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## Copolymers based on *N*-acryloyl-L-leucine and urea methacrylate with pyridine moieties

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**Abstract:** By using free radical polymerization of 2-(((4-pyridinylmethyl)amino)carbonyl)aminoethyl methacrylate (MAcPU) and *N*-acryloyl-L-leucine (AcLeu), an optically active copolymer, poly{2-(((4-pyridinylmethyl)amino)carbonyl)aminoethyl methacrylate-*co*-*N*-acryloyl-L-leucine}, MAcPU-*co*-AcLeu (1.86:1 mole ratio), was prepared and subsequently functionalized at the pyridine-*N* with (1*R*/*S*)-(±)-10-camphorsulfonic acid (*R*/*S*-CSA) and at the carboxyl group with (*R*)-(+)- $\alpha$ -ethylbenzylamine (*R*-EBA) or *trans*-4-stilbene-methanol (*t*-StM). The structures, chemical compositions and chiroptical activities of the monomers and the copolymers were characterized by spectral analysis (FTIR, <sup>1</sup>H- and <sup>13</sup>C-NMR, <sup>1</sup>H,<sup>1</sup>H-COSY and UV–Vis), thermal methods (TGA and DSC), fluorescence spectroscopy, gel permeation chromatography and specific rotation measurements. The influence of the optical activity of the monomer and modifier on the modified copolymers suggested a good correlation between the experimental data obtained ( $[\alpha]_{589}^{23} = +12.5^\circ$  for AcLeu and MAcPU-*co*-AcLeu,  $[\alpha]_{589}^{23} = 0^\circ / +27.5^\circ$  for (MAcPU-*co*-AcLeu)-*R*/*S*-CSA,  $[\alpha]_{589}^{23} = +25^\circ$  for (MAcPU-*co*-AcLeu)-*R*-EBA, and  $[\alpha]_{589}^{23} = 0^\circ$  for (MAcPU-*co*-AcLeu)-St). In addition, the photobehavior of the stilbene copolymer (MAcPU-*co*-AcLeu)-St in film was investigated by UV–Vis spectroscopy. The fluorescence quenching of the stilbene species in the presence of aliphatic/aromatic amines in DMF solution was evaluated, more efficient being 4,4'-dipyridyl (detection limit:  $7.2 \times 10^{-6}$  mol L<sup>-1</sup>).

**Keywords:** amino acid; radical polymerization; post-modification; photochemistry.

### INTRODUCTION

In the past few decades, great attention has been devoted to the preparation of chiroptical poly(acryloyl amino acids) or poly(meth)acrylamides bearing amino acids, because of their wide utilization in the field of synthetic non-biological macromolecules with biomimetic structures and properties,<sup>1</sup> biomed-

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ical and biocompatible materials,<sup>2,3</sup> controlled drug delivery systems,<sup>4-6</sup> polyelectrolytes<sup>7</sup> and optical resolution.<sup>8</sup> The chiroptical effects in such polymers are determined by the presence of an asymmetric carbon atom in the main<sup>9</sup> or side chain,<sup>4,10</sup> which plays a fundamental role in the polymerization process, the connection mode of hydrogen bonds between the amide groups of the amino acid moieties and their capacity to adopt stable conformations.<sup>11</sup> The conformation of the chain segments is sensitive to external stimuli, in alkaline medium being disordered random coils and in acid medium swelled ordered coil conformations, while temperature variations induce the Cotton effect.<sup>12</sup> Therefore, the incorporation of amino acids into synthetic polymers is essential for their amphoteric nature, low toxicity, chiroptical and optoelectronic properties,<sup>13</sup> excellent biocompatibility and quick biodegradability,<sup>14</sup> response to stimuli,<sup>7,15</sup> chiral recognition ability,<sup>16-18</sup> and enabling the formation of various chiral hierarchical superstructures, especially  $\alpha$ -helix structures.<sup>11,19</sup> For instance, leucine-based poly(meth)acrylamides were prepared by conventional free-radical polymerization<sup>12,20,21</sup> and the RAFT technique achieving enantiopure homopolymer and amphiphilic block copolymers, respectively.<sup>10,22</sup> Recently, some types of biomimetic poly(*N*-methacryloyl-L-leucine) coatings were used in the medical and biotechnological areas for isolation of cancer cells as well as protein capture agents or functional replacements of antibodies ("plastic antibodies").<sup>23</sup>

On the other hand, the introduction of photosensitive groups into optically active polymer chains can generate chiroptical properties and sensing functionality. Thus, the synthesis of optically active photochromic polymethacrylates based on L-valine, L-leucine and L-proline having in each repeat unit both an asymmetric carbon atom and a *trans*-azobenzene moiety was reported.<sup>24</sup> Moreover, the photochromic behavior of some optically active polymeric systems with azobenzene groups in the side chain spaced by pendent L-alanine and L-lactic acid sequences were examined in order to elucidate the potential conformational transitions of the macromolecules induced by *trans-cis* photo-isomerization of the chromophore, a property exploited in the design of optical data storage devices.<sup>25</sup> Other studies focused on the development of responsive (pH, *T*) and fluorescent soft materials based on covalently cross-linked 3D polymeric gels, which comprised a tryptophanyl moiety as the amino acid.<sup>26</sup>

Previously, controlled/free radical polymerization techniques were used in the preparation of various optically active copoly(meth)acrylates with amino acid moieties and different fluorescent groups in the side chain.<sup>27-29</sup> In this article, the synthesis of a new chiroptical copolymer through the classical polymerization of 2-[[[(4-pyridinylmethyl)amino]carbonyl]amino]ethylmethacrylate (MAcPU) with *N*-acryloyl-L-leucine (AcLeu), followed by functionalization of the pyridine-*N* with (1*R*/*S*)-(±)-10-camphorsulfonic acid (*R*/*S*-CSA), is described. In the same manner, the carboxyl group of the copolymer was functionalized with (*R*-

-(+)- $\alpha$ -ethylbenzylamine (*R*-EBA) or *trans*-4-stilbenemethanol (*t*-StM) to yield optically active copolymers. Previously, the study of the chiroptical properties and the photobehavior of stilbene units in polymer solutions or in thin films suggested the potential applications of these materials as chemosensors in analytical techniques, in optical devices and in biochemical/biomedical investigations.

