taking into account that an E/S ratio higher than 2 afforded hydroxyethylacrylamide as the main product, as reported previously [17]. The reactions were performed at 25ºC for 72 h. The enzyme:substrate ratio had a profound effect on the monomer conversion rate As shown in Figure 2 the conversion of the monomer increased with increasing E/S ratio. However, the addition of CAL B to an E/S higher than 0.5 did not led to increasing these values. Therefore, in subsequent experiments, enzyme:substrate ratio of 0.5 was employed in the polymerization reaction.

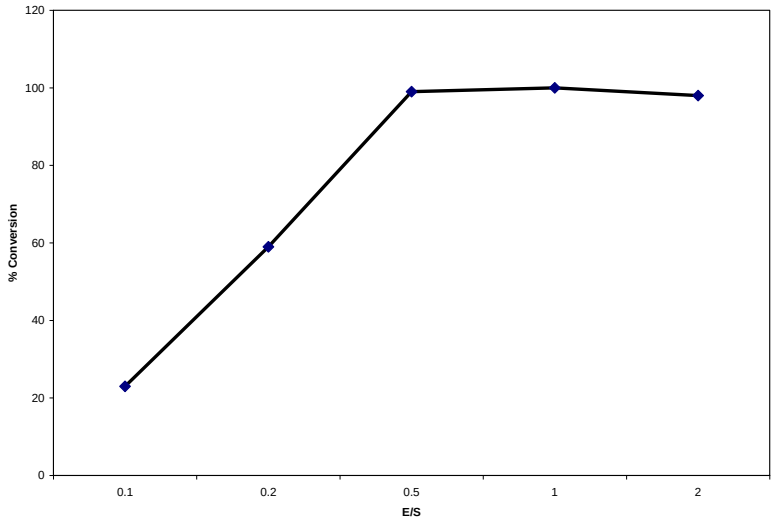


Figure 2. Monomer conversion as a function of E/S ratio. The reactions were carried out in standard conditions.

B. Effect of solvent and temperature

Considering that the solvent plays an important role in determining enzyme activity and selectivity, we studied the effect of various organic solvents on the reaction of oligomerization at 25ºC for 72 h. The reaction in a solvent free system was unsuccessful, recovering the starting materials. Low conversion was observed using non-.polar solvents such as hexane (12%) and toluene (11%) and slightly higher in ethers such as tetrahydrofurane (23%), diisopropylether (31%) and dioxane (29%). The best results in the polymerization correspond to the process occurring in acetonitrile that afforded 100% conversion and completely dissolved the reactants and the product of reaction.

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The effect of temperature on the reaction was investigated over a range of temperatures between 15°C and 55°C in the presence of CAL B and acetonitrile as solvent during 72 h. The best results were obtained at 25°C. As there was no increase in terpolymer production above this temperature, it was chosen as the optimum reaction temperature.

C**.** *Synthesis of terpolymers 1-3*

Taking into account the above mentioned results, CAL B was the enzyme of choice for the polymerization of ethyl acrylate at 25°C, in an E/S ratio 0.5 and using acetonitrile as solvent. Using variable amounts of ethanolamine, three different polymeric products were obtained: 1 to 3, according to the ethanolamine/ethyl acrylate ratios showed in Figure 1. The analysis of the compounds 1 to 3 showed that, besides ethyl carboxylate, the hydroxyethylamido and carboxylic acid moieties were present as pendant groups in the products.

D. Structural characterization of the terpolymers 1-3

The products were characterized by spectroscopic methods.

*^{1}H-NMR*The ^{1}H-NMR spectra of the products are quite complex. As an example, Figure 3 shows the spectrum of 3 (ethanolamine: ethyl acrylate: 0.25), recorded at 500 MHz using DMSO as solvent. The assignments of resonances to various types of protons are shown directly in the spectrum in Figure 3.

The different regions in the complex ^{1}H NMR spectrum of **3** has been completely analyzed using 2D COSY and HSQC spectra (Supplementary material, Fig. 1S and 2S).

In this compound, polymeric chains having two different chain-ending groups have been observed: hydroxyethylamino group (Ea) and ethoxy group (Eb). The protons of methylene of the polymer chain (i) and (k) gave resonance signals between 2.34 and 2.93 ppm. The protons of the methylene (g) of the first unit in the polymer chain with ethanolamine chain-ending group show signals between 2.84 and 3.27 ppm, the same protons with ethoxy chain-ending (g') group gave resonance signals between 3.73.and 3.89 ppm. The protons of methyne (h, j and l) show signals in the range 2.82-3.50 ppm. A signal can be also observed at 1.24 and 4.20 ppm corresponding to methyl (a) and methylene (b) respectively in the ethyl group of ethyl carboxylate of ester pendant group.

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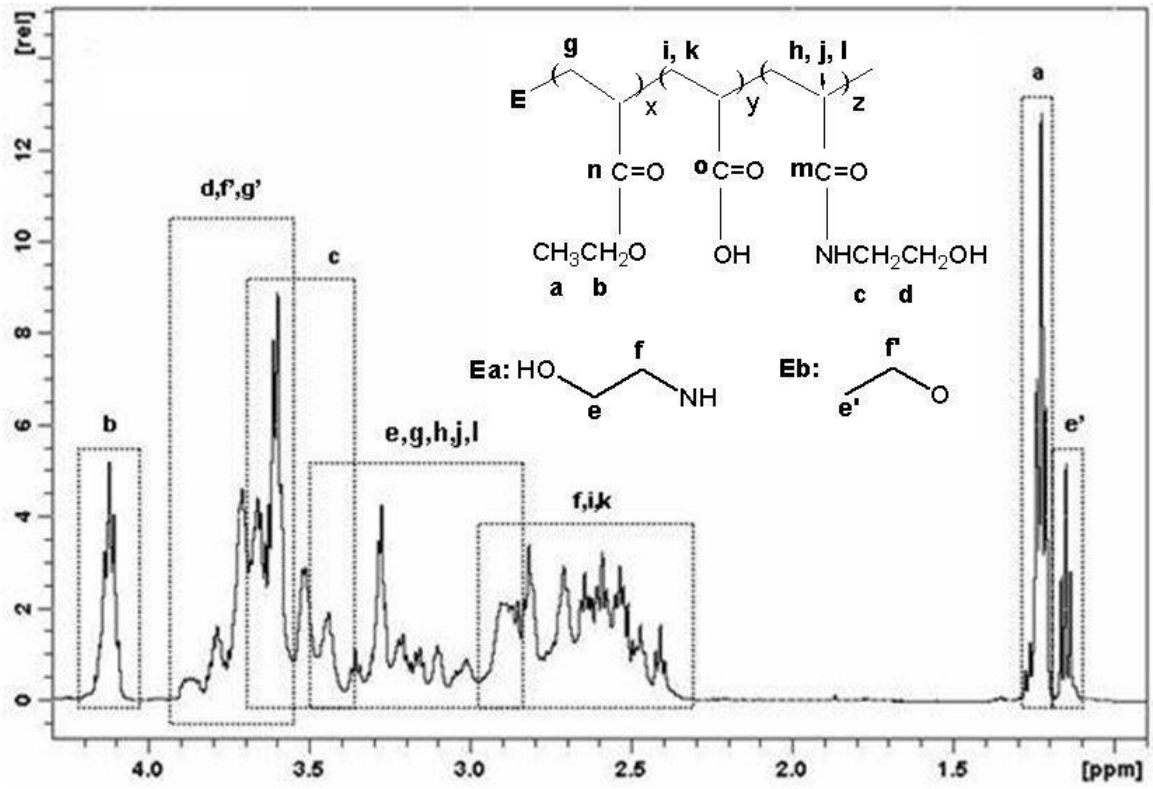

Figure 3. [1]H NMR of product 3 at 500 MHz, solvent DMSO.

