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Polymer Chemistry

Synthesis of Biomimetic Polymers Based on Nonproteinogenic α-Amino Acids

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Nonproteinogenic amino acids (NPAAs) represent promising building blocks for constructing biologically active and functional polymers. From this point, one of the most promising are NPAAs containing unsaturated bonds in the lateral chains. Among biologically active and functional polymers an increased attention was attracted by pseudoproteins (PPs) – a family of biomimetic biodegradable polymers made from bis-(amino acid) alkylene diesters (diamine-diesters, DADEs). The present work deals with the first successful synthesis of PPs of poly(ester amide) (PEA) class (PP-PEAs) on the basis of unsaturated NPAAs such as allylglycine (AlG) and propargylglycine (PrG). The high-molecular-weight PP-PEAs (M_w up to 51,300) were obtained using a method of step growth polymerization - Interfacial Polycondensation (IP) of AlG/PrG based di-p-toluenesulfonic acid salts of DADEs (TDADEs) with sebacoyl chloride (SC). The obtained polymers were insoluble in water but soluble in a number of organic solvents. The PP-PEAs synthesized are of interest as prolonged acting biologically active materials (e.g. nanoparticles) as well as precursors for subsequent chemical modifications, e.g. to prepare biodegradable hydrogels by photo-crosslinking or via alkyne-azide click reactions, etc. In general, the construction of biomimetic polymers on the basis of NPAAs substantially expands a set of available functionalities which are less accessible for naturally occurring polymers – proteins. *© 2021 Bull. Georg. Natl. Acad. Sci.*

Biomimetic polymers, pseudoproteins, poly(ester amide)s, nonproteinogenic amino acid, allylglycine, propargylglycine

Amino acids (AAs), playing a central role in all processes of live cells, are widely used for construting molecules with specific biological activity. In the synthesis of peptides/polypeptides and other biopolymers naturally occuring (proteinogenic) AAs are typically used [1-3]. However, during the last 20 years enantiomerically enriched nonproteinogenic amino acids (NPAAs) containing different functional lateral groups attracted an increased attention. The NPAA showed various activities suh as antimicrobial and

antiherbivory activity, protection from stress, cell signaling, nitrogen storage, as toxins against invertebrates and vertebrates and as allelochemicals [4].

Among NPAAs those ones containing unsaturated chemical bonds in the lateral chains (unsaturated NPAAs) are of special interest. Such amino acids were used as selective inhibitors of endothelin-converting enzymes, inhibitors of thrombin and cathepsin B, inactivators of pyridoxalphosphate-dependent γ-cystathionase, growth inhibitors of B. Subtillis B-50, etc. [5-11].

Pseudoproteins (PPs) represent a relatively new family of biomimetics - artificial amino acis based biodegradable polymer made by step growth polymerization of diamine-diesters (DADEs) with various bis-electrophiles [12-14]. The present paper deals with the synthesis of new PPs on the basis of unsaturated NPAAs such as AlG and PrG. Such kind of PPs are of interest as both prolonged acting bioactive materials and precursors for subsequent transformations. We have focused on the PPs of poly (ester amide) (PEA) class which were synthesized using a method of step growth polymerization – Interfacial Polycondensation (IP).

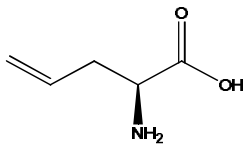
Materials and Methods

Materials. 1,6-hexanediol, p-toluenesulfonic acid monohydrate (TosOH.H_2O), cyclohexane, sebacic acid (SA), phosphorous pentachloride, dichloromethane (DCM), 1,6-hexanediol (HD) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification; NPAAs – L-Allylglycine (ALG) and L-propargylglycine (PrG) were synthesized at the Institute of Pharmacy of Yerevan State University.

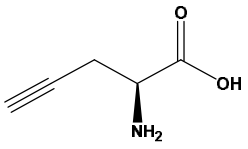
General instrumentation. Thermo Nicolet Avatar 370 FT-IR spectrophotometer (coupled with EZ OMNIC software) was used for IR analysis. FT-IR spectra were recorded using Avatar Multi-Bounce Flat Plate 45 degree Ge. Molecular weight measurements were performed by gel permeation chromatography (GPC) using Waters 1525 system equipped with three consecutive Styragel columns (HR4, HR3, HR0.5 all 7.8 mm x300 mm) calibrated by PMMA standards (from Aldrich), and refractive index detector (Waters 2414). GPC measurements were carried out in a 0.1 M solution of LiBr in DMF at a flow rate of 1.0 $mL \cdot min^{-1}$, injection volume 100 μL, and sample concentration 5.0 $mg \cdot mL^{-1}$.

