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ORIGINAL ARTICLE

Synthesis, characterization and in vitro antimicrobial and biodegradability study of pseudo-poly(amino acid)s derived from *N,N′*-(pyromellitoyl)-*bis*-L-tyrosine dimethyl ester as a chiral bioactive diphenolic monomer

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Abstract In this investigation *N,N′*-(pyromellitoyl)-*bis*-L-tyrosine dimethyl ester (**7**) as a chiral bioactive diphenolic monomer was prepared in three steps. The aim of this work was to obtain novel optically and biologically active pseudo-poly(amino acid)s (**PAA**)s that are more soluble in common organic solvents while maintaining their high thermal stability. Thus, several new, highly soluble, thermally stable, optically active and biodegradable **PAA**s containing different amino acid moieties in the main chain were prepared with moderate molecular weights via direct polycondensation using tosyl chloride, pyridine and *N,N′*-dimethylformamide as a condensing agent. The resulting novel polymers were characterized with FT-IR, ^{1}H-NMR, elemental and thermogravimetric analysis techniques. In addition, in vitro toxicity and biodegradability behavior of the diphenolic monomer **7**, different synthetic diacids (**3a**–**3e**) and obtained **PAA**s, which were investigated in culture media, showed that the synthesized compounds and polymers derived from them are biologically active and biodegradable under a natural environment.

Keywords Pseudo-poly(amino acid)s · Nontoxic aromatic diol · L-tyrosine · Biodegradable

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Introduction

Bisphenols (**BP**s) are a group of chemical materials, which consist of two phenolic rings joined together through a bridging carbon or other chemical structures (Chen et al. 2002). **BP**s such as bisphenol-A (**BPA**, 2,2-*bis*(4-hydroxydiphenyl)propane) are frequently used in manufacturing high-performance materials, since their aromatic backbone structures can significantly increase the stiffness and mechanical strength of obtained polymers. It is used primarily as a raw material for the production of polycarbonate plastics, epoxy resins and lacquer coatings (Sajiki and Yonekubo 2004; Pressman et al. 2005). It is also contained in thermographic and pressure-sensitive papers and is used as the material for dental composites and sealants (Joskow et al. 2006). The annual production of **BPA** in the world exceeded 910 million pounds in the beginning of the 1990s, and the amount has increased to nearly 1 billion pounds in 2007 (Saal et al. 2007). However, due to its increased application, its demand has greatly increased year by year. Therefore, the discharge of a variety of **BP**s into the environment is estimated to increase, and it seems that various **BP**s as well as **BPA** will become widespread environmental pollutants in the near future. As mentioned above, several researchers have focused on the toxicity of **BPA**, especially its estrogenic activity, and a number of data are available at present (Vandenberg et al. 2007; Nakagawa and Tayama 2000; Tsai 2006). Chen et al. (2002) paid much attention to the toxicity of **BP**s. In their study, acute toxicity, mutagenicity and estrogenicity of **BP**s were investigated. However, **BPA** and other industrially used diphenols are cytotoxic and may not be suitable candidate as monomers in medical implant and biodegradable materials. So, there is a significant need for a non-cytotoxic diphenolic monomer that could be used

as a building block in the design of biodegradable materials. This need was addressed by the development of some tyrosine-based monomers (Pulapura and Kohan 1992; Parth et al. 2009; Tangpasuthadol et al. 1997; Acunzo and Kohn 2002; Aamer et al. 2009; Jack et al. 1996). L-Tyrosine is the only major natural nutrient amino acid containing an aromatic hydroxyl group. L-Tyrosine is itself used as a common dietary supplement, primarily because of anecdotal reports of its ability to stimulate brain activity for improved memory and mental alertness, to act as an appetite suppressant, to control depression and anxiety and to enhance physical performance. It has promise as a valuable precursor compound for various industrial and pharmaceutical applications (Eversloh et al. 2007; Shah et al. 2009).

Poly(amino acid)s (**PAA**)s have been used as a simple synthetic model for proteins in investigations relating to antigenicity, enzyme specificity, DNA protein interactions, protein folding and tertiary structure. In addition, **PAA**s have been explored as high-performance textile fibers and materials with nonlinear optical properties, as well as for other specialty applications. During the 1980s, **PAA**s were extensively explored as potential implant materials for clinical applications including sutures and drug delivery devices (Brocchini et al. 1997). When degradable polymers are used as implant materials in patients, the potential toxicity of the polymer degradation products and their subsequent metabolites becomes a major anxiety. For this reason, **PAA**s were particularly attractive candidates for biomedical applications (Uhrich et al. 1999). Synthetic **PAA**s that contain one or at most two different amino acid residues were virtually non-immunogenic and were found to degrade in vitro and in vivo to their respective amino acid building blocks, which are nontoxic and natural metabolites (Anderson et al. 1985). Furthermore, due to their structural variability, a large number of **PAA**s were available. Based on these considerations, it is not astonishing that **PAA**s were intensively investigated as potential implant material and biodegradable commercial polymers (Anderson et al. 1974; Sidman et al. 1980). In spite of their structural variability, however, **PAA**s tend to share a number of common physicomechanical properties: most **PAA**s are insoluble in common organic solvents and degrade thermally in the molten state (Bamford et al. 1956). Due to the combination of these two properties, most **PAA**s are unprocessable by conventional polymer fabrication techniques, a disadvantage that has severely limited their practical applicability. Since the insolubility of **PAA**s and their high melting points are a reflection of the strong interchain hydrogen bonds created by the recurring amide backbone linkages, it is proposed that it should be possible to improve the physicomechanical and thermal properties of some **PAA**s by replacing the amide backbone linkages with nonamide bonds, such as iminocarbonate, ester and carbonate bonds. This approach represents a logical extension of "pseudo"-**PAA**s chemistry, in which amino acids have been used as monomeric building blocks in polymers that do not have the conventional backbone structure found in peptides (Spatola 1983). L-tyrosine-based 'pseudo'-**PAA**s were introduced as a novel class of polymeric biomaterials by Kohn and Langer (1984, 1986). In view of the non-processibility of conventional poly (L-tyrosine), which cannot be used as an engineering plastic, recently, the synthesis of polymers was reported using derivatives of tyrosine. These polymers are degradable under physiological conditions and have significantly improved engineering properties as compared to most conventional **PAA**s (Tangpasuthadol et al. 2000). Biodegradable 'pseudo'-**PAA**s derived from tyrosine may be used as an alternative to conventional non-degradable polymers, such as polyethylene and polypropylene in the fabrication of packaging films in the near future, and may be a solution to the environmental problem (Hooper et al. 1998).