The signals of the protons corresponding to the methyl group (e') and methylene group (f ') of the ending chain ethoxy group can be observed at 1.17 and 3.74 ppm. The peaks in the range 3.35-3.70 ppm can be assigned to the methylene group (c) contiguous to the amino group from hydroxyethylamide pendant group and the peaks in the range 3.53-3.91 to the methylene group (d) contiguous to the hydroxyl from hydroxyethylamide pendant group. Finally, the protons of methylene group attached to the amino group (f) and those belonging to the methylene group attached to the hydroxyl group (e) in ethanolamine chain-ending group gave resonance signals between 2.35-2.80 ppm (f) and 3.60-3.94 ppm (e).

[13]C NMR

The [13]C NMR spectra of the compounds 1-3 were also recorded. As an example, the proton decoupled [13]C NMR spectrum of 3 is shown in Figure 4.

The chemical shift assignments were made from the off-resonance decoupled spectra of the polymer and based on DQF-COSY and HSQC correlations (Supplementary material, Figures 3S and 4S).

The ester carbonyl carbon resonance of the pendant carboxyethyl unit (n) appears at 176.8-179.1 ppm, the amide carbonyl carbons of the hydroxyethylamide unit (m) at 169.5-171.06 ppm

ISSN No:-2456 –2165

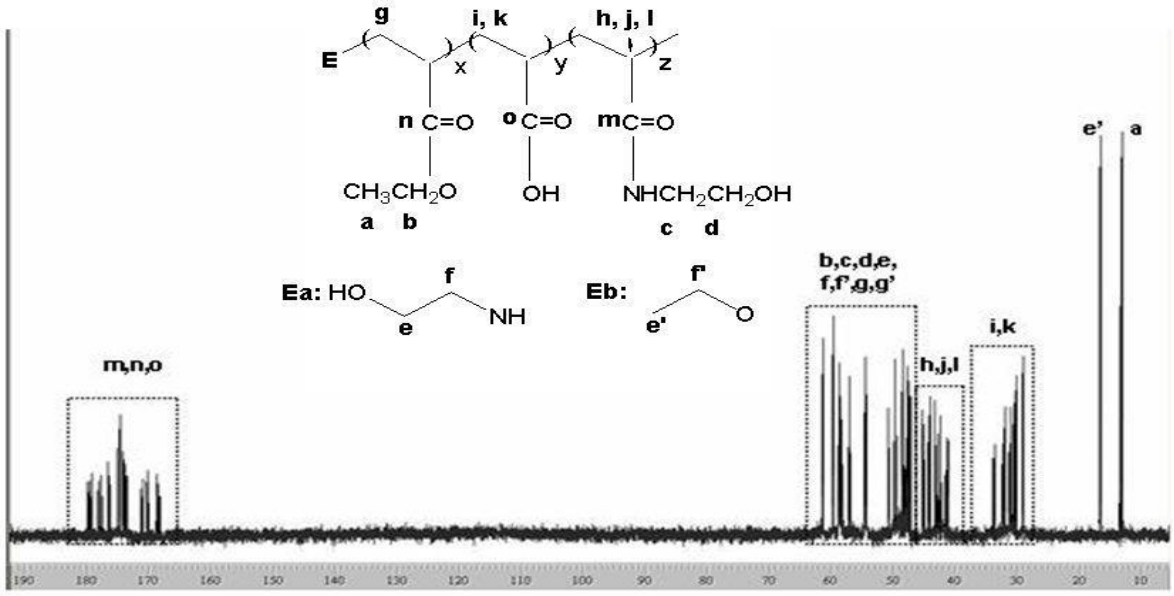

Figure 4. Proton-decoupled ^{13}C NMR of product 3 at 500 MHz, solvent DMSO.

and the carbonyl carbon resonance of the pendant carboxylic acid unit (o) appears at 180.9-181.9 ppm. In the carboxyethyl unit of pendant ester group the methyl group gave a resonance signal at 14.2 (a) and the methylene (b) at 62.5 ppm. The methylene group attached to –OH (d) gave resonance signals between 52.5 and 59.6 ppm and the methylene group attached to –NH in amide (c) between 49.0 and 51.5 ppm. Accordingly the polymer backbone –CH- signal (h, j and l) appeared between 41.5 and 46.0 ppm and the $–CH_2-$ (i and k) between 29.0 and 34.8 ppm. In ethanolamine chain–ending group, the carbon of the methylene (g) of the first unit in the polymer chain gave resonance signals between 49.0 and 50.5 ppm and with ethoxy chain-end group the carbon of this methylene (g') gave resonance signals between 56.9 ppm and 59.7 ppm. The carbon of methylene adjacent to nitrogen of ethanolamine (f) gave resonance signals between 50.5 and 53.6 ppm and that belonging to methylene group attached to the hydroxyl group in ethanolamine (e) between 58.0 and 60.8 ppm. In the polymeric chains containing ethoxy chain-ending group the carbon of methyl group (e') in ethoxy gave resonance signals at 17.7 ppm and the carbon of methylene group (f ') at 51.3 ppm. The assignments of the signals at 14.2 and 17.7 ppm corresponding to methyl groups belonging to the ethyl of ester pendant groups (a) and ethyl of chain ending groups (e') respectively, were made taking into account a proton coupled-^{13}C spectrum of compound 3. (This spectrum is shown in Supplementary material: Fig. 3 and 4).

E. UV-MALDI-TOF-MS

Among mass spectrometry techniques UV-MALDI-MS has the great advantage that is resistant to the presence of salts in the samples to be analyzed as well as allows using low volatile solvents such as dimethylsulfoxide (DMSO) and dimethylformamide (DMF) among others. Currently, untreatable polymeric materials can be conveniently transferred onto the UV-MALDI probe and analyze. Thus, in the present study solutions of polymers 1 and 2 were prepared in NaOH 5% in water and that of polymer 3 was prepared in DMSO. (Tables with results from UV-MALDI- TOF-MS analysis of the terpolymers 1-3 and discussion of behavior of different matrices used are described in Supplementary material, Schemes 1S and 2S, Tables 1S, 2S and 3S and Figure 5S).

Using ^1H and ^{13}C NMR as characterization techniques, we observed that the polymers 1-3 are a mixture of structures containing ethyl carboxylate, carboxylic acid and N-(2-hydroxyethyl)amide functional moieties as pendant groups. The use of UV-MALDI-TOF-MS enabled us to confirm the presence of these functional groups

ISSN No:-2456 –2165

and to compare the calculated theoretical m/z values for each polymer molecule with the experimental m/z values measured.