Synthesis of bis-nucleophilic monomers – DADEs composed of NPAAs. For synthesizing new bis-nucleophilic DADE monomers we have employed two unsaturated NPAAs α-monosubstituted AlG and PrG (Fig. 1). The DADEs were synthesized by direct condensation of NPAA with a diol (HD) in an refluxed organic solvent (cyclohexane).

TDADE from AlG and HD - di-p-toluenesulfonic acid salt of bis-(allylglycine)-1,6-hexylen diester (AlG6). For the synthesis of AlG6 (Scheme 1) a mixture of 1.84 g (0.016 mol) of AlG, 0.95g (0.008 mol) of HD and 3.35g (0.0176 mol - a slight excess) of TosOH·H_2O was refluxed in 175 mL of cyclohexane in the presence of 50 mg of copper powder for 24 h. 0.6 mL of water (0.0336 mol) liberated after the reaction was collected in a Dean-Stark apparatus. The precipitated white sticky product was filtered off, dried in a vacuum at 60°C. The goal product – AlG6 was recrystallized from acetone/ethanol mixture two times and had m.p.154-155°C. The yield was 71%. FT-IR (fine powder on Ge plate), cm^{-1}: (2900-3230 C-H, NH_3^+), 1736 (C=O ester), 1173 (C-O-C) .



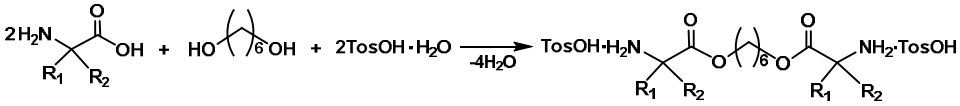
L-Allylglycine (AlG)



L-Propargylglycine (PrG)

Fig. 1. NPAAs used for synthesizing the new bis-nucleophilic monomers – DADEs.

TDADE from PrG and HD - di-p-toluenesulfonic acid salt of bis-(propargylglycine)-1,6-hexylen diester (PrG6). For the synthesis of PrG6 (Scheme 1) a mixture of 1.81 g (0.016 mol) of PrG, 0.95g (0.008 mol) of HD and 3.35g (0.0176 mol – 10% mole excess) of TosOH·H_2O was refluxed in 175 mL of cyclohexane in the presence of 50 mg of copper powder for 24 h. 0.6 mL of water (0.0336 mol) liberated after the reaction was collected in a Dean-Stark apparatus. The precipitated white sticky product was filtered off, dried in a vacuum at 60°C. The goal product – PrG6 was recrystallized from ethanol two times and had m.p. 181-185ºC. The yield was 47%. FT-IR (fine powder on Ge plate) cm^{-1}: 2900-3230 (C-H, NH_3^+), 1736 (C=O ester), 1180 (C-O-C).

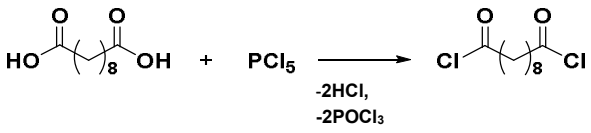


Where: $R_1 = H$, $R_2 = CH_2CH{=}CH_2$ (AlG)

$R_1 = H$, $R_2 = CH_2C{\equiv}CH$ (PrG)

Scheme 1. The synthesis of bis-nucleophilic monomers TDADEs based on NPAAs.

Synthesis of a bis-electrophilic monomers - sebacoylcchloride (SC). For the synthesis of sebacoyl chloride (SC) (Scheme 2) 2.02g (0.01 mol) of powdery SA was mixed with 4.16 g (0.02 mol) of a solid phosphorus pentachloride. The mixture was thoroughly ground, heated and stirred when the liquid phase was arisen. The mixture was stirred at 40°C for 6 h, then cooled to r.t. and kept overnight. Next day the mixture was heated to 40°C and the released $POCl_3$ was completely removed under reduced pressure. The obtained SC, without further purification, was used for synthesizing the target PPs of PEAs class (PP-PEAs) by IP [12,13].