Most general methods for preparation of ester bonds comprise bulk polycondensation under elevated temperature and solution step-growth polymerization in high-boiling aprotic organic solvents (Fu and Liu 2008). Solution polyesterification using tosyl chloride (TsCl/dimethylformamide (DMF)/pyridine (Py) as a condensing agent produces rather high-molecular weight polymers from aromatic and aliphatic dicarboxylic acids and bisphenols (Higashi and Tobe 2001; Mallakpour and Kolahdoozan 2006).

In a previous work (Mallakpour et al. 2010), we reported the synthesis of a derivative of tyrosine which can be regarded as biological active diphenolic monomer and may be employed as replacements for the industrially used diphenols in the design of biodegradable and biological materials. This study describes successful synthesis and characterization of optically active PAAs via step-growth polymerization reactions of different aliphatic diacids derived from natural α-amino acids (**3a–3e**) with non-toxic diphenolic monomer 7 under step-growth polymerization. The use of dicarboxylic acids in direct polycondensation, instead of their significantly more toxic though more reactive derivatives, such as diacid chlorides, is one of the main and important prerequisites for the study of such polymer syntheses. To test this hypothesis, we synthesized a series of pseudo-**PAA**s polymers in which natural α-amino acids were linked together by nonamide bonds such as ester linkage. In connection with our interest in preparing optically active thermally modified polymers (Mallakpour and Seyedjamali 2008; Mallakpour and Zadehnazari 2010; Mallakpour and Rafiee 2008), herein we also would like to describe the synthesis and characterization of **PAA**s with main-chain chirality through the

polycondensation reactions of *N,N′*-(pyromellitoyl)-*bis*-dimethyl ester tyrosine (**7**) as a nontoxic aromatic diol with *N,N′*-(pyromellitoyl)-*bis*-(L-phenylalanine) (**3a**), *N,N′*-(pyromellitoyl)-*bis*-(L-leucine) (**3b**), *N,N′*-(pyromellitoyl)-*bis*-(L-methionine) (**3c**), *N,N′*-(pyromellitoyl)-*bis*-(L-valine) (**3d**) and *N,N′*-(pyromellitoyl)-*bis*-(L-alaine) (**3e**). In vitro toxicity of **BPA**, synthetic aromatic diol **7** and different aliphatic diacids (**3a**–**3c**) as well as obtained novel **PAA**s were evaluated with Petri plate containing potato dextrose agar (PDA).

Experimental

Chemicals

All chemicals were purchased from Fluka Chemical Co. (Buchs, Switzerland), Aldrich Chemical Co. (Milwaukee, WI) and Riedel–deHaen AG (Seelze, Germany). Pyromellitic dianhydride (benzene-1,2,4,5-tetracarboxylic dianhydride) (1) (from Merck Chemical Co) was purified by recrystallization from a mixture of acetic anhydride and acetic acid (1:4). *N,N′*-dimethylformamide (DMF) was dried over BaO and then was distilled under reduced pressure.

Instruments

Nuclear magnetic resonance (^{1}H-NMR, 500 MHz) spectra and also carbon nuclear magnetic resonance (^{13}C-NMR, 125 MHz) spectrum were recorded in DMSO-d_6 solution using a Bruker (Germany) Avance 500 instrument, at Sharif University of Technology, Tehran, Iran. The FT-IR adsorption spectra were recorded on a Nicole Impact 400_D IR spectrophotometer with KBr pallets. Vibration bands were reported as wave number (cm^{-1}). The band intensities were classified as weak (w), medium (m), strong (s) or broad (br). Inherent viscosities of polymer solution (0.2% w/v) in DMF were determined at 25°C by a standard procedure using a Cannon–Fenske Routine Viscometer (Cannon, Mainz, Germany). Specific rotations were measured by a Jasco polarimeter (Japan). Solubility of the polymers was tested in various polar and non-polar solvents. As much as 5 mg of the polymer was added to 1 mL of different solvents. Thermogravimetric analysis (TGA) data for the polymers were taken on Perkin-Elmer TGA (Perkin-Elmer, Jugeshein, Germany) in nitrogen atmosphere at a heating rate of 10°C min^{-1} at the Research Institute of Petroleum Industry (Tehran, Islamic Republic of Iran). Differential scanning calorimetry (DSC) data were recorded on a DSC-PL-1200 instrument under nitrogen atmosphere at a heating rate of 20°C min^{-1} under N_2 atmosphere. Elemental analyses were performed by the Iran Polymer and Petrochemical Research Institute, Tehran, Iran. Melting points were taken with a Gallenham melting point apparatus. Cultured samples in Petri plate were imaged with a confocal digital camera (Canon DS126181, Japan).

Synthesis of monomers

N,N′-(Pyromellitoyl)-*bis*-(L-α-amino acid)s (**3a**–**3e**) were reported in our previous work (Faghihi et al. 2004a, b; Mallakpour et al. 2002; Mallakpour and Habibi 2003; Mallakpour and Shahmohammadi 2005).

The brief procedure is as follows. Into a 25-mL, round-bottomed flask, 4.58×10^{-2} mol of pyromellitic dianhydride (**1**), 9.16×10^{-2} mol of L-α-amino acids (L-phenylalanine, L-leucine, L-methionine, L-valine and L-alanin), 15 mL of acetic acid and a stirring bar were placed. The mixture was stirred at room temperature for 3 h and then refluxed for 10 h. The solvent was removed under reduced pressure and then the mixture was poured into a mixture of 50 mL/5 mL of cold distillated water/concentrated HCl and finally stirred for 1 h. A white precipitate was formed, which was filtered off, washed with water and dried under vacuum at 80°C to give a compound (**3a**–**3e**). Recrystallization from methanol/water gave white crystals.

N,N′-(pyromellitoyl)-*bis*-L-tyrosine dimethyl ester (**7**) was introduced for the first time for the application of a new membrane-selective electrode based on the potentiometric method for the determination of phenazopyridine as a drug (Ensafi et al. 2010). The preparation and characterization of this non-toxic and biologically active diol **7** have been completely reported in a previous work (Mallakpour et al. 2010).