## EXPERIMENTAL

### Materials

4-(Aminomethyl)pyridine (AMP) 98 %, 2-isocyanatoethyl methacrylate (IEMA) 98 %, L-leucine (Leu)  $\geq 98$  %, acryloyl chloride (Ac-Cl) 97 %, 1,1'-azobis(cyclohexanecarbonitrile) 98 %, (1*S*)-(+)-10-camphorsulfonic acid (*S*-CSA) 99 %, (1*R*)-(-)-10-camphorsulfonic acid 98 %, (*R*)-(+)- $\alpha$ -ethylbenzylamine (*R*-EBA)  $\geq 99$  %, *N,N'*-dicyclohexylcarbodiimide 99 %, *trans*-4-stilbenemethanol (*t*-StM) 99 %, triethylamine (TEA) 99.5 %, (*R*-CSA)2-(methylamino)-ethanol (MAE)  $\geq 98$  %, 4-(dimethylamino)pyridine (dMAP)  $\geq 99$  %, 4,4'-dipyridyl (4,4'-DP) 98 %, dimethylformamide (DMF), deuterated dimethyl sulfoxide (DMSO-*d*<sub>6</sub>) 99.96 at. % D and deuterated chloroform (CDCl<sub>3</sub>) 99.96 at. % D were purchased from Sigma–Aldrich (Taufkirchen, Germany). All reagents were used without further purification.

### Equipment

The structures of all compounds were verified by their <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and <sup>1</sup>H,<sup>1</sup>H-COSY (correlation spectroscopy) spectra, which were recorded in DMSO-*d*<sub>6</sub> and CDCl<sub>3</sub> at room temperature on a Bruker Avance DRX 400 spectrometer with TMS as an internal standard. A 2D <sup>1</sup>H,<sup>1</sup>H-COSY study was performed using standard pulse sequences in the version with z-gradients, as given in Bruker with TopSpin 2.1 PL6 operating software. Fourier transform infrared (FTIR) spectra were registered on a Bruker Vertex 70 spectrometer at room temperature, using KBr pellets. The UV absorption spectra were measured in DMF solution, in quartz cells and thin film with a Specord 200 Analytik Jena spectrophotometer. The polymer film was obtained by casting a polymer solution in DMF onto a quartz plate and then drying at 60 °C under reduced pressure. The samples were exposed to irradiation using UV light with an intensity of 30 mW cm<sup>-2</sup> generated by a high-pressure Hg–Xe lamp (Hamamatsu Lightningcure Type LC58, Model L9588). The average molecular weight of the copolymer was determined by gel permeation chromatography (GPC) performed with a PL EMD-950 instrument (Polymer Laboratory) fitted with an evaporative mass detector and two PL gel 5  $\mu$ m mixed columns. Measurements were realized using a polymer solution in DMF + 0.04 M LiBr as the mobile phase and a calibration plot constructed with polystyrene standards of known molecular weight. The TGA and DSC analyses were performed on a STA 449F1 Jupiter model (Netzsch, Germany) and a DSC 200 F3 Maia device (Netzsch, Germany), respectively, in alumina crucibles. The samples (10 mg) were heated from room temperature to 700 °C under a nitrogen atmosphere at a flow rate of 50 mL min<sup>-1</sup> at a heating rate of 10 °C min<sup>-1</sup>. All thermograms were studied with Proteus software. The onset on the TG curve was considered as the beginning of decomposition or the initial decomposition temperature. Likewise, the maximum signal in the differential thermogravimetry (DTG) curves was registered as the temperature of the maximum rate of decomposition. The fluorescence measurements were performed at room temperature in DMF solution, on a Perkin-Elmer LS 55 spectrophotometer using triethylamine (TEA), 2-(methylamino)ethanol (MAE), 4-(dimethylamino)pyridine (dMAP), and 4,4'-dipyridyl (4,4'-DP) as quenchers. The specific optical

rotation was measured at 23 °C in DMF solution on an OPTIK Pol 1 polarimeter with cell path length of 20 cm ( $\lambda = 589$  nm).

#### Monomer syntheses

*Synthesis of N-acryloyl-L-leucine, AcLeu.* The synthesis of the vinyl monomer with L-leucine (AcLeu) was performed in accordance with literature data.<sup>30</sup> Shortly, optically pure L-leucine (10.0 g, 76.2 mmol) was dissolved in a solution of sodium hydroxide (6.1 g, 152.4 mmol) in water (35 mL), then acryloyl chloride (6.2 mL, 76.2 mmol) was added dropwise over a period for 30 min under vigorous stirring, keeping the reaction mixture at about 0 °C. Then, the stirring was continued for 1 h at room temperature. The solution was acidified to pH 1 by the addition of concentrated HCl (6.3 mL, 76.2 mmol) and after 2 h, the formed product was filtered and dried.

*Synthesis of 2-[[[(4-pyridinylmethyl)amino]carbonyl]amino]ethyl methacrylate, MAcPU.* The 2-[[[(4-pyridinylmethyl)amino]carbonyl]amino]ethylmethacrylate monomer was obtained by the dropwise addition of 2-isocyanatoethyl methacrylate (3.92 mL, 27.74 mmol) in 3 mL CH<sub>2</sub>Cl<sub>2</sub> to a stirred solution of 4-(aminomethyl)pyridine (2.82 mL, 27.74 mmol) in 15 mL CH<sub>2</sub>Cl<sub>2</sub> in a round-bottomed flask placed in a bath of cold-water. The resulting product was reacted at room temperature for 24 h and subsequently, the solvent was removed under reduced pressure. Yield: 7 g (97.4 %)

#### Polymer synthesis

*Synthesis of poly{2-[[[(4-pyridinylmethyl)amino]carbonyl]amino]ethyl methacrylate-co-N-acryloyl-L-leucine}, MAcPU-co-AcLeu.* Into a polymerization ampoule equipped with a magnetic stirrer, 3.0 g (16.2 mmol) AcLeu, 4.26 g (16.2 mmol) MAcPU, and 14.52 mg 1,1'-azobis(cyclohexanecarbonitrile) (2 ‰ initiator), dissolved in 40 mL DMF, were introduced and polymerized under an argon atmosphere at 80 °C for 72 h. After the heating was stopped, the formed copolymer was purified by precipitation into distilled water, filtered and dried in an oven at 60 °C for 24 h. Yield: 5.4 g (77.5 %).