Thus, by analysis of the UV-MALDI-TOF mass spectra of the compounds 1 to 3, it was possible to determine the number average molecular weight (M_n), weight average molecular weight (M_w) and polydispersity (PD). Moreover it was possible to estimate the proportion of carboxylic molar fraction of each sample. The results are shown in Table 1.

All the products are low molecular weight polymers. The molecular weight varies with the ratio ethanolamine/ethyl acrylate in the polymerization feed. The highest molecular weight was obtained for a ratio ethanolamine/ethyl acrylate of 0.25.

It is remarkable the high selectivity showed by the enzyme in the presence of ethanolamine. In the reaction of ethanolamine with the pendant esters groups only hydroxyethylamide was obtained by an aminolysis reaction through the amino group of ethanolamine and not the corresponding aminoester through a transesterification involving the hydroxyl group. The same highly chemoselective behaviour was observed in the synthesis of hydroxyalkylacrylamides reported by our laboratory [18].

Product	Ethanolamine/ Ethyl acrylate	Mw	Mn	PD	Carboxylic acid molar fraction
1	0.05	1000	905	1.09	0.50
2	0.10	1206	1106	1.04	0.26
3	0.25	1661	1590	1.04	0.19

Table 1. Molecular weight and Polidispersity of products 1-3.

The composition of the products was also dependent on the ethanolamine/ethyl acrylate ratio. It was observed that a maximum content of carboxylic acid in the pendant groups occurs for an ethanolamine/ethyl acrylate ratio of 0.05. At this point we could suppose that the enzyme shows its maximum hydrolytic activity. It could be possible that the presence of a small amount of water in the solvent would be enough to trigger the hydrolysis.
Finally, it is remarkable the high monodispersity shown by the teroligomers 1-3 (1.04 to 1.09). This fact was independent of the ethanolamine/ethyl acrylate ratio and it is difficult to achieve by conventional polymerization procedures.

Regarding the solubility, the products are soluble in polar solvents. The terpolymers have functional groups of different polarity as pendant groups such as ethyl carboxylate (low polarity), hydroxyethylamide and carboxylic acid (high polarity). Therefore, in this system there is a variety of hydrophilic/hydrophobic balances as well as many inter- and intramolecular interactions. Although the distribution of pendants groups in the products is at random, the presence of carboxylic group influences the solubility and compound 1 with a molar fraction of carboxylic acid of 0.50 is soluble in an aqueous solution of sodium hydroxide. By increasing the ethanolamine:ethyl acrylate ratio in the feed, the proportion of carboxylic acid in the pendant groups decreases almost to the half and compounds 2 and 3 are soluble not only in sodium hydroxide solution but also in DMSO.

ISSN No:-2456 –2165

III. Conclusions

This work reports the synthesis and characterization of three new acrylic terpolymer obtained from ethyl acrylate as the only monomer starting material. The lipase of *Candida antarctica* not only catalyzed the chain polymerization of ethyl acrylate forming the polyacrylate chain but it was also active in aminolysis and hydrolysis of the pendant ester groups using ethanolamine and water respectively as nucleophiles. As a consequence of the triple action developed by the lipase in the presence of ethyl acrylate and ethanolamine, and depending on the ratio ethanolamine/ethyl acrylate in the reaction feed, it was possible to obtain polymers of poly(ethyl acrylate), poly(acrylic acid) and poly(*N*-(2-hydroxyethyl)acrylamide). The products, characterized by ^{1}H and ^{13}C NMR and UV-MALDI-TOF-MS, showed low molecular weight and high monodispersity. The enzymatic approach allows obtaining new acrylic polymers becoming useful the well known advantages of the biocatalytic process: mild reaction conditions, easy handling and recovery of the enzymatic catalyst and, in this particular case, the use of only one monomeric material.

IV. Experimental

General Ethyl acrylate, ethanolamine, methanol, tetrahydrofurane, dioxane and acetonitrile (Sigma-Aldrich), dimethylsulfoxide and trifluoroacetic acid (Merck) were used as purchased without further purification. Water of very low conductivity (Milli Q grade; 56-59 nS/cm with PURIC-S, ORUGANO Co., Ltd., Tokyo, Japan) was used. Lipase from *Candida rugosa* (CRL) (905 U/mg solid) and type II crude from porcine pancreas (PPL) (190 U/mg protein) were purchased from Sigma Chemical Co.; *Candida antarctica* lipase B (CAL B): Novozym® 435 (7400 PLU/g) and Lipozyme RM 1M (LIP) (7800 U/g) were generous gifts of Novozymes Latinoamerica Ltda and Novozymes A/S. All enzymes were used "straight from the bottle". Calibrating chemicals for UV-MALDI-TOF-MS analysis: Proteins: aprotinin (A1153, m.w. 6512), bovine insulin (I5500, m.w. 5733.5), angiotensin 1 (m.w. 1296.49) and angiotensin 2 (m.w. 1046.21) were obtained from Sigma. Carbohydrates: (neutral cyclic oligosaccharides) □-cyclodextrin (cyclohexaamylose, m.w. 972.9), □-cyclodextrin (cycloheptaamylose, m.w. 1135) and □-cyclodextrin (cyclooctaamylose, m.w. 1297) were purchased from Sigma-Aldrich. Matrices for UV-MALDI-TOF-MS: □-carboline (9H-pyrido[3,4-*b*]indole), *nor*-harmane (nor-Ho), 2,5-dihydroxybenzoic acid (gentisic acid (GA)), *trans*-3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid (SA)) and 2-(4-hydroxyphenylazo)-benzoic acid (HABA) were obtained from Aldrich Chemical Co.

A. Synthesis of acrylic terpolymers 1-3

To a 3 M solution of ethyl acrylate in acetonitrile, variable amounts of ethanolamine (ethanolamine/ethyl acrylate ratio: 0.05, 0.1 and 0.25) and lipase (enzyme/substrate ratio: 0.5) were added. The suspension was stirred (200 rpm) at 25º C and the progress of the reaction was monitored by ^{1}H NMR. After 72 h, the enzyme was filtered off and washed four times with dioxane. The solvent of reaction was evaporated. Three different polymeric products (1-3) were obtained depending on the ethanolamine/ethyl acrylate ratio as follows: (ratio: product): 0.05: 1; 0.1: 2 and 0.25: 3.

Analysis of polymers

NMR spectroscopy

^{1}H and ^{13}C NMR mono- and bidimensional spectra were recorded on a Bruker AM-500 MHz NMR instrument operating at 500.14 and 125.76 MHz for ^{1}H and ^{13}C. NaOD 5% D_2O was used as solvent for 1 and d_6-DMSO as solvent for 2 and 3. ^{1}H-^{13}C heteronuclear chemical shift correlation spectrum (HSQC) of product 3 was

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recorded in D_2O using the standard pulse sequence. A total of 32 scans were accumulated with a relaxation delay of 2 s for each of the 512 t_1 experiments. The double quantum filter homonuclear shift correlated spectroscopy (DQF-COSY) experiment of product 3, with 32 scans being collected for each t_1 value, was carried out in D_2O. A total of 256 spectra, each containing the 1 K data points, were accumulated.