Polymerization reactions

PAA8a–**PAA8e** were prepared by the following procedure: For synthesis of **PAA8a**, a Py (0.20 mL, 2.4×10^{-3} mol) solution of TsCl 0.185 g (9.75×10^{-4} mol), after 30 min stirring at room temperature, was treated with DMF 0.07 g (0.1 mL, 9.57×10^{-4} mol) for 30 min and the resulting solution was added dropwise to a solution of diacid **3a**, 0.10 g (1.95×10^{-4} mol) in Py (0.40 mL). The mixture was maintained at room temperature for 30 min and then to this mixture, 0.11 g (1.92×10^{-4} mol) diphenolic monomer 7 was added as yellow powder and the whole solution was stirred at room temperature for 30 min and then at 120°C for 4 h, respectively. As the reaction proceeded, the solution became viscous. Then the viscous liquid was precipitated in 15 mL of methanol to give 0.2 g (95%) of **PAA8a**. **PAA8b**–**PAA8e** were prepared by a similar

procedure. The structures of some polymers were confirmed as **PAAs** with elemental analysis, FT-IR and ^{1}H-NMR spectroscopy techniques. FT-IR spectra of all polymers indicated the characteristic absorption peaks for the imide ring at 1,775, 1,384 and 720 cm^{-1} because of the symmetrical and asymmetrical carbonyl stretching.

In vitro toxicity test

In vitro toxicity assessment has been widely used for recent toxicity studies. Such assays provide rapid, cost-effective and reliable results (Hayes et al. 2007; Bastioli 2005). In vitro toxicity of **BPA**, optically active diphenolic monomer **7**, different chiral aliphatic diacids (**3a–3c**) and **PAA**s were evaluated based on the growth of different aliphatic diacids (**3a–3c**), **PAA8c** and air-borne spores were co-cultivated on the same Petri plates containing potato dextrose agar (PDA) in three replications. Plates were incubated at 23–25°C for 4 weeks. Then colonial growth of fungal saprophytes were noted and visually observed on each Petri plate by digital camera (Canon DS126181).

Results and discussion

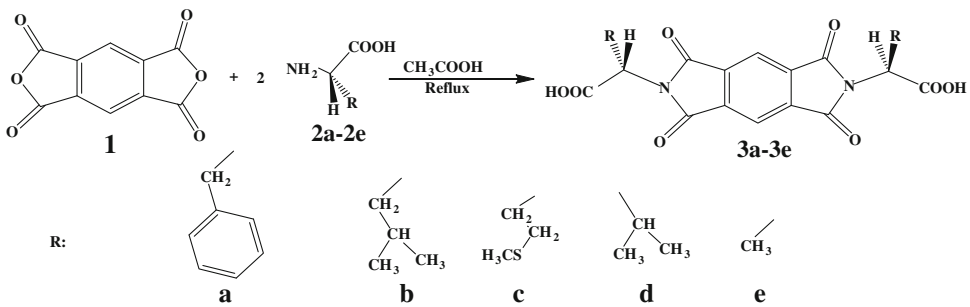
Monomer synthesis

Optically active diacids (**3a–3e**) were prepared according to previous studies (Faghihi et al. 2004a, b; Mallakpour et al. 2002; Mallakpour and Habibi 2003; Mallakpour and Shahmohammadi 2005) and are shown in Scheme 1.

N,N′-(pyromellitoyl)-*bis*-L-tyrosine dimethyl ester (**7**) was prepared according to a previous study (Mallakpour et al. 2010) and is shown in Scheme 2.

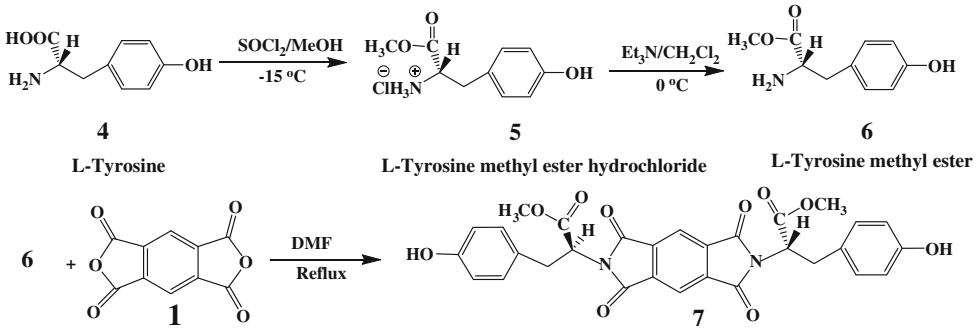
Polymer synthesis

PAA8a–PAA8e were synthesized via direct polycondensation reactions of an equimolar mixture of monomer **7** with several different optically active diacids, such as **3a**, **3b**, **3c**, **3d** and **3e** in a system of TsCl/Py/DMF. In this study for the polycondensation reaction of diphenolic monomer **7** and optically active diacids, Vilsmeier adduct was used as a condensing agent for the polymerization reaction of novel chiral diacids **3a–3e** with aromatic diphenolic monomer **7**, of which a detailed mechanism is illustrated in Scheme 3. Thus, sulfonium salt (**I**) was prepared by dissolving TsCl in Py and stirring for 30 min (aging time) followed by the addition of DMF and stirring for 30 min until the Vilsmeier adduct (**II**) was formed, as suggested previously (Higashi and Mitani 2000; Mallakpour and Kowsari 2006). The reaction mixture was added to a solution of diacid in Py to produce activated diacid (**III**). After a period of time, a powder of diphenolic monomer **7** was added and the whole solution was maintained at elevated temperature for several hours. Polycondensation reaction was performed by varying the amount of DMF, the molar ratio of TsCl/diacid, Py/diacid and the time of heating. Under optimized conditions, polyesterification of different chiral diacids with diphenolic monomer **7** was carried out as follow: TsCl was dissolved in Py at room temperature and kept at this temperature for 30 min according to previously reported procedures (Mallakpour and Kowsari 2006; Mallakpour and Tirgir 2009). The yield and viscosity of the resulting **PAA**s were affected by the amount of DMF. From this data, it is clear that a ratio of DMF/diacid should be about 7 (0.1 mL DMF) in order to obtain polymers with high yield and moderate inherent viscosity. Furthermore, addition of DMF did not improve the molecular weights and the yields (Fig. 1). The effect of ratio of TsCl/diacid on inherent viscosity and yield of the resulting **PAA8a** is shown in Fig. 2. From this information, an appropriate ratio of TsCl/diacid is 5 mol/mol. The effect of reaction times on inherent viscosity and yield of the obtained **PAA8a** is shown in Fig. 3. From this information, a suitable reaction time of 4 h is required to get **PAA8a** with high yield and inherent viscosity. The step-growth polymerization reactions were also carried out at various temperatures and a suitable temperature of 120°C was obtained. The optimum conditions for the preparation of **PAA8a** are summarized in Table 1. The synthesis and some physical properties of these novel optically active