#### Functionalization of the copolymer with (1R/S)-(±)-10-camphorsulfonic acid (MAcPU-co-AcLeu)-R/S-CSA

The reaction at pyridinic nitrogen atom was performed in a round-bottomed glass flask equipped with a stirrer and thermometer. The MAcPU-co-AcLeu copolymer (1.0 g) was dissolved in 10 mL DMF, and then 0.38 g (1.6 mmol) R/S-CSA solubilized in 1 mL DMF was added dropwise under vigorous stirring. The mixture was kept at 35 °C for 24 h and then the polymer was precipitated in diethyl ether, collected by filtration and dried in oven at 60 °C for 48 h. Yield: 1.1 g (79.5 %).

#### Functionalization of copolymer with (R)-(+)- $\alpha$ -ethylbenzylamine, (MAcPU-co-AcLeu)-R-EBA

For the functionalization of MAcPU-co-AcLeu with the R-EBA chiral compound, a solution of 1.0 g of copolymer solubilized in 10 mL DMF was cooled on an ice water bath for 1 h and afterwards, in order to activate the carboxyl group of the amino acid, 0.3 g (1.49 mmol) N,N'-dicyclohexylcarbodiimide was added. The reaction mixture was stirred at room temperature for another 1 h, when 0.22 mL (1.49 mmol) R-EBA was added. The resultant polymer ((MAcPU-co-AcLeu)-R-EBA) was isolated by precipitation into diethyl ether, collected by filtration and vacuum dried. Yield: 0.81 g (67.7 %).

#### Functionalization of the copolymer with trans-4-stilbenemethanol (MAcPU-co-AcLeu)-St

To a solution of 1.0 g of MAcPU-co-AcLeu in 10 mL DMF cooled on an ice water bath for 1 h, 0.3 g (1.49 mmol) N,N'-dicyclohexylcarbodiimide was added as a coupling agent. The

reaction was maintained at a temperature below 0 °C for 1 h, and then the system was brought to room temperature, when 0.31 g (1.49 mmol) *trans*-4-stilbenemethanol was added, and the stirring continued for 24 h. The polymer was precipitated into distilled water, filtered and dried in an oven at 60 °C for 48 h. Yield: 0.87 g (66.7 %).

The functionalization degree (*FD*) of the copolymers was calculated from the peak areas measured by <sup>1</sup>H-NMR spectroscopy of the as-functionalized MAcPU-*co*-AcLeu using Eq. (1):

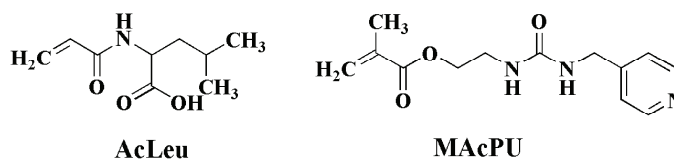
$$FD = 100 \frac{A / N}{A / N + B / 4} \quad (1)$$

where *A* is the peak areas of methyl protons from CSA (0.85 and 1.04 ppm), aromatic/unsaturated protons of stilbene (7.3 and 7.7 ppm) or aromatic protons from EBA (7.31–7.4 ppm); *N* is the corresponding number of protons from CSA (6H), stilbene (11) and EBA (5); *B* is the area of peaks at 7.51 and 8.61 ppm, which were assigned to pyridine protons (4H).

## RESULTS AND DISCUSSION

### Synthesis and characterization

The synthesis of 2-[[[(4-pyridinylmethyl)amino)carbonyl]amino]ethyl methacrylate (MAcPU, Scheme 1) involved an addition reaction between 4-(aminomethyl)pyridine and 2-isocyanatoethyl methacrylate, and the chemical structure and purity of this monomer was proven by spectral methods.



Scheme 1. The structures of *N*-acryloyl-L-leucine and 2-[[[(4-pyridinylmethyl)amino)carbonyl]amino]ethyl methacrylate.

The FTIR spectrum of the monomer MAcPU (not shown here) presented absorption bands characteristic for NH units (3348–3341 cm<sup>-1</sup>), C–H linkages (2956–2910 cm<sup>-1</sup>), C=O carbonyl groups (1710 cm<sup>-1</sup>) and the amide I stretching vibration (1628 cm<sup>-1</sup>). The aromatic structure could be identified at 1585 and 1470 cm<sup>-1</sup> and the amide II deformation at 1557 cm<sup>-1</sup>. Other important absorption bands that appeared in the spectrum are those at 1298 cm<sup>-1</sup> (C–N vibration), 1171 cm<sup>-1</sup> (C–O vibration) and at 820 cm<sup>-1</sup> (C=C double bond from methacrylate). In the <sup>1</sup>H-NMR spectrum of MAcPU (Fig. 1a) in CDCl<sub>3</sub>, signals attributed to the aromatic protons of the pyridine ring from the  $\alpha$  and  $\beta$  positions could be detected as doublets at 8.50 and 7.18 ppm, respectively. The resonance signals of the *trans*- and *cis*-protons from methacrylate were visible at 6.08 (singlet) and 5.57 ppm (singlet), respectively, while the presence of two triplets at 5.28 and 5.09 ppm was ascribed to the urea NH protons. The methylene protons from the ester group provided a doublet centered at 4.35 ppm and the  $\gamma$ -position to the pyridine ring supplied a triplet at 4.24 ppm. Other signals located at 3.51 ppm (as