B. UV-MALDI-TOF Mass Spectrometry

Measurements were performed using a (i) Shimadzu Kratos, Kompact MALDI III laser-desorption/ionization time-of-flight mass spectrometer (Shimadzu, Kyoto, Japan) equipped with a pulsed nitrogen laser (λ_{em}=337 nm; pulse width=3 ns) and (ii) a Shimadzu Kratos, Kompact MALDI 4 (pulsed extraction) laser-desorption/ionization time-of-flight mass spectrometer (Shimadzu, Kyoto, Japan) equipped with a pulsed nitrogen laser (λ_{em}=337 nm; pulse width=3 ns), tunable PDE, PSD (MS/MS device), and both with a secondary electron multiplier (SEM) detector. Experiments were performed using firstly the full range setting for laser firing position in order to select the optimal position for data collection, and secondly fixing the laser firing position in the sample sweet spots. The samples were irradiated just above the threshold laser power for obtaining molecular ions and with higher laser power for studying matrix cluster formation. Thus, the irradiation used for producing a mass spectrum was analyte-dependent with an acceleration voltage of 20 kV. Usually 50-100 spectra were accumulated. All samples were measured in the linear and the reflectron modes, in both positive- and negative-ion mode. The spectra obtained in the linear, positive ion-mode were the best, and only the peaks clearly distinguishable from the matrix baseline were taken into account. The stainless steel polished surface twenty-sample-slides were purchased from Shimadzu Co., Japan (P/N 670-19109-01). Polished surface slides were used in order to get better images for morphological analysis with a stereoscopic microscope (NIKON Optiphot, Tokyo, Japan; magnification x 400) and with a high-resolution digital microscope (Keyence VH-6300, Osaka, Japan; magnification x 800).

V. ACKNOWLEDGMENTS

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A. References

[1]. Bayer, I. S.; Biswas, A.; Szczech, J. B.; Suhir, E.; Norton, M. G. Radio frequency functional capacitors made of all-organic composites of thiourea in field-responsive polymers. *Appl. Phys. Lett*., 2008, 92, (3 pages). http://scitation.aip.org/getpdf/servlet/GetPDFServlet?filetype=pdf&id=APPLAB000092000000808330200000 01&idtype=cvips&doi=10.1063/1.2888767&prog=normal&bypassSSO=1

[2]. Farrugia, V. M.; Qi, Y.; Gerrior, P. J.; Duque, R. M.; Asfaw, B.; Hawkins, M.S. Toners including carbon nanotubes dispersed in a polymer matrix. *Pat. Appl. Publ.*, US 20100124713A1, 2010.

[3]. Hann, W. M.; Bardsley, J. H.; Robertson, S. T.; Shulman, J. E. Silica Scales Inhibition. *U.S. Pat*., US005277823A, 1994.

[4]. Krishna Pai, R.; Hild, S.; Ziegler, A.; Marti, O. Water-soluble terpolymer mediated calcium carbonate crystal modification. *Langmuir*, 2004, 20, 3123-3128.

ISSN No:-2456 –2165
[5]. Story, H. G. Synthetic based self seal adhesive system for packaging. *Pat. Appl. Publ*., US20050095436A1, 2005.
[6]. Yang, Q.; Song, C.; Chen, Q.; Zhang, P.; Wang, P. Synthesis and aqueous solution properties of hydrophobically modified anionic acrylamide copolymers. *J. Polym. Sci. B* 2008, *46*, 2465-2474.
[7]. Turan, E.; Demirci, S.; Caykara, T. Preparation of polyacrylamide hydrogels at various charge densities by postmodification. *J. Appl. Polym. Sci.* 2009, *111*, 108-113.
[8]. Mc Cormick, C. L.; Lowe, A. B. Aqueous RAFT Polymerization: Recent Developments in Synthesis of Functional Water-Soluble (Co)polymers with Controlled Structures. *Acc. Chem. Res.* 2004, *37*, 312-325.
[9]. Uyama, H.; Kobayashi, S. Enzyme-catalyzed polymerization to functional polymers *J. Mol. Catal. B: Enzym*. 2002, 117, *19-20*.
[10]. Kobayashi S.; Ritter H.; Kaplan D. in *Enzyme-catalyzed synthesis of polymers*, Advances in Polymer Science Series; Springer-Verlag: Heidelberg, 2006, Vol.194; (b) Gross R. A.; Cheng H. N. in *Biocatalysis in Polymer Science*; ACS Symposium Series, ACS: Washington, 2002 (Vol. 840)
[11]. Lavalette A.; Lalot, T.; Brigodiot, M.; Marechal, E. Lipase-Catalyzed Synthesis of a Pure Macrocyclic Polyester from dimethyl terephthalate and diethylene glycol. *Biomacromolecules* 2002, *3*, 225.
[12]. Matsumura, S. Enzymatic synthesis of polyesters via ring-opening polymerization. In: *Enzyme-catalyzed synthesis of polymers. Advances in polymer science series*, Vol. 194, Berlin, Heidelberg: Springer-Verlag; 2006, p. 95-132.
[13]. Mahapatro, A.; Kumar, A.; Gross, R. A. Mild, Solvent-Free ω-Hydroxy Acid Polycondensations Catalyzed by Candida antarctica Lipase B. *Biomacromolecules*, 2004, *5*, 62-68.
[14]. Namekawa, S.; Uyama, H.; Kobayashi, S. Enzymatic Synthesis of Polyesters from Lactones, Dicarboxylic Acid Divinyl Esters, and Glycols through Combination of Ring-Opening Polymerization and Polycondensation. *Biomacromolecules*, 2000, *1*, 335-338.
[15]. Iglesias, L.E.; Fukuyama, Y.; Nonami, H.; Erra-Balsells, R.; Baldessari, A. A simple enzymatic procedure for the synthesis of a hydroxylated polyester from glycerol and adipic acid. *Biotechnol. Techniques*, 1999, *13*, 923-926.
[16]. Rustoy, E. M.; Sato, Y.; Nonami, H.; Erra-Balsells, R.; Baldessari, A. Lipase-catalyzed synthesis and characterization of copolymers from ethyl acrylate as the only monomer starting material *Polymer*, 2007, *48*, 1517-1525.
[17]. Monsalve, L. N.; Fatema, M. K.; Nonami, H.; Erra-Balsells, R.; Baldessari, A. Lipase-catalyzed synthesis and characterization of a novel linear polyamidoamine oligomer *Polymer* , 2010, *51*, 2998-3005.
[18]. Rustoy, E. M.; Baldessari, A. Chemoselective enzymatic preparation of N-hydroxyalkylacrylamides, monomers for hydrophilic polymer matrices. *J. Mol. Catal. B: Enzym.*, 2006, *39*, 50-54.
[19]. (a) Nonami, H.; Fukui, S.; Erra-Balsells, R. β-Carboline Alkaloids as Matrices for Matrix-assisted Ultraviolet Laser Desorption Time-of-flight Mass Spectrometry of Proteins and Sulfated Oligosaccharides: a Comparative Study Using Phenylcarbonyl Compounds, Carbazoles and Classical Matrices *J. Mass. Spectrom.* 1997, *32*, 287-296. (b) Nonami, H.; Tanaka, K.; Fukuyama, Y.; Erra-Balsells, R. β-Carboline alkaloids as matrices for UV-matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in positive and negative ion modes. Analysis of proteins of high molecular mass, and of cyclic and acyclic oligosaccharides. Rapid *Commun. Mass. Spectrom.* 1998, *12*, 285-296. (c) Erra-Balsells, R.; Nonami, H. *Environ. Control in Biol.* 2002, *40*, 55.