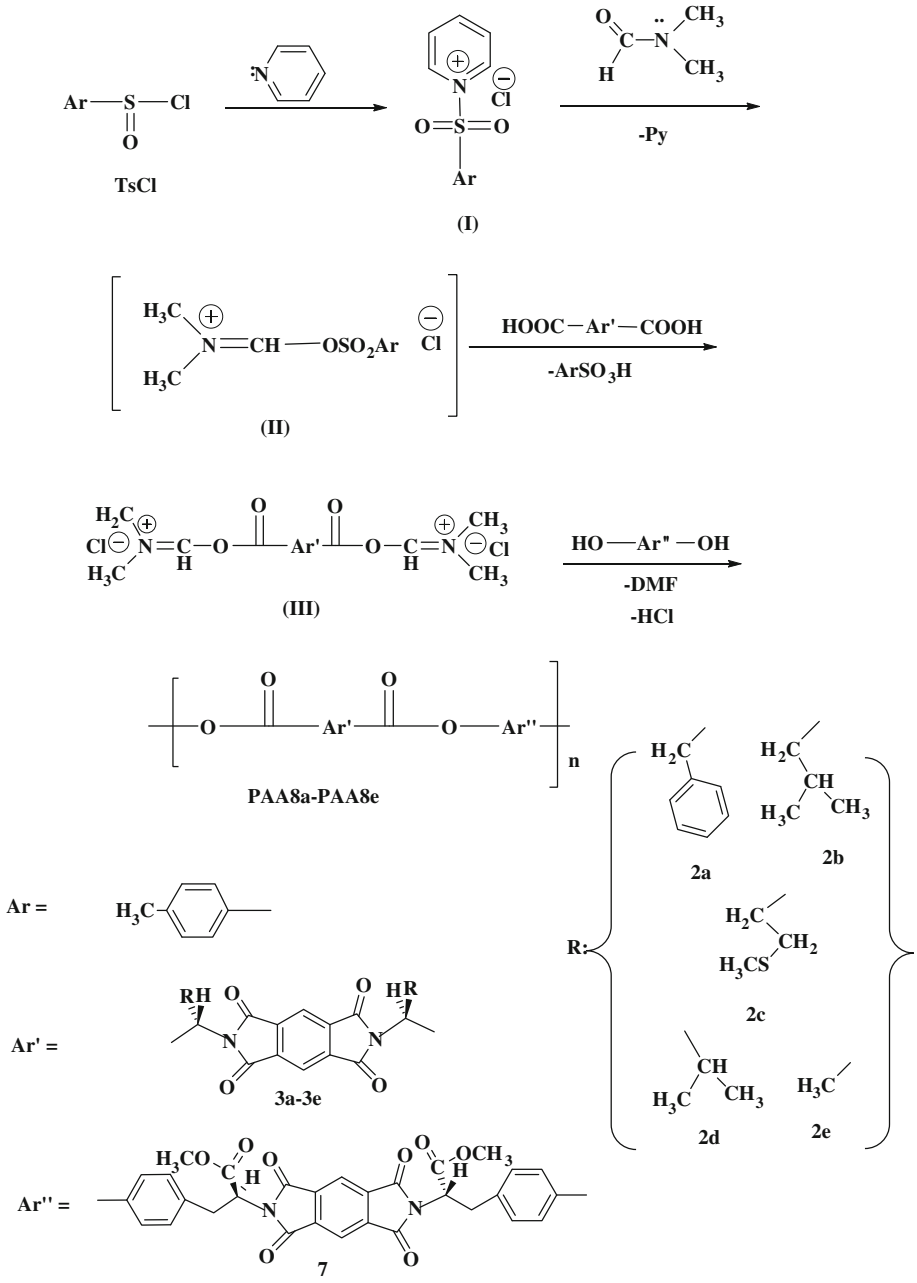


Scheme 1 Synthesis of optically active diacids (**3a–3e**)

Scheme 2 Synthesis of *N,N′*-(pyromellitoyl)-*bis*-L-tyrosine dimethyl ester



Scheme 3 Mechanistic representation of polycondensation reaction of diphenolic monomer **7** with different chiral diacids using TsCl/Py/DMF as a condensing agent



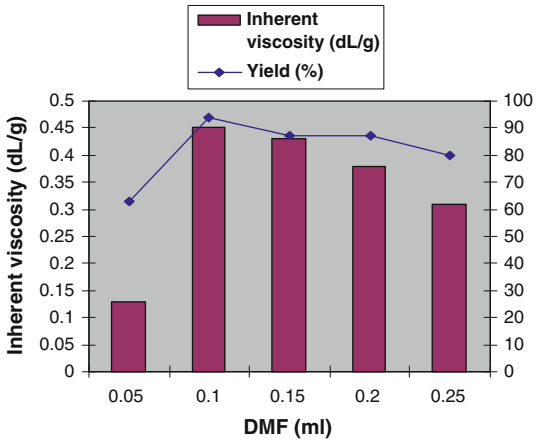


Fig. 1 Effect of the amount of DMF added to TsCl on the inherent viscosity and yield of **PAA8a**

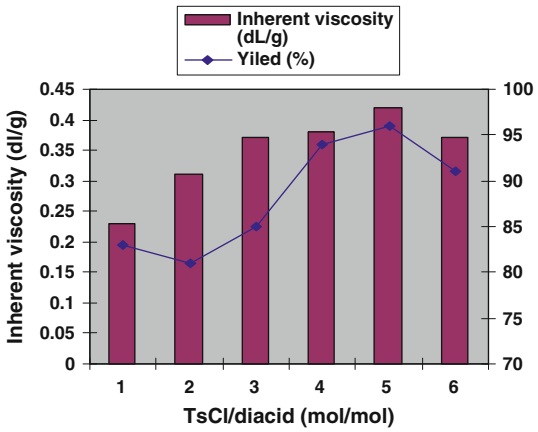


Fig. 2 Effect of the amount of TsCl added to diacid on the inherent viscosity and yield of **PAA8a**

