Express Polymer Letters Vol.16, No.9 (2022) 933–938
Available online at www.expresspolymlett.com
https://doi.org/10.3144/expresspolymlett.2022.68

Research article

Enzyme-catalyzed amine-functionalization of poly(ethylene-glycol)

Judit E. Puskas[1*], *Enikő Krisch*[1], *Aswathy Sasidharan Pillai*[1], *Prajakatta Mulay*[2]

[1]The Ohio State University, 281 W Lane Ave, 43210 Columbus, OH, USA
[2]Cleveland Clinic, 9500 Euclid Ave, 44195 Cleveland, OH, USA

Received 3 January 2022; accepted in revised form 19 April 2022

Abstract. This paper presents a new method for the amine-functionalization of poly(ethylene glycol) (PEG) of M_n = 2050 g/mol via *Candida antarctica* lipase B (CALB)-catalyzed esterification of *tert*-butyloxycarbonyl (*t*BOC)-protected β-Alanine and L-Alanine. NMR showed full conversion for protected β-Alanine, and MALDI-ToF demonstrated the purity of the product. After deprotection, the desired diamine-functionalized PEG was obtained. Protected L-Alanine did not reach full conversion by NMR, likely due to the steric hindrance of its methyl side group.

Keywords:*tailor-made polymers, polymer molecular engineering, biocompatible polymers, poly(ethylene glycol) diamine, enzyme catalysis*

1. Introduction

Poly(ethylene glycol) diamine (PEG-diamine) is a versatile intermediate that plays an important role in biomedical applications such as the development of controlled drug release systems [1]. It can also be used for the surface modification of hemoglobin or other proteins for pharmacological use [2] and as a crosslinking agent in the preparation of hydrogels [3]. Several methods have been published in the literature for the synthesis of PEG-diamine. In general, the HO– terminal groups of PEG are converted first to a good leaving group, followed by nucleophilic displacement and other modifications to yield the desired product. [4–24]. We found two reports for using butyloxycarbonyl (BOC)-protected amino acids for PEG-diamine synthesis: N-(BOC)-11-aminoundecanoic acid and N-(BOC)-phenylalanine were reacted with PEG activated using DCC/EDC/DMAP, yielding di-BOC functionalized PEGs that on deprotection produced PEG-diamines [25, 26]. All reactions had multiple steps and used chemical catalysts.

In the spirit of green chemistry, we developed a new synthetic strategy for the synthesis of H_2N-PEG-NH_2. Our group pioneered enzyme-catalyzed polymer functionalization, leading to quantitative reaction under mild conditions [27–30]. Based on the successful transesterification of lipoic acid with tetraethylene glycol (TEG) [31], we theorized that the carboxylic acid end-group of an amino acid would react with the hydroxyl end groups of TEG and PEG using *Candida antarctica* lipase B (CALB) catalyst. Figure 1 shows the reaction.

The proposed approach using CALB catalysis would be a 'greener' method when compared to chemical catalysis [25, 26]. We have shown earlier that CALB-catalyzed