ISSN No:-2456 –2165

NMR analysis of terpolymers

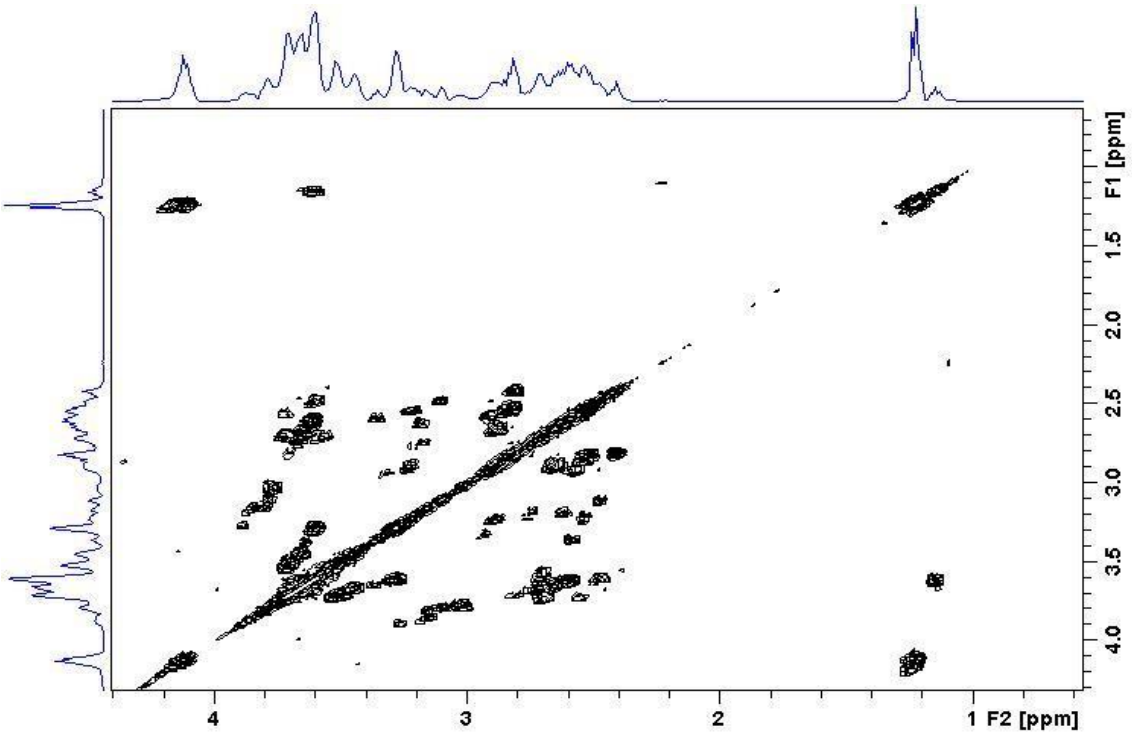

Figure 1S. 2D COSY NMR spectrum of product 3 at 500 MHz, solvent DMSO.

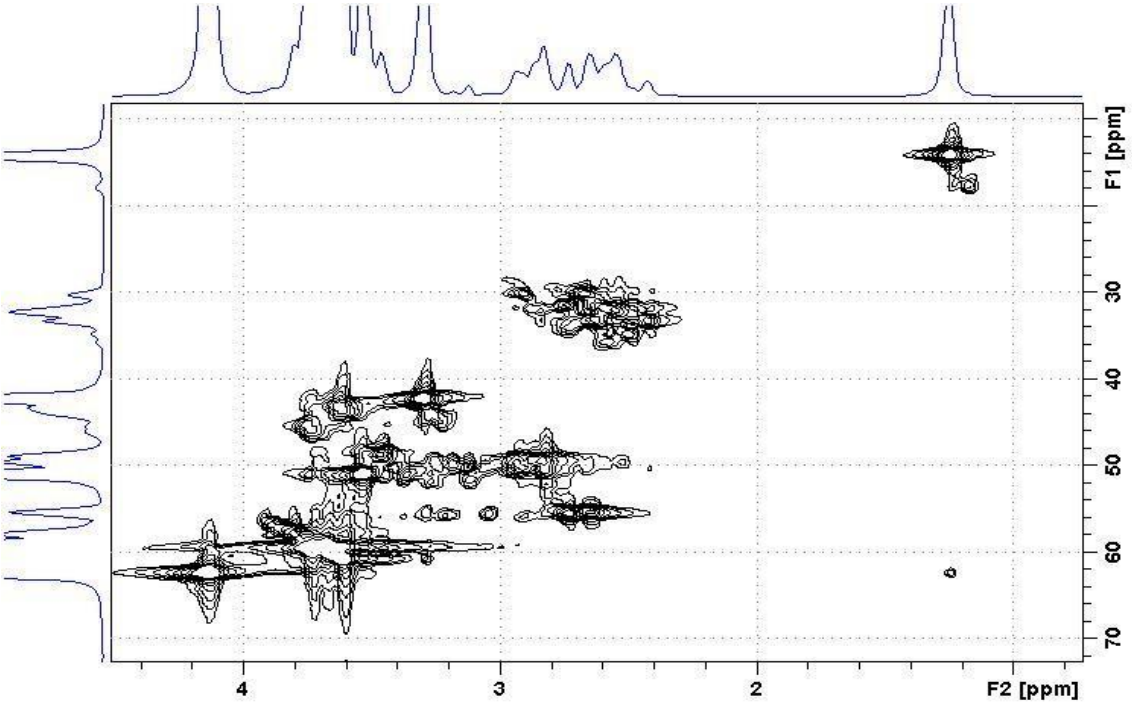

Figure 2S. 2D HSQC NMR spectrum of product 3 at 500 MHz, solvent DMSO.