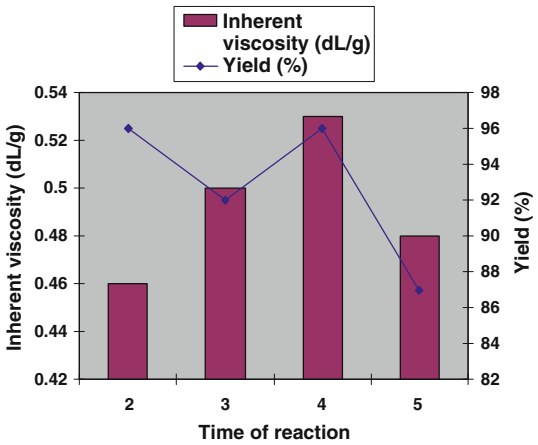


Fig. 3 Effect of reaction time on the inherent viscosity and yield of **PAA8a**

PAA8b–PAA8e are listed in Table 2. The inherent viscosities of the polymers under optimized condition were in the range of 0.43–0.63 dL g^{-1} and the yields were 83–96%.

Table 1 The optimum conditions for the preparation of **PAA8a**

Optimum condition	
TsCl/diacid (mol/mol)	5
Py/diacid (mol/mol)	31
DMF/diacid (mol/mol)	7
Aging time (min)	30
Reaction time (h)	4

Table 2 Synthesis and some physical properties of **PAA8a–PAA8e** prepared using TsCl/DMF/Py

Diacid	Polymer	Yield (%)	η (dL g^{-1})[a]	$[\alpha]_D^{25,a}$	Color
3a	**PAA8a**	96	0.53	−11.36	W
3b	**PAA8b**	95	0.63	−18.91	W
3c	**PAA8c**	87	0.43	−24.33	OW
3d	**PAA8d**	83	0.46	−13.80	OW
3e	**PAA8e**	92	0.44	−25.41	W

W white, *OW* Off-white

[a] Measured at a concentration of 0.5 dL g^{-1} in DMF at 25°C and consisting of 2%W/V LiCl

The incorporation of chiral units into the polymer backbone was confirmed by measuring the specific rotations of polymers with sodium source lamps (Table 2). The specific rotation of polymers based on different diacids showed random changes. As shown in Table 2, all of the polymers show optical rotations and are therefore optically active.

Polymer characterization

The obtained polymers were characterized by FT-IR, ^{1}H-NMR spectroscopy techniques and elemental analyses. The results are shown in Tables 3 and 4. All of these **PAAs** exhibited absorption at 1,380 and 728 cm^{-1} showing the presence of the imide heterocycle in these polymers (Table 3). The ^{1}H-NMR spectrum (500 MHz) of **PAA8c** is shown in Fig. 4. In the ^{1}H-NMR spectrum of this polymer, appearance of the methoxy protons (OCH_3) at 3.66 ppm as a single peak indicates the presence of ester groups in the polymer side chain. The protons of the two chiral centers appeared as two peaks in the range of 5.25–5.26 and 5.37–5.38 ppm, respectively. The resonance of aromatic protons appeared in the range of 6.80–7.24 ppm. Elemental analysis data of the resulting polymers are also in good agreement with calculated values of carbon, hydrogen and nitrogen in the polymers (Table 4).

Thermal properties

The thermal properties of some **PAA**s were evaluated by means of thermal gravimetric analysis (TGA). Thermal stability of the polymers was studied based on 5 and 10%

Table 3 ^{1}H-NMR and FT-IR characterization of **PAA**s

Polymer	Spectra data	
PAA8a	FT-IR peaks (cm^{-1}): 3,031 (w), 1,776 (m), 1,725 (s), 1,605 (w), 1,508 (m, sh), 1,455 (m), 1,383 (s), 1,363 (s), 1,195 (m, br), 1,113 (m), 917 (w), 728 (m), 701 (m)	^{1}H-NMR peaks (500 MHz, DMSO-d_6, TMS) (ppm): δ 3.20–3.23 (m, 8H), 3.68 (s, 6H), 5.28–5.29 (dd, 2H, J_1 = 10.73 Hz, J_2 = 3.77 Hz), 5.54–5.58 (dd, 2H, J_1 = 10.73 Hz, J_2 = 4.80 Hz), 6.80–6.82 (m, 2H, Ar–H), 6.90 (s, 4H, Ar–H), 7.14–7.24 (m, 12H, Ar–H), 8.19 (s, 4H, Ar–H)
PAA8b	FT-IR Peaks (cm^{-1}): 2,959 (m), 1,777 (m), 1,725 (s), 1,508 (m), 1,456 (w,br), 1,382 (s), 1,362 (m), 1,198 (m, br), 1,114 (w), 1,018 (m), 917 (w), 728 (m)	^{1}H-NMR Peaks (500 MHz, DMSO-d_6, TMS) (ppm): δ 0.85–0.90 (s, 6H), 0.95–0.97 (s, 6H), 1.51 (s, 2H), 1.93 (s, 2H), 2.11 (s, 2H), 3.31 (s, 4H), 3.66 (s, 6H), 5.25 (s, 4H), 6.86 (s, 4H, Ar–H), 7.17 (s, 4H, Ar–H), 8.15 (s, 2H, Ar–H), 8.30 (s, 2H, Ar–H)
PAA8c	FT-IR Peaks (cm^{-1}): 2,917 (m), 1,776 (m), 1,725 (s), 1,507 (m), 1,437 (w, br), 1,382 (s), 1,362 (m), 1,196 (m, br), 1,169 (w, br), 1,112 (m), 1,018 (w), 728 (m)	^{1}H-NMR Peaks (ppm): (500 MHz, DMSO-d_6, TMS): δ 1.99–2.04 (m, 10H), 2.37–2.58 (m, 4H), 3.31 (s, 4H), 3.66 (s, 6H), 5.25 (m, 2H), 5.37–5.38 (m, 2H), 6.84 (s, 4H, Ar–H), 7.18 (s, 4H, Ar–H), 8.16 (s, 2H, Ar–H), 8.33 (s, 2H, Ar–H)
PAA8d	FT-IR Peaks (cm^{-1}): 2,917 (m), 1,776 (m), 1,725 (s), 1,508 (m), 1,438 (w, br), 1,383 (s), 1,363 (m), 1,196 (m, br), 1,112 (w), 1,018 (w), 917 (w), 728 (m)	
PAA8e	FT-IR Peaks (cm^{-1}): 2,924 (w), 2,853 (w), 1,776 (w), 1,725 (s), 1,594 (w), 1,508 (m), 1,436 (m, br), 1,384 (m), 1,235 (s), 1,116 (m), 1,017 (m), 917 (w), 834 (m), 728 (m), 701 (m)	