a quartet) could be attributed to the methylene protons from NH-CH<sub>2</sub>-CH<sub>2</sub>-CO and the methyl proton from methacrylate (as singlet at 1.92 ppm). Additional proof for the structure of the MACPU monomer was provided by the spectral assignment from the <sup>13</sup>C-NMR spectrum. In Fig. 1b, the peaks at 167.71 and 158.44 ppm were assigned to the ester carbon and the carbonyl carbon from the urea group, respectively. The occurrence of other peaks at around 149.91, 149.19 and 122.17 ppm were associated with the aromatic carbons from the pyridine ring, and those positioned at 136.12 and 126.27 ppm were attributed to the carbons of the double bond (>C=CH<sub>2</sub>). Furthermore, the presence of signals at 64.20 ppm was due to the methylene carbons from the ester group and those near to the pyridine ring/urea group (43.19 and 39.72 ppm) as well as the methyl carbons (18.45 ppm) could also be detected. A complete analysis of the monomer MACPU required the use of <sup>1</sup>H,<sup>1</sup>H-COSY spectroscopy, where the homonuclear correlations occur only if there is a coupling between spin-spin adjacent atoms. Thus, the 2D NMR spectrum presented in Fig. 1c shows that there was an accurate correlation of each pair of directly coupled protons (H<sub>d</sub>-H<sub>e</sub>, H<sub>e</sub>-H<sub>n</sub> and H<sub>h</sub>-H<sub>m</sub>) in the urea methacrylate.

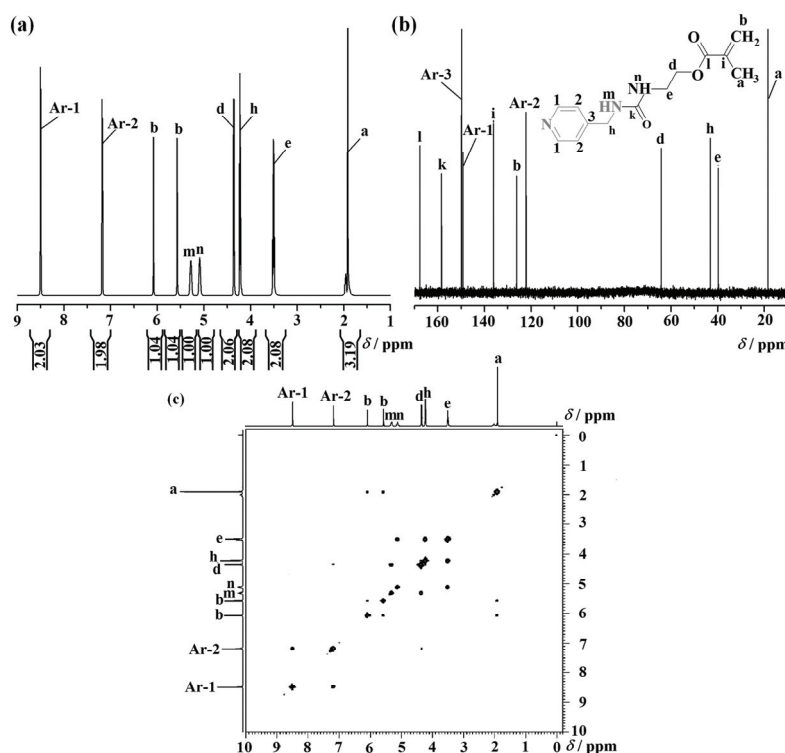
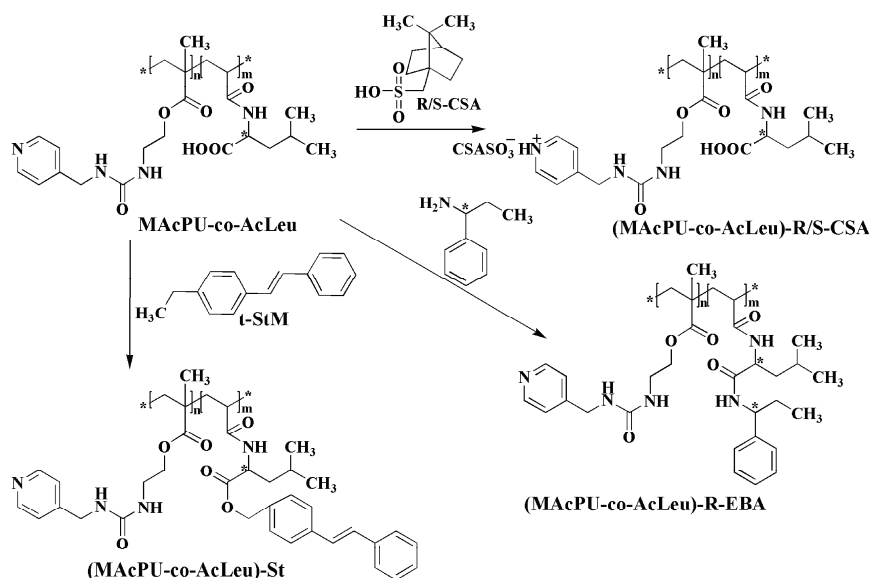


Fig. 1. <sup>1</sup>H-NMR (a), <sup>13</sup>C-NMR (b) and <sup>1</sup>H,<sup>1</sup>H-COSY (c) spectra of 2-(((4-pyridinylmethyl)amino)carbonyl)aminoethyl methacrylate (MACPU) in CDCl<sub>3</sub>.

Next, the copolymer with leucine and pyridine moieties in the side chain was prepared through a conventional radical polymerization of MAcPU with AcLeu using 1,1'-azobis(cyclohexanecarbonitrile) as the initiator. For a better understanding of the optical properties caused of chiroptical effects, three copolymers modified with *R/S*-CSA at the pyridine-N, and with *R*-EBA or *t*-StM at the carboxyl group from leucine were synthesized. The molecular structures of these copolymers, MAcPU-*co*-AcLeu, (MAcPU-*co*-AcLeu)-*R/S*-CSA, (MAcPU-*co*-AcLeu)-*R*-EBA and (MAcPU-*co*-AcLeu)-St (Scheme 2) were characterized by FTIR,  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ ,  $^1\text{H},^1\text{H-COSY}$  and UV-Vis spectroscopies, as well as through GPC measurements, differential scanning calorimetry (DSC) and fluorescence spectroscopy. It should be noted that all copolymers were soluble in organic solvents, such as DMF and DMSO, and could form thin films.



Scheme 2. Structures of the copolymers with leucine and pyridine moieties functionalized with *R/S*-CSA, *R*-EBA or *t*-StM.