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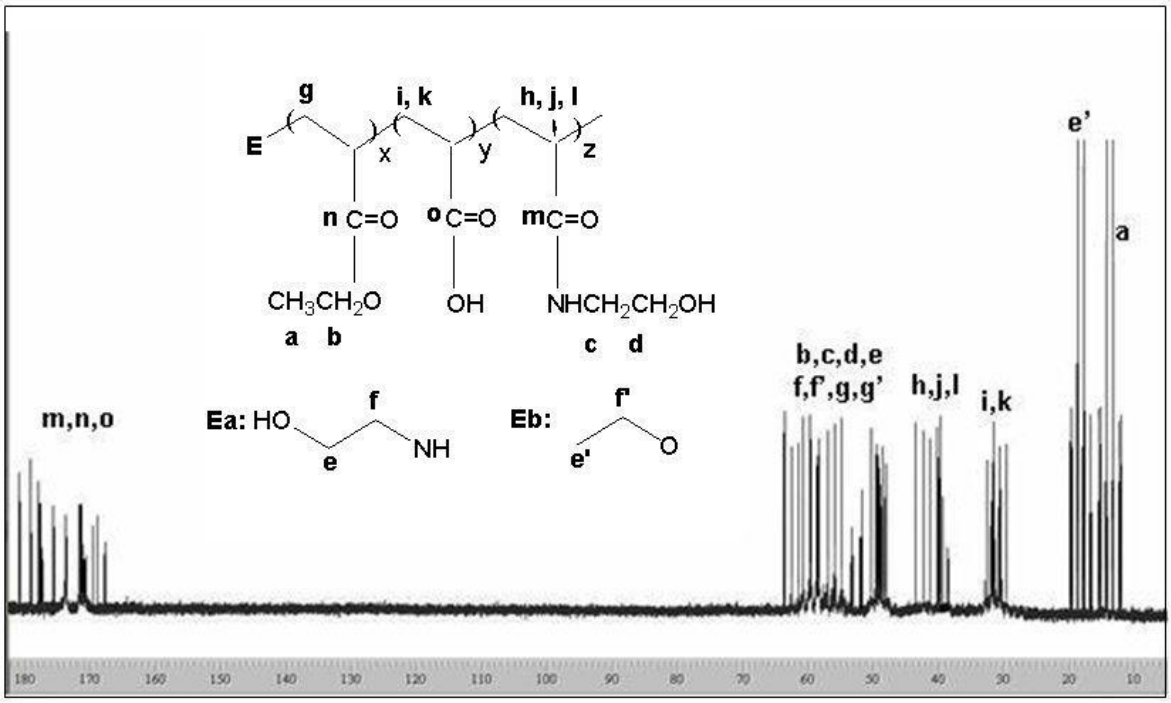


Figure 3S. Proton-coupled NMR of product 3 at 500 MHz, solvent DMSO.

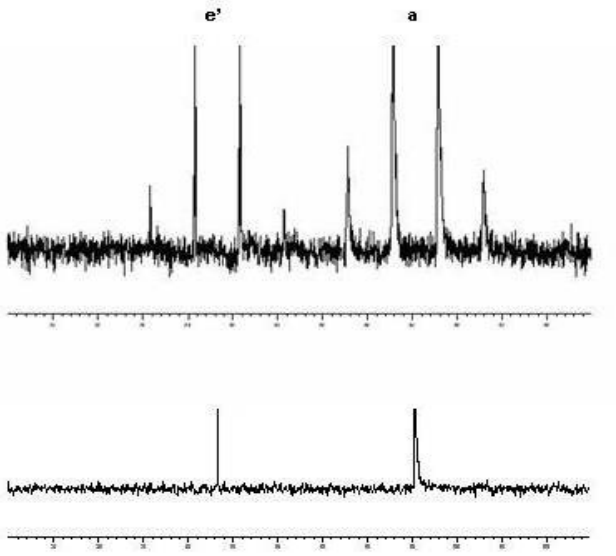


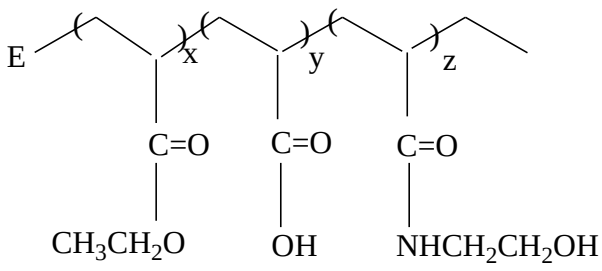
Figure 4S. Amplification of signals a and e' in proton-coupled ^{13}C NMR of product 3 at 500 MHz, solvent DMSO.

MS ANALYSIS OF TERPOLYMERS

UV-MALDI- TOF-MS analysis of the polymers was conducted in comparative way by using HABA, GA and nor-Ho as matrices in positive and negative ion mode. Reproducible spectra were obtained only in the former

ISSN No:-2456 –2165

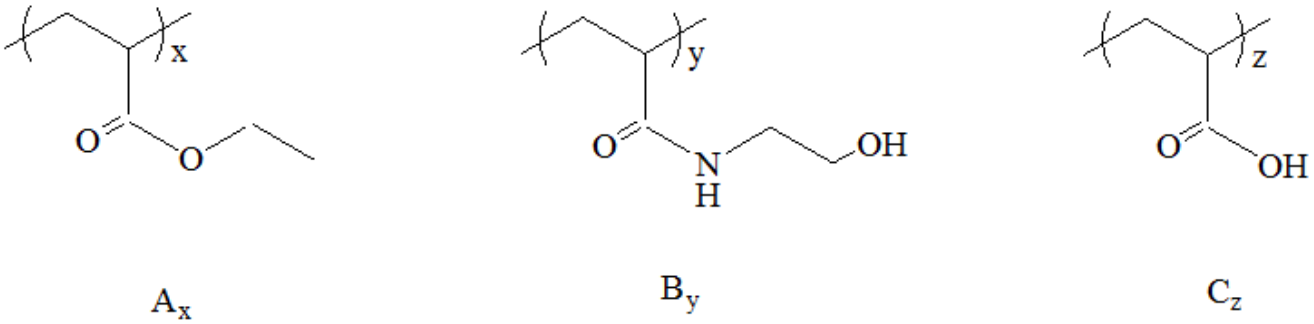
mode, preparing the samples by mixture method (method B). When GA was used as matrix, sample signals were only obtained in the same sweet spot after the first and second laser shot. After that, signals could not be obtained anymore by shotting the same position.



Ea: T= $HO(CH_2)_2NH$- **Eb**: T'= CH_3CH_2O-

Ethanolamine/ Ethyl acrylate	0.05	0.1	0.25
Product	**1**	**2**	**3**

Scheme 1S. General formulae of the products 1-3 prepared through lipase-catalyzed vinyl polymerization of ethyl acrylate. The sequence of x, y and z is at random



A_x B_y C_z

T T'

Scheme 2S. Structure moieties (A, B, C, T and T') present in the products 1-3.

ISSN No:-2456 –2165

Scheme 2 shows the schematic representation of the structural moieties A, B, C, T and T', present in the different products obtained. The stoichiometric numbers x, y and z (odd numbers) of each unit in **1**-**3** are also indicated in Scheme 2 and in Tables 1-3, where n = x+y+z for compounds **1**-**3**.

Thermal decomposition of the pendant ester (A_x) and/or amide groups (B_y) (Schemes 1 and 2) assisted by GA as acid catalyst during the ablation process, changing the chemical structure of the sample surface, would be the reason of this behaviour. Thus, only nor-Ho yielded reproducible spectra in positive ion mode showing the molecules as $[M+H]^+$ or $[M+Na]^+$ species for compounds **1** and **2** (see details in Tables 1 and 2) and as $[M+H]^+$ or $[M+Na]^+$ species sometimes including one DMSO unity for compound **3** (see details in Table 3). As can be seen in Table 3, these DMSO clusters are just formed twice and this fact does not affect the majority of the signals obtained.