Table 4 Elemental analysis of **PAA8a** and **PAA8b**

Polymer	Formula		Elemental analysis (%)		
			C	H	N
PA8Aa	($C_{58}H_{40}N_4O_{16}$)	Calculated	66.41	3.83	5.34
	MW(1,048.96)	Found	65.34	3.77	5.47
PAA8b	($C_{52}H_{44}N_4O_{16}$)	Calculated	63.67	4.52	5.71
	MW(980.92)	Found	62.81	4.44	5.66

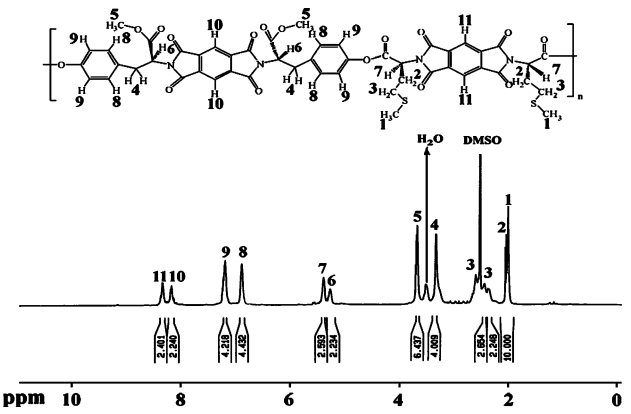


Fig. 4 ^{1}H-NMR (500 MHz) spectrum of **PAA8c** in DMSO-d_6 at RT

weight loss (T_5, T_{10}) of the polymers. The thermoanalysis data of these polymers are summarized in Table 5. According to Table 5, it can be concluded that the resulting polymers are thermally stable. The differential scanning calorimetry (DSC) technique was used to determine the T_g of the polymers. The heating rate was kept at 20°C min^{-1} under a flow of nitrogen. Figure 5 shows the DSC curves for **PAA8a** and **PAA8b**. These polymers showed T_g values in

Table 5 Thermal behavior of **PAA8a** and **PAA8b**

Polymer	Decomposition temperature (°C)		Char yield[c] (%)	T_g[d](°C)
	T_5[a]	T_{10}[b]		
PAA8a	363	390	30	116
PAA8b	328	341	15	80

[a] Temperature at which there was 5% weight loss

[b] Temperature at which there was 10% weight loss recorded by TGA at a heating rate of 10°C min^{-1} in a nitrogen atmosphere

[c] Percentage weight of material left undecomposed after TGA analysis at a maximum temperature of 800°C in a nitrogen atmosphere

[d] Glass transition temperature was recorded at a heating rate of 20°C min^{-1} in a nitrogen atmosphere

the range of 80–116°C, respectively (Table 5). The pure poly(L-tyrosine) showed a slight slope change around 185°C (Gupta and Lopina 2004), which implied that it hardly underwent glass transition. For **PAA8a** and **PAA8b**, thermal degradation temperatures were observed above 390 and 341°C, respectively. Compared to pure poly(L-tyrosine) (200°C), these obtained polymers showed potential for avoiding the risk of thermal degradation. The lower T_g values and high thermal degradation temperature values suggested a broad thermal processing temperature range. Hence, the L-tyrosine-based **PAA**s could be considered as biomaterial with significant engineering advantages.

Solubility of **PAA**s

The solubility of **PAA8a**–**PAA8e** was tested quantitatively in various solvents. All of the **PAA**s are soluble in organic polar solvents such as DMF, *N,N′*-dimethyl acetamide

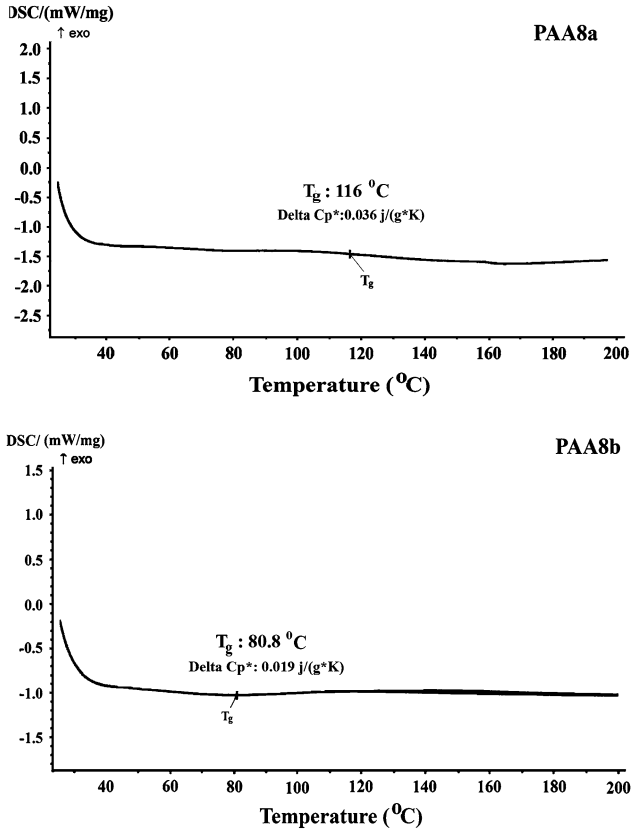


Fig. 5 DSC thermograms of **PAA8a** and **PAA8b** under N_2 atmosphere and a heating rate of 20°C min^{-1}

(DMAc), dimethyl sulfoxide (DMSO), *N*-methyl-2-pyrrolidone (NMP) and H_2SO_4 at room temperature and are insoluble in solvents such as chloroform, methylene chloride, methanol, ethanol and water (Table 6).