The  $^1\text{H-NMR}$  spectra for all copolymers (Fig. 2a–d) confirmed the expected structure by exhibiting resonance signals of the characteristic protons as follows: the aromatic protons in the  $\alpha$  or  $\beta$  positions of pyridine (8.44 and 7.20 ppm), urea protons (6.75 and 6.39 ppm), methine protons ( $\text{H}_r$ ) linked to carboxyl group and ester methylene protons (4.22–4.10 ppm), methylene protons adjacent to pyridine (3.88 ppm) and methylene protons connected to urea groups (3.35–2.93 ppm). Other signals belong to the methyl, methylene, and methine protons from backbone and those connected to an isopropyl group (2.15–1.27 ppm) along with the methyl protons from isopropyl (1.01–0.68 ppm). Additionally, the disappearance

of the signals characteristic for the acryl protons at 6.51 and 5.40 ppm suggested the absence of unreacted monomers in the copolymers.

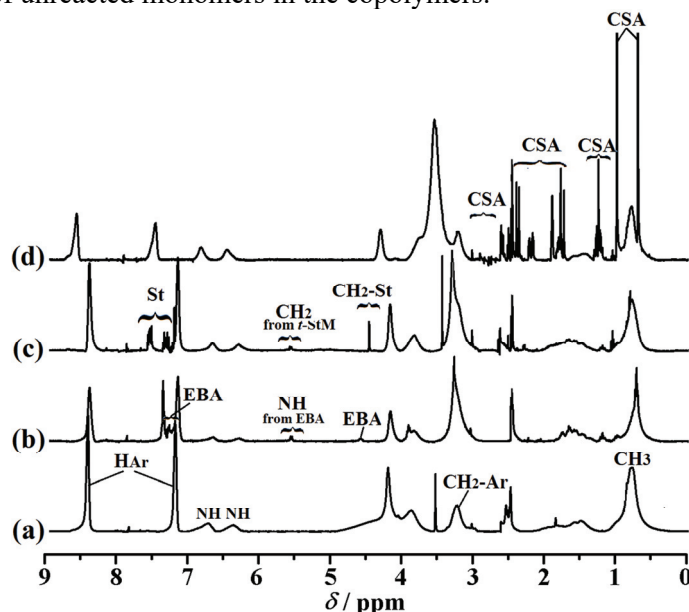


Fig. 2.  $^1\text{H-NMR}$  spectra of poly{2-[[[(4-pyridinylmethyl)amino]carbonyl]amino] ethyl methacrylate-*co-N*-acryloyl-L-leucine} (MAcPU-*co*-AcLeu) (a) and modified copolymers: (MAcPU-*co*-AcLeu)-*R*-EBA (b), (MAcPU-*co*-AcLeu)-St (c) and (MAcPU-*co*-AcLeu)-*R/S*-CSA (d) in  $\text{DMSO-}d_6$ .

All these signals could be unambiguously assigned using the  $^1\text{H}, ^1\text{H-COSY}$  spectrum by simple analysis of the 2D map of homonuclear through-bond interactions to correlate protons that are directly coupled, as illustrated in Fig. S-1a of the Supplementary material to this paper. The structural composition of MAcPU-*co*-AcLeu was determined from the integration ratio of peak areas of the aromatic protons from MAcPU to the methine and methylene aliphatic protons at 4.22 ppm. Accordingly, the mole ratio between monomeric units of MAcPU and AcLeu in copolymers corresponds to a value of about 65:35. The structure of the copolymer was further confirmed by  $^{13}\text{C-NMR}$  spectral data, and the well-resolved signals of carbon atoms contained in each of the structural moiety are presented in Fig. S-1b. The average molecular weight of MAcPU-*co*-AcLeu ( $\bar{M}_n$ ) determined by GPC was  $21500 \text{ g mol}^{-1}$  with a polydispersity index of 1.58.

When the copolymer was functionalized at the pyridinic *N* with *R/S*-CSA, in the NMR spectrum of the resulting copolymer, additional signals appeared at 8.61 and 7.51 ppm (aromatic protons), 2.95 ppm ( $\text{CH}_2\text{-SO}_3\text{H}$ ), 2.71, 2.53, 2.30 ppm ( $\text{CH}_2$ ), 2.18 ppm (CH), 1.06 and 0.85 ppm (methyl protons). The functionalization of MAcPU-*co*-AcLeu at the carboxyl group from leucine with *R*-EBA



was reflected by new signals at 7.40–7.31 ppm (aromatic protons), 5.6 ppm (NH protons), and at 4.63 ppm (benzylic CH protons). Attachment of *t*-StM yields supplementary peaks at 7.7–7.3 ppm (aromatic protons) and at 5.6 ppm (methylene protons from *t*-StM). The content of *R/S*-CSA, *R*-EBA or *t*-StM units present in the post-modified copolymers was calculated by comparison of the integrated intensity of the aromatic protons of pyridine with methyl protons of *R/S*-CSA, aromatic protons of *R*-EBA or aromatic protons of *t*-StM, as was described in the Experimental.

The results of the NMR measurements suggested that the degree of functionalization of the pyridinic *N* with *R/S*-CSA was of 45 mol % for (MAcPU-*co*-AcLeu)-*R/S*-CSA, whereas the degree of carboxyl group functionalization with *R*-EBA or stilbene units on the repetitive monomeric sequence was of about 22 mol % for (MAcPU-*co*-AcLeu)-*R*-EBA and of 9 mol % for (MAcPU-*co*-AcLeu)-*St*. Taking into consideration the mole ratio of co-monomers in MAcPU-*co*-AcLeu (65:35), the percentage of copolymer (yield) converted into one functionalized product was 69.2 mol % (MAcPU-*co*-AcLeu)-*R/S*-CSA, 25.7 mol % (MAcPU-*co*-AcLeu)-*St* and 62.8 mol. % (MAcPU-*co*-AcLeu)-*R*-EBA.

The FTIR spectra of all copolymers (not shown) exhibited absorption bands characteristic of NH amide stretching vibration (3353–3325 cm<sup>-1</sup>), C–H stretching (2956–2926 cm<sup>-1</sup>), C=O ester stretching (1728–1710 cm<sup>-1</sup>), C=O stretching (amide I, 1670–1650 cm<sup>-1</sup>), N–H and C–N (amide II, 1585–1560 cm<sup>-1</sup>), and C–O–C ester stretching (1171–1151 cm<sup>-1</sup>).