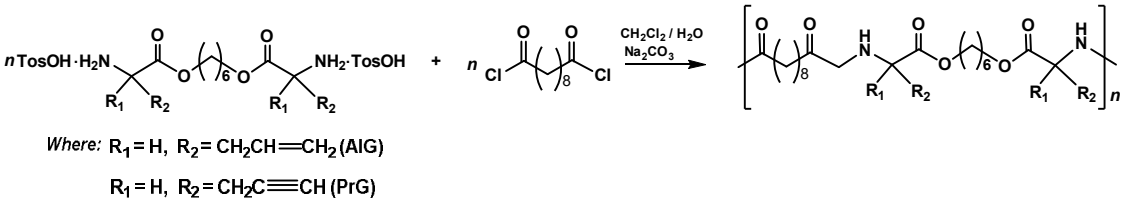


Scheme 2. The synthesis of bis-electrophilic monomer – SC.

Polymer Synthesis

Synthesis of PP-PEAs via IP. Two NPAA-based PP-PEAs were synthesized by IP according to Scheme 3 [12,13]. For this, AlG (or PrG) based TDADE - AlG6 (or PrG6) was interacted with SC in two phase system CH_2Cl_2/water + Na_2CO_3. In brief, 0.42 mL (2 mmol) SC was dissolved in anhydrous DCM (6 mL) and then the solution was added dropwise to an aqueous solution (6 mL) of 1.3136 g (2.0 mmol) AlG6 (or 1.3096 g (2.0 mmol) PrG6) and 0.553 g (4.0 mmol) potassium carbonate. The reaction mixture was vigorously stirred

for additional 15-20 min and left quiet until the two phase systems were clearly formed. After that, the DCM layer was separated using funnel, washed several times with water and dried over anhydrous sodium sulfate. Afterwards, sodium sulfate was filtered off and dried polymer solutions were poured onto the weighed Teflon Petri dishes and kept in a hood at r.t. for 3 days. The Petri dishes then were dried in a vacuum at 80°C up to constant weights. The resulted PEAs films were removed from the Teflon Petri dishes. The PEAs were characterized by FT-IR: for this small peaces of the films were dissolved in DCM and cast on NaCl plates; the main absorption bands, cm^{-1}: 2850-3330 (C-H, -CO-NH.), 1735 (C=O ester), 1650 (CO amide), 1196 (C-O-C).



Scheme 3. The synthesis of the PP-PEAs *via* IP.

Results and Discussion

Monomer synthesis. The syntheses of new bis-nucleophilic monomers – DADEs composed of NPAAs AlG and PrG were carried out by direct condensation of an AA (2 moles) with 1,6-hexanediol (1 mole) in the presence of *p*-toluenesulfonic acid monohydrate (TosOH·H_2O) (2.2 moles) which was used as both amino groups protector and the condensation reaction catalyst (Scheme 1). Accordingly, the DADEs were obtained in the form of stable di-*p*-toluenesulfonic acid salts - TDADEs. The synthesis of TDADE-monomers has been modified by replacing toxic benzene and toluene (which were previously used in such kind of synthesis [12,13,15]) with by far less toxic cyclohexane. The condensation reation according to Scheme 1 was carried out in the presence of copper powder which was used to inhibit undesirable polymerization of lateral unsaturated substituents. The assumed structures of the NPAAs-based monomers were in accordance with the data of FTIR spectra and elemental analysis.

Long-chain dichloride such as SC was selected as a bis-electrophilic counter partner for the synthesis of the goal PP-PEAs *via* the IP. The polycondensation grade SC of a high purity was obtained by gentle heating 1 mole of sebacic acid with 2 moles of phosporus pentachloride (Scheme 2).

Polymer synthesis. PPs of poly(ester amide) class (PP-PEAs) were obtained on the basis of crystalline NPAA-based TDADEs such as AlG6 and PrG6 by their IP with SC, according to Scheme 3. The PP-PEA - 8AlG6 showed excellent molecular weight characteristics (M_w 51,300, M_n 25,900, M_w/M_n 1.97). The PP-PEA - 8PrG6 also had rather good molecular weight (M_w 10,300, M_n 6,600, M_w/M_n 1.55) enough to fabricate stable nanoparticles on its basis. Both PP-PEAs showed good solubility in organic solvens (DMF, DMSO, DCM, ethanol) but were insoluble in water that restrcits examination of their biological activities. However, solubility of the PP-PEAs in organic solvents makes them promising for fabricating nanoparticles with anticipated biological activities.