Fungal biodegradation

Figure 6a–c shows an overview of diacids **3a**, **3b** and **3c** cultured on Petri plates and colonized by saprophytic fungi. Figures 6d, f, 7g–i, show colonial growth of fungi on the diacid derived from L-phenylalanine (**3a**) and L-methionine (**3b**), respectively. This may indicate biodegradability of these diacids in the presence of fungal saprophytes. On the contrary, the diacid derived from L-leucine was not invaded by the fungi under the same condition (Fig. 6j–l). Interestingly, the L-leucine-derived diacid (**3b**) is intensively hydrophobic (Fig. 6k) possibly due to alkyl groups (isopropyl groups) preventing enzymatic activity and growth of microorganisms.

Fungal growth profiles of **BPA** as a blank (Mallakpour et al. 2010), diphenolic monomer **7** and **PAA8c** in Petri plate containing culture media are shown in Fig. 7a–c after

Table 6 Solubility properties of **PAA**s

Solvent	**PAA8a**	**PAA8b**	**PAA8c**	**PAA8d**	**PAA8e**
DMF	+	++	++	++	++
2%LiCl-DMF	+++	+++	+++	+++	+++
NMP	++	++	+++	+++	++
DMAC	++	+++	+++	+++	+++
CH_2Cl_2	−	−	−	−	−
$CHCl_3$	−	−	−	−	−
H_2O	−	−	−	−	−
DMSO	+++	+++	+++	+++	+++
CH_3CN	−	−	−	−	−
HOAC	±	±	±	±	±
EtOAC	−	−	−	+	−
MeOH	−	−	−	−	−
EtOH	−	−	−	−	−
Acetone	−	−	−	−	−
THF	−	−	−	−	−
H_2SO_4	++	++	+++	+++	+++

Solubility: measured at a polymer concentration of 5 mg mL^{-1}

+, Soluble at boiling temperature of the solvent; ++, soluble at boiling temperature of the water bath; +++, soluble at RT; ±, partially soluble at RT; −, insoluble

a period of 2, 3 and 4 weeks, respectively. Figure 7g–i on culture media PDA shows that synthetic diphenolic monomer **7** was almost completely covered and colonized by saprophytic fungi and they formed densely populated areas after 2, 3 and 4 weeks, respectively. As shown in Fig. 7j, k, **PAA8c** was wholly colonized by saprophytic fungi similar to diphenolic monomer **7** after 2 and 4 weeks, respectively. Figure 7l shows very clear images of fungal colonial growth in the presence of **PAA8c**. In contrast, **BPA** on the media was not invaded after 2, 3 and 4 weeks (Fig. 7d, h and f, respectively). It seems that **BPA** has toxic properties for the mentioned fungal growth than synthetic diphenolic monomer **7** at the period of experimental time, and it may have lower degradation rate under the soil burial conditions (Mallakpour et al. 2010).

Conclusions

The challenge of this research is the use of aromatic hydroxyl group of L-tyrosine amino acid as a new chiral nontoxic diphenolic monomer **7** candidate for the preparation of optically active **PAA**s. In designing of this monomer, some notification is considered. The protecting groups used to block the NH_2 and COOH of tyrosine have a significant impact on the properties of the obtained diphenolic monomer **7** and then on the resulting