#### *Thermal behavior*

The thermal behavior of all copolymers were assessed by thermogravimetric (TGA) and differential scanning calorimetry (DSC) analyses. The values registered from the former experiments are presented in Table S-I of the Supplementary material. In agreement with another report,<sup>31</sup> the TGA thermograms (Fig. 3) presented a three-stage degradation pattern, which could be related to the decomposition of the ester groups of the synthesized copolymers, and the presence of groups *R/S*-CSA, *R*-EBA and *t*-StM did not significantly influence the decomposition temperatures.

Thus, the initial thermal decomposition at which the samples began to lose weight ( $T_{\text{onset}}$ ) was between 161–195 °C, with a weight loss around 9.6–12.8 %, while the temperatures of 10 % weight loss were registered around 190 °C. These processes were completed at decomposition temperatures between 451–500 °C, the results being evidence for the decomposition of the main polymer chains. Furthermore, the thermal data gathered from the DSC thermograms showed that the glass-transition temperatures ( $T_g$ ) of MAcPU-*co*-AcLeu and (MAcPU-*co*-AcLeu)-*St* were of 58 and 50 °C, respectively. Such results are consistent with

the  $T_g$  values of 56 and 55 °C for poly(Boc-L/D-leucine methacryloyloxyethyl ester), as reported in the literature.<sup>32</sup>

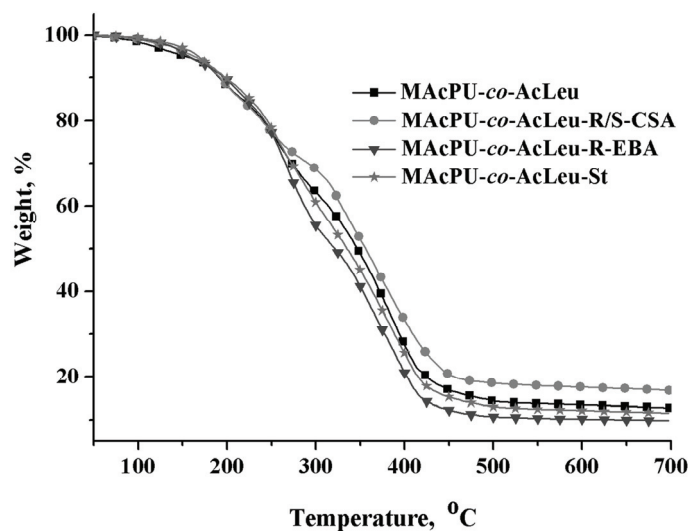


Fig. 3. TG curves obtained for copolymers MAcPU-*co*-AcLeu, (MAcPU-*co*-AcLeu)-*R*-CSA, (MAcPU-*co*-AcLeu)-*R*-EB and (MAcPU-*co*-AcLeu)-*St*.

#### Optical activity

The presence of an asymmetric carbon atom (chiral) in the structure of the copolymers allowed the recording of the rotation angle of the plane of polarized light in DMF solution using a polarimeter with a sodium vapor lamp emitting light of wavelength 590 nm. The functionalization of pyridine-*N* with *R/S*-ACS or of the carboxyl group with *R*-EBA from the initial copolymer influenced the optical activity, indicating a good correlation between the experimental data obtained. The fact that the specific optical rotation value ( $[\alpha]_{589}^{23} = +12.5^\circ$ ) in the optically active monomer with leucine moieties was identical to that of MAcPU-*co*-AcLeu ( $[\alpha]_{589}^{23} = +12.5^\circ$ ) at a concentration of 0.5 mg mL<sup>-1</sup> suggested that the polymerization reaction did not affect the chirality of the asymmetric carbon atom of the amino acid sequence. After the functionalization of the pyridinic *N* with the dextro isomer of ACS, which at a concentration of 1 mg mL<sup>-1</sup> rotates the plain of polarized light by  $-10^\circ$ , it was found that the optical activity decreased to zero, while in the copolymer with *S*-ACS ( $+10^\circ$ ), the value of the optical activity increased to  $+27.5^\circ$ . In the copolymer post-modified with *R*-EBA, the optical activity increased to  $+25^\circ$  due to the contribution of *R*-EBA ( $+7.5^\circ$ ) registered at the same concentration (1 mg mL<sup>-1</sup>).

*UV-induced trans-cis photo-isomerization of the stilbene molecule*

It is well known that stilbene is one of the most studied fluorophores from the perspective of photochemistry and *trans-cis* photoisomerization,<sup>33,34</sup> its main features being intense absorption, fluorescence properties and other dynamic processes. After direct photo-excitation, the stilbene molecule undergoes the fluorescence emission process (see Scheme S-1a of the Supplementary material), which is in competition with the *trans-cis* isomerization reaction (Scheme S-1b). In addition, photo-excitation of the *cis*-isomer (Scheme S-1c) practically does not produce fluorescence and it loses its ability to brighten material, and it undergoes cyclization to form dihydrophenanthrene<sup>35</sup> rather than *cis-trans* isomerization.<sup>36</sup> Since the (MAcPU-*co*-AcLeu)-St contains stilbene units in the side chain, its photochemical response in the film state to UV irradiation was analyzed. The UV spectral changes of this photopolymer are presented in Fig. 4a, in which, two peaks and one shoulder corresponding to the *trans*-isomer localized at 304, 318 and 330 nm were registered. It is clear that the strong absorption maximum at 318 nm decreased gradually with irradiation time up to a limit, the mole fraction of *cis*-isomer formed being around 0.72 (after 750 s of UV exposure). It should be noted that the photo-process follows kinetics with a rate constant value of about  $4.2 \times 10^{-2} \text{ s}^{-1}$ , determined according to Eq. (2):

$$k = \ln \left( \frac{A_0 - A_\infty}{A_t - A_\infty} \right) \frac{1}{t} \quad (2)$$

where  $k$  is the rate constant and  $A_0$  and  $A_t$  represent the values of the absorbance of the *trans*-stilbene moiety before and after irradiation, *i.e.*, at time  $t_0$  and  $t$ , respectively. The photo-induced process presented first-order kinetics (Fig. 4b), which was estimated from the kinetic representation achieved by plotting the logarithm of the reduced absorption *vs.* irradiation time.