ISSN No:-2456 –2165

Table 1S. Assignment of Peaks of UV-MALDI-TOF mass spectra for product

m/z (exp.)	Species	n	*m/z* (theor.+ H^+)	*m/z* (theor.+ Na^+)
527.4	TA_3C_2H	5		528.6
	$T'A_2BC_2H$	5		528.5
563.6	$T'A_3C_3H$	6		
595.6	TAC_6H	7	594.6	
	$T'BC_6H$	7	594.6	
624.3	TB_3C_3H	6	623.7	
659.8	$TABC_5H$	7		659.6
	$T'B_2C_5H$	7		659.6
681.3	TBC_7H	8	681.6	
738.9	TAC_8H	9	738.7	
	$T'BC_8H$	9	738.7	
797.5	TB_2C_7H	9	796.8	
854.9	$TC_{11}H$	11	854.8	
912.4	TB_3C_7H	10	911.9	
971.4	TA_7BCH	9		972.1
	$T'A_6B_2CH$	9		972.1
1030.1	TA_6B_3H	9		1030.2
	$T'A_5B_4H$	9		1030.2
1086.9	TA_7B_2CH	10		1087.2
	$T'A_6B_3CH$	10		1087.2
1145.4	TA_6B_4H	10		1145.3
	$T'A_5B_5H$	10		1145.3
1203.1	TA_7B_3CH	11		1202.4
	$T'A_6B_4CH$	11		1202.4
1261.7	TA_3B_7CH	11		1262.4
	$T'A_2B_8CH$	11		1262.4
1319.7	$TA_4B_6C_2H$	12		1319.4
	$T'A_3B_7C_2H$	12		1319.4
1377.6	TA_3B_8CH	12		1377.5
	$T'A_2B_9CH$	12		1377.5
1436.3	$T'A_{11}C_4H$	15	1436.6	
1493.9	$TA_3B_4C_9H$	16		1493.5
	$T'A_2B_5C_9H$	16		1493.5
1551.8	$TA_2B_6C_8H$	16		1551.6
	$T'AB_7C_8H$	16		1551.6
1609.7	TAB_8C_7H	16		1609.6
	$T'B_9C_7H$	16		1609.6
1668.6	$TA_{11}C_7H$	18	1667.8	
	$T'A_{10}BC_7H$	18	1667.8	
1728.4	$TA_7B_4C_7H$	18	1727.9	
	$T'A_6B_5C7H$	18	1727.9	
1785.9	$TA_5B_{10}CH$	16	1786.1	
	$T'A_4B_{11}CH$	16	1786.1	
1843.5	$TA_7B_5C_7H$	19	1843.0	
	$T'A_6B_6C_7H$	19	1843.0	
1900.8	$TA_5B_{11}CH$	17	1901.2	
	$T'A_4B_{12}CH$	17	1901.2	
1963.6	$TAC_{25}H$	26	1963.7	
	T'BC25H	26	1963.7	

ISSN No:-2456 –2165

Table 2S. Assignment of Peaks of UV-MALDI-TOF mass spectra for product 2[a]

m/z (exp.)	Species	N	*m/z* (theor.+ H^+)	*m/z* (theor.+ Na^+)
335.6	$T'C_4$	4	335.3	
485.6	$T'A_2C_3H$	5		485.5
559.4	TBC_5H	6		559.5
600.9	TA_3C_3H	6		600.6
	$T'A_2BC_3H$	6		600.6
628.4	TA_4C_2H	6		628.7
	$T'A_3BC_2H$	6		628.7
683.7	TA_6H	6		684.8
	$T'A_5BH$	6		684.8
701.1	TA_4C_3H	7		700.7
	$T'A_3BC_3H$	7		700.7
715.6	TA_3BC_3H	7		715.7
	$T'A_2B_2C_3H$	7		715.7
745.0	TA_2B_4H	6		744.8
	$T'AB_5H$	6		744.8
795.2	TA_2B_4CH	7	794.9	
	$T'AB_5CH$	7	794.9	
817.4	$T'AC_9H$	10		817.7
831.3	$TA_3B_2C_3H$	8		830.9
	$T'A_2B_3C_3H$	8		830.9
842.6	TA_7CH	8		842.0
	$T'A_6BCH$	8		842.0
886.9	TA_5B_2CH	8		887.0
	$T'A_4B_3CH$	8		887.0
916.9	TA_3B_4CH	8		917.0
	$T'A_2B_5CH$	8		917.0
923.9	$TA_3B_3C_3H$	9	924.0	
	$T'A_2B_4C_3H$	9	924.0	
932.4	TA_2B_5CH	8		932.0
	$T'AB_6CH$	8		932.0
1003.8	TA_3BC_7H	11		1004.0
	$T'A_2B_2C_7H$	11		1004.0
1039.3	TA_3BC_8H	12	1039.0	
	$T'A_2B_2C_8H$	12	1039.0	
1048.4	$TA_{10}H$	10	1048.3	
	$T'A_9BH$	10	1048.3	
1061.9	$TA_3B_4C_3H$	10		1061.1
	$T'A_2B_5C_3H$	10		1061.1
1093.1	TA_8B_2H	10	1093.3	
	$T'A_7B_3H$	10	1093.3	
1105.1	TAB_8H	9		1105.2
	$T'B_9H$	9		1105.2
1146.5	$TA_5B_3C_3H$	11		1146.2
	$T'A_4B_4C_3H$	11		1146.2
1204.2	$TA_4B_5C_2H$	11		1204.3
	$T'A_3B_6C_2H$	11		1204.3
1246.7	$TA_6B_3C_3H$	12		1246.4
	$T'A_5B_4C3H$	12		1246.4
1262.8	TA_3B_7CH	11		1262.4
	$T'A_2B_8CH$	11		1262.4
1321.5	$TA_2B_4C_8H$	14		1321.2

ISSN No:-2456 –2165

Table 2S. Continuation

	$T'AB_5C_8H$	14		1321.3
1335.6	$TAB_{10}H$	11		1335.5
	$T'B_{11}H$	11		1335.5
1379.2	$TA_{12}BH$	13	1378.7	
	$T'A_{11}B_2H$	13	1378.7	
1392.4	$TA_3B_5C_6H$	14		1392.4
	$T'A_2B_6C_6H$	14		1392.4
1466.0	$TB_{12}H$	12		1465.6
1495.8	$TA_{10}C_6H$	16	1495.7	
	$T'A_9BC_6H$	16	1495.7	
1516.8	$TA_{11}BC_3H$	15		1516.7
	$T'A_{10}B_2C_3H$	15		1516.7
1553.8	TA_8B_6H	14	**1553.8**	
	$T'A_7B_7H$	14	**1553.8**	
1582.6	$TA_8B_5C_2H$	15	1582.8	
	$T'A_7B_6C_2H$	15	1582.8	
1612.9	$TA_6B_7C_2H$	15	1612.8	
	$T'A_5B_8C_2H$	15	1612.8	
1671.7	$TA_4B_8C_4H$	16	1671.9	
	$T'A_3B_9C_4H$	16	1671.8	
1729.6	$TA_3B_{10}C_3H$	16	1729.9	
	$T'A_2B_{11}C_3H$	16	1729.9	
1787.0	$T'A_{15}C_3H$	18		1787.0
1843.0	$TA_6B_9C_2H$	17	1843.1	
	$T'A_5B_{10}C_2H$	17	1843.1	
1902.6	$TA_{16}C_3H$	19		1902.2
	$T'A_{15}BC_3H$	19		1902.2
1964.6	$TA_{19}H$	19	1964.4	
	$T'A_{18}BH$	19	1964.4	