Conclusion

New pseudoproteins (PPs) of poly(ester amide) class (PP-PEAs) composed of the PP-PEAs were obtained. For this the NPAAs were transformed into corresponding TDADE monomers by direct condensation with

1,6-hexanediol in refluxed cyclohexane. The PP-PEAs were synthesized by IF of TDADE monomer with SC. The obtained PP-PEAs had good molecular mass characteristics (M_w within 10,300-51,300, Mn within 6,600-25,900). The polymers were insoluble in water but were soluble in organic solvents that makes them promising for fabricating nanoparticles with anticipated biological activities. Besides, the new PP-PEAs are of interest as precursors for preparing useful biodegradable materials *via* subsequent chemical modifications, e.g. hydrogels by photo-crosslinking in the case of AlG-based polymers, or *via* alkyne-azide click reactions in the case of PrG-based polymers.

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პოლიმერების ქიმია

ბიომიმეტიკური პოლიმერების სინთეზი არაპროტეინოგენული α-ამინომჟავების საფუძველზე

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[#]*სომხეთის რესპუბლიკის სამეცნიერო და საწარმოო ცენტრი "არმბიოტექნოლოგია", ერევანი, სომხეთის რესპუბლიკა*

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არაპროტეინოგენული ამინომჟავები (NPAA) წარმოადგენს პერსპექტიულ საშენ ბლოკებს ბიოლოგიურად აქტიური და ფუნქციური პოლიმერების შესაქმნელად. ამ თვალსაზრისით, ერთ-ერთი ყველაზე პერსპექტიულია გვერდით ჯაჭვებში უჯერი ბმების შემცველი NPAA. ბიოლოგიურად აქტიურ და ფუნქციურ პოლიმერებს შორის განსაკუთრებულ ყურადღებას იპყრობს ფსევდოპროტეინები (PPs) – ბიომიმეტიკური ბიოდეგრადირებადი პოლიმერების ახალი კლასი ბის-(ამინომჟავა) ალკილენ დიესტერების (დიამინო დიესტერები, DADE) საფუძველზე. წინამდებარე ნაშრომი ეძღვნება პოლი (ესტერამიდების) კლასის ფსევდოპროტეინების (PP-PEA) პირველ წარმატებულ სინთეზს უჯერი NPAAs-ის ალილგლიცინისა (AlG) და პროპარგილგლიცინის (PrG) საფუძველზე. მაღალმოლეკულური PP-PEA (Mw 51,300-მდე) მიღებულია ჯაჭვური პოლიმერიზაციის ერთ-ერთი მეთოდის – ფაზათაშორისი პოლიკონდენსაციის (IP) გამოყენებით, კერძოდ, ალილ-გლიცინის/პროპარგილგლიცინის საფუძველზე მიღებული დიამინო დიესტერების დი-პ-ტოლუოლსულფონატების (TDADE) ურთიერთქმედებით სებაცოილ ქლორიდთან (SC). დასინთეზებული პოლიმერები PP-PEAs წყალში უხსნადია, თუმცა იხსნება მთელ რიგ ორგანულ გამხსნელებში; აქედან გამომდინარე, აღნიშნული ფსევდოპროტეინები საინტერესოა როგორც ბიოლოგიურად აქტიური მასალები (მაგ. ნანონაწილაკები), აგრეთვე შესაძლებელია მათი ქიმიური მოდიფიკაცია, მაგ. ბიოდეგრადირებადი ჰიდროგელების მიღება ფოტო-შეკერვით, აზიდ/ალკინურ კლიკ-რეაქციებში მონაწილეობა და სხვ. ზოგადად, ბიომიმეტიკურ ფსევდოპროტეინებს არაპროტეინული ამინომჟავების საფუძველზე გააჩნიათ საკმაოდ გაზრდილი ფუნქციონალურობა ბუნებრივი წარმოშობის პროტეინებთან შედარებით.

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