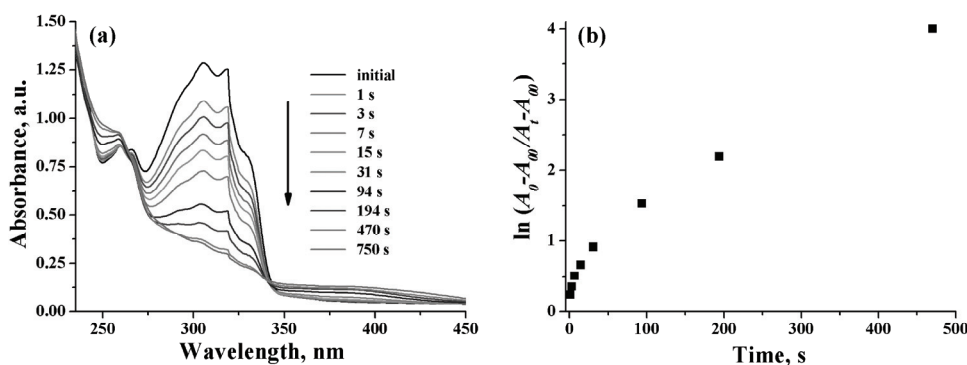


Fig. 4. a) Changes in the UV-Vis spectrum of (MAcPU-*co*-AcLeu)-St in thin film after different irradiation periods and b) kinetic evaluation of the stilbene photo-isomerization.

### Fluorescence study

The fluorescence properties of the stilbene molecule from (MAcPU-*co*-AcLeu)-St arise as a result of a configuration transition of an S<sub>1</sub> excited singlet state of the *trans*-isomer to the S<sub>0</sub> initial ground state thereby emitting a photon during a fast radiative process (Scheme S-1b). Notable modifications in the fluorescence spectra of the (MAcPU-*co*-AcLeu)-St in DMF solution (freshly prepared) were observed on excitation with light of different wavelengths (317, 328, 334, 353 and 365 nm) (Fig. S-2 of the Supplementary material). For the sample excited with 317 and 328 nm radiation, there was only monomer emission with a strong fluorescence maximum (373 nm) and two shoulders (341 and 372 nm), whereas on excitation with 334 nm light, a maximum at 358 nm and a larger shoulder (374 nm) were detected. In these cases, the Stokes shift was about 40 nm due to the energy losses during the transition period into the excited state. At higher excitation wavelengths (353 and 365 nm), the photo-process was followed by the suppression of monomer fluorescence and the appearance of excimer emission (392 and 413 nm) due to the interaction between the excited singlet state of the fluorophore and a molecule in the ground state. In the literature, such complexes were evidenced in stilbene compounds with a relatively polar substituent (chloro, hydroxyl or aryloxymethyl) in the *para* position.<sup>37</sup> In order to understand the fundamental processes that occur in such structures, it is necessary to study the intermolecular interactions of the excited fluorophore in solution through the fluorescence technique. In general, the fluorescence quenching process may be the result of a variety of molecular interactions, which include excited state reactions, energy transfer, electron transfer, molecular rearrangements, *etc.*<sup>38</sup> Any process in which the fluorescence intensity of a sample decreases is usually evaluated by the Stern–Volmer (SV) Equation:

$$\frac{I_0}{I} = 1 + K_{SV}[Q] \quad (3)$$

where  $I_0$  and  $I$  are the fluorescence intensities in the absence and presence of a quencher, respectively,  $[Q]$  is the quencher concentration, and  $K_{SV}$  is the Stern–Volmer quenching constant, which represents a direct measure of the sensitivity of a fluorophore to a quencher.

As reported,<sup>27,39</sup> the absorption and emission properties of the stilbene fluorophore were very sensitive to the addition of known amounts of aliphatic/aromatic amines owing to electron transfer from the ground state amine (electron donor) to the excited stilbene molecule (acceptor molecules). By comparing the fluorescence spectra of (MAcPU-*co*-AcLeu)-St in the presence/absence of different amines, such as 2-(methylamino)ethanol, 4-(dimethylamino)pyridine, 4,4'-dipyridyl, and triethylamine, their abilities to quench the fluorescence of stilbene molecules using an excitation wavelength of 317 nm were investigated. The plots

of the emission spectra for (MAcPU-*co*-AcLeu)-St in DMF solution in the presence/absence of MAE are given in the Supplementary material (Fig. S-3a). A decrease in the fluorescence intensity of about 53.9 % was observed on addition of  $92 \times 10^{-4}$  mol L<sup>-1</sup> of the quencher, the minimal concentration exhibiting an effect was  $0.8 \times 10^{-4}$  mol L<sup>-1</sup>. A similar behavior of the photopolymer was obtained on addition of dMAP (Fig. S-3b), when for a concentration of  $82 \times 10^{-4}$  mol L<sup>-1</sup>, the fluorescence emission decreased by 54.3 %, and the detection limit was of  $0.28 \times 10^{-4}$  mol L<sup>-1</sup>. Moreover, this effect was more pronounced upon addition of 4,4'-DP into the copolymer solution (Fig. 5a), because such an amine allows stronger interactions between its ground state and the photo-excited singlet state of the *trans*-stilbene from the copolymer. Thus, the addition of  $61.2 \times 10^{-4}$  mol L<sup>-1</sup> 4,4'-DP afforded a fluorescence quenching of 57.6 % with a detection limit  $7.2 \times 10^{-6}$  mol L<sup>-1</sup> 4,4'-DP.