ISSN No:-2456 –2165

Table 3S. Assignment of Peaks of UV-MALDI-TOF mass spectra for product 3[a]

m/z (exp.)	Species	N	*m/z* (theor.+ H^+)	*m/z* (theor.+ Na^+)
631.4	TBC_6H	7		631.6
837.6	TA_4B_2H	6	837.0	
	$T'A_3B_3H$	6	836.9	
893.9	TA_6B_2H	8	893.1	
	$T'A_5B_3H$	8	893.1	
1005.3	TB_8H	8		1005.1
1106.3	TAB_3C_8H	12		1106.1
	$T'B_4C_8H$	12		
1258.9	$TA_8B_2C_2H$	12		1259.4
	$T'A_7B_3C_2H$	12		1259.4
1315.2	$TA_{10}B_2H$	12		1315.5
	$T'A_9B_3H$	12		1315.5
1432.1	TA_7B_5CH	13		1432.6
	$T'A_6B_6CH$	13		1432.6
1491.0	$TA_5B_6C_3H$	14		1491.6
	$T'A_4B_7C_3H$	14		1491.6
1533.4	TA_8B5CH	14		1532.7
	$T'A_7B6CH$	14		1532.7
1548.7	$TA_4B_8C_2H$	14		1549.7
	$T'A_3B_9C_2H$	14		1549.7
1607.2	$TA_3B_{10}CH$	14		1607.8
	$T'A_2B_{11}CH$	14		1607.8
1621.5	TA_5B_9H	14		1620.8
	$T'A_4B_{10}H$	14		1620.8
1665.5	$TA_2B_{12}H$	14		1665.9
	$T'AB_{13}H$	14		1665.9
1722.9	$TA_3B_{11}CH$	14		1722.9
	$T'A_2B_{12}CH$	14		1722.9
1739.3	$TA_{11}B_5H$	16	1739.1	
	$T'A_{10}B_6H$	16	1739.1	
1781.7	$TA_2B_{13}H$	15		1781.0
	$T'AB_{14}H$	15		1781.0
1811.5	$TB_{15}H$	15		1811.0
1839.5	$TA_{12}B_5H$	17	1839.2	
	$T'A_{11}B_6H$	17	1839.2	
1872.0	$TA_{15}B_2H$(DMSO)	17	1872.3	
	$T'A_{14}B_3H$(DMSO)	17	1872.3	
1896.4	$TA_2B_{14}H$	16		1896.1
	$T'AB_{15}H$	16		1896.1
1956.6	$TA_{10}B_4C_6H$	20	1956.2	
	$T'A_9B_5C_6H$	20	1956.2	
2014.0	$TA_8B_{10}H$	18	2014.4	
	$T'A_7B_{11}H$	18	2014.4	
2189.8	$TA_3B_{14}C_3H$	20	2190.4	
	$T'A_2B_{15}C_3H$	20	2190.4	
2286.1	$TA_{11}B_6C_6H$	22	2286.6	
	$T'A_{10}B_7C_6H$	22	2286.6	
2381.8	$TA_8B_{13}H$	21		2381.7

ISSN No:-2456 –2165

Table 3S. Continuation

2445.4	$T'A_7B_{14}H$	21		2381.7
	$TA_9B_{11}C_3H$	23	2445.8	
	$T'A_8B_{12}C_3H$	23	2445.8	
2636.1	$TA_4B_{17}C_3H$	24	2636.0	
	$T'A_3B_{18}C_3H$	24	2636.0	

[a] n = x + y + z; DMSO: dimethyl sulfoxide

As an example the Figure 5S shows the UV-MALDI-TOF mass spectrum of the product 3 and the assignment of the main peaks is displaced in Table 3.

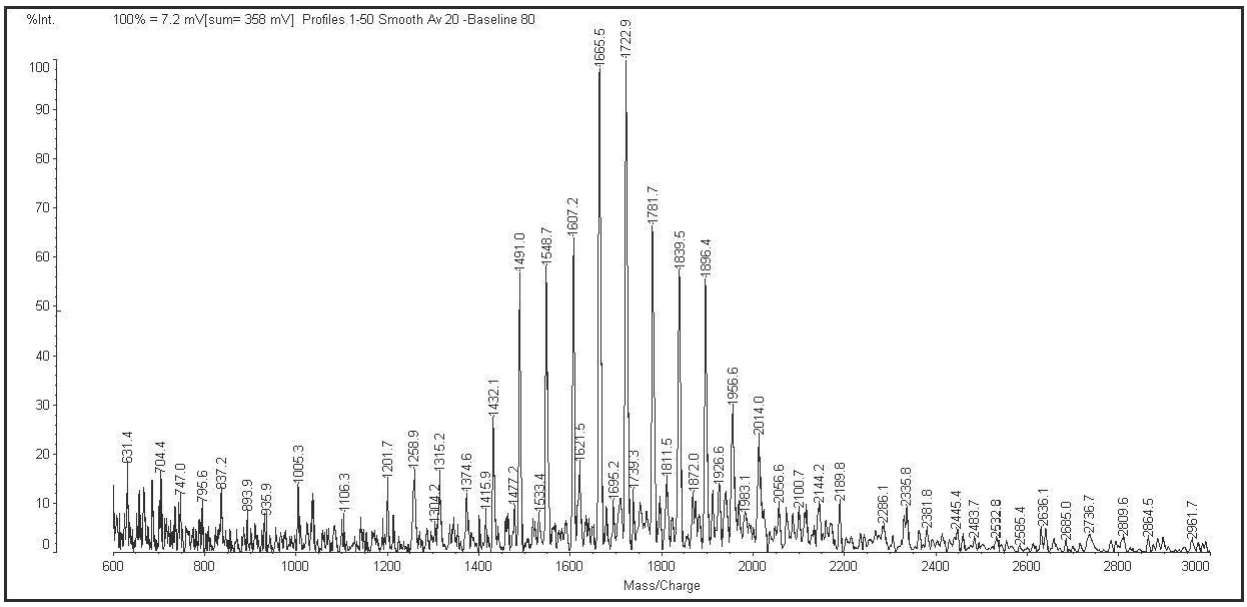


Figure 5S. UV-MALDI-TOF-MS of product 3 (matrix: nor-Ho, positive ion mode, sample preparation method: sandwich).