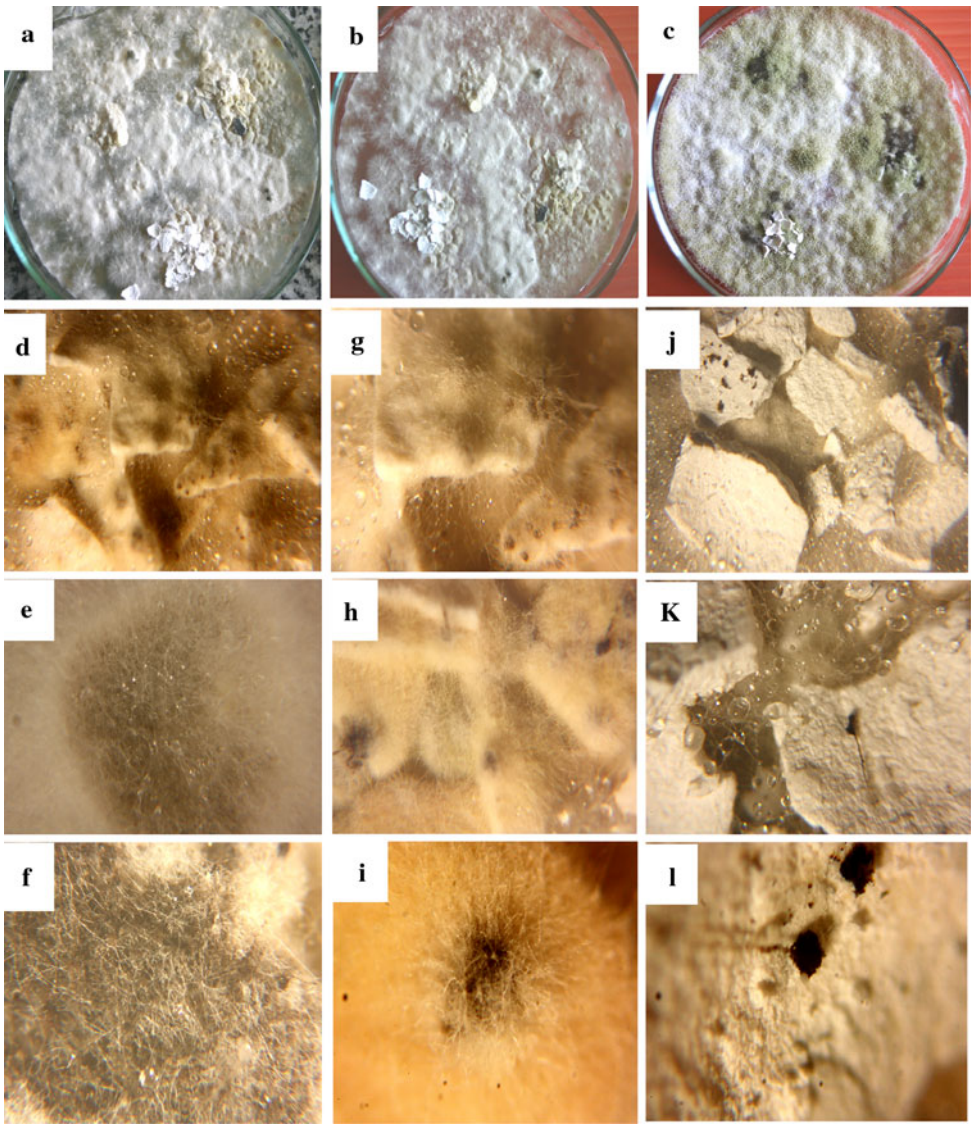


Fig. 6 Whole Petri plates containing diacids **3a**, **3b** and **3c** cultivated and incubated with saprophytic fungi at 23–25°C after 1 (**a**), 2 (**b**) and 4 weeks (**c**), respectively. Colonial growth around synthetic diacid **3a** (**d**, **e** and **f**) after 2, 3 and 4 weeks in Petri plates, respectively, and colonial growth around **3c** (**g**, **h** and **i**) at 23–25°C, after 2, 3 and weeks, respectively. Also inhibited growth of saprophytic fungi incubated with **3b** (**j**, **k** and **l**) after 2, 3 and 4 weeks in Petri plates, respectively

polymers, such as thermal stability due to the presence of imide linkages, solubility because of the existing pendant and polar methyl ester group in the side chain, and biodegradability and biological activity due to the presence of L-tyrosine amino acids in the main chain of the synthetic materials. Therefore, the combination of these different properties within one single design in the aromatic diol backbone was used to synthesis *N,N′*-(pyromellitoyl)-*bis*-L-tyrosine dimethyl ester, which may be considered as a nontoxic diphenolic monomer derived from L-tyrosine in three steps. This monomer is more thermally stable than **BPA** because its melting point (mp) is 168°C, while the MP of **BPA** is 158°C. This monomer is soluble in most organic solvents, while **BPA** has lower solubility; diphenolic monomer **7** is biologically active, while **BPA** has toxic properties and is not biodegradable under microbial effect. This type of biologically active monomer has a valuable potential for development of new bioactive polymers with biodegradable properties. Here also, a series of new optically active **PAA**s having *N,N′*-(pyromellitoyl)-*bis*-L-tyrosine dimethyl ester and *N,N′*-(pyromelitoyl)-*bis*-L-α-amino acids moieties were synthesized by the direct polycondensation method. Polymerization was performed by the reaction of chiral diacids containing some α-amino acids in the main chain with diphenolic monomer **7** using TsCl/DMF/pyridine as condensing agent. The resulting PAAs are thermally stable and are readily soluble in common organic solvents. In vitro toxicity studies also suggest that polymeric products have no inhibition effect against microbial growth at lower and high concentrations. Hence, these multiblock copolymers with good cytocompatibility are

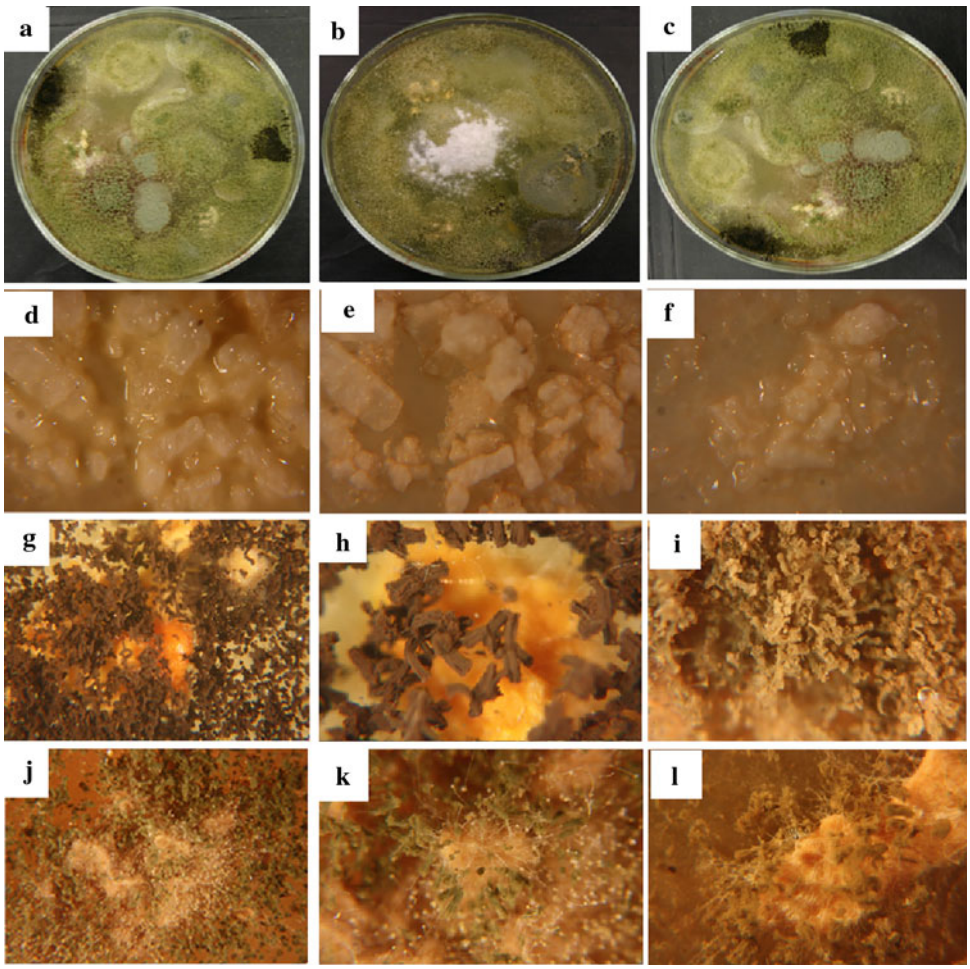


Fig. 7 Whole Petri plates containing diphenolic monomer **7**, **PAA8c** and **BPA** cultivated and incubated with saprophytic fungi at 23–25°C after 1 (**a**), 2 (**b**) and 4 weeks (**c**), respectively. Also inhibited growth of saprophytic fungi incubated with **BPA** (**d**–**f**) versus their colonial growth around synthetic diphenolic monomer **7** (**g**, **h** and **i**) after 2, 3 and 4 weeks in Petri plates, respectively, and colonial growth around **PAAc** (**j** and **k**) at 23–25°C, after 2 and 4 weeks, respectively. High resolution photographs of colonial growth of fungal on **PAA8c** (**l**)

favorable candidates as biodegradable and biologically active polymers.

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