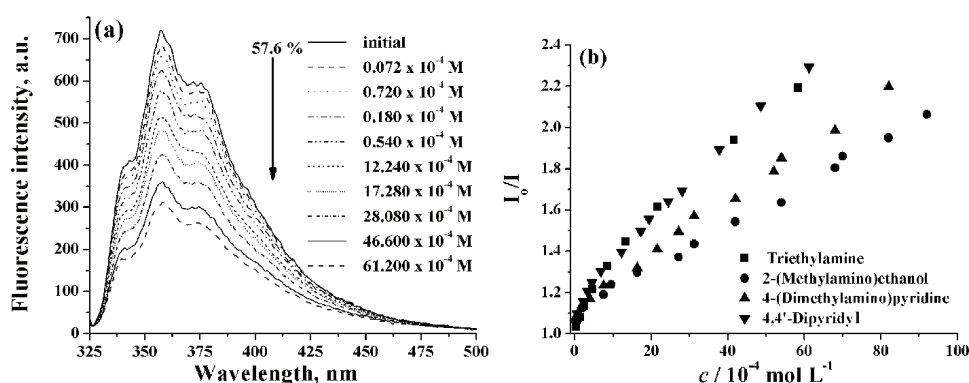


Fig. 5. Fluorescence spectra of (MAcPU-*co*-AcLeu)-St in DMF solution in the absence/presence of 4,4'-DP (a) and Stern–Volmer plots in presence of MAE, dMAP, 4,4'-DP and TEA at  $\lambda_{\text{ex}} = 317$  nm (b).

Analogous results were registered for the gradual addition of TEA into the polymer solution, whereby the shape of the emission bands did not modify throughout the quenching process, and the monomer emission was suppressed up to 57.62 % at an amine concentration of  $61.2 \times 10^{-4}$  mol L<sup>-1</sup>. Furthermore, the value of the minimal concentration detected was of  $0.5 \times 10^{-4}$  mol L<sup>-1</sup>. Using the same concentration of quencher ( $16.3 \times 10^{-4}$  mol L<sup>-1</sup>), the fluorescence intensity dropped to 36.4 % (TEA), 32.5 % (4,4'-DP), 24.5 % (dMAP) and 22.9 % (MAE). For a higher concentration ( $61.1 \times 10^{-4}$  mol L<sup>-1</sup> amine), the quenching effect was quantified as 56.3 % (TEA), 48.2 % (dMAP) and 40.3 % (MAE) and the efficiency of fluorescence quenching occurred in the next order: 4,4'-DP > TEA > > dMAP > MAE, according to the Stern–Volmer diagrams (Fig. 5b).

From the Stern–Volmer plots, a linear dependence between the concentration of the added reactant and  $I_0/I$  for (MAcPU-*co*-AcLeu)-St was deduced

over the investigated concentration range, suggesting the dynamic nature of quenching process. Analyzing the behavior of (MAcPU-*co*-AcLeu)-St in the presence and absence of aliphatic/aromatic amine, a dual nature of its properties, photosensitivity and luminescence, was observed, which is of interest for chemosensors and optoelectronics.

#### CONCLUSIONS

A series of optically active copolymers starting from 2-(((4-pyridinylmethyl)amino)carbonyl)aminoethyl methacrylate and *N*-acryloyl-L-leucine were obtained through conventional free-radical polymerization and subsequent modification of the resulting copolymer with (*R/S*)-( $\pm$ )-10 camphorsulfonic acid, (*R*)--(+)- $\alpha$ -ethylbenzylamine or *trans*-4-stilbene methanol. The presence of a stilbene moiety on side chain of the copolymer created the possibility of preparing optically active and photoluminescent materials, with possible applications in the field of chemosensors for the detection of aliphatic/aromatic amines in polymer solutions.

#### SUPPLEMENTARY MATERIAL

Thermal characteristics, photobehavior and some additional spectral data of the synthesized optically active copolymers are available electronically from <http://www.shd.org.rs/JSCS/>, or from the corresponding author on request.

#### ИЗВОД

#### КОПОЛИМЕРИ НА БАЗИ *N*-АКРИЛОИЛ-*L*-ЛЕУЦИНА И УРЕА-МЕТАКРИЛАТА СА ПИРИДИНСКИМ ОСТАЦИМА

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Полимеризацијом преко слободних радикала мономера: 2-(((4-пиридилметил)-амино)карбонил)амино)етил-метакрилата (MAcPU) и *N*-акрилоил-*L*-леуцина (AcLeu), синтетисан је оптички активни кополимер, поли{2-(((4-пиридилметил)амино)карбонил)амино)етил-метакрилата-*ко*-*N*-акрилоил-*L*-леуцин}, MAcPU-*co*-AcLeu (молски однос 1,86:1). Након тога, хемијском модификацијом оптички активног кополимера, на пиридин-*N* остатку са (*R/S*)-( $\pm$ )-10-камфорсулфонском киселином (*R/S*-CSA) и на карбоксилној групи са (*R*)--(+)- $\alpha$ -етилбензиламином (*R*-EBA) или *trans*-4-стилбенметанолом (*t*-STM) су добијена три различито функционализована оптички активна кополимера. Структура, хемијски састав и хирална активност мономера и кополимера је потврђена спектроскопским (FTIR,  $^1\text{H}$ - и  $^{13}\text{C}$ -NMR,  $^1\text{H}$ ,  $^1\text{H}$ -COSY и UV-Vis) и термичким (TGA и DSC) методама, флуоресцентном спектроскопијом, гелпропусном хроматографијом, као и мерењем углава ротације поларизоване светлости. Утицај оптичке активности мономера и модификатора на оптичку активност функционализованих кополимера је експериментално потврђен ( $[\alpha]_{589}^{23} = +12,5^\circ$  за AcLeu и MAcPU-*co*-AcLeu,  $[\alpha]_{589}^{23} = 0^\circ/+27,5^\circ$  за (MAcPU-*co*-AcLeu)-*R/S*-CSA,  $[\alpha]_{589}^{23} = 25^\circ$  за (MAcPU-*co*-AcLeu)-*R*-EBA, а  $[\alpha]_{589}^{23} = 0^\circ$  за (MAcPU-*co*-AcLeu)-St). Поред тога, изучавано је и фотохемијско понашање танких филмова кополимера са стилбенским остацима (MAcPU-*co*-AcLeu)-St помоћу UV-Vis спектроскопије. Учено је гашење флуоресценције стилбенских остатака

у присуству алифатских/ароматских амина у раствору DMF, најефикасније у присуству 4,4'-дипиридила (лимит детекције:  $7,2 \times 10^{-6}$  mol L<sup>-1</sup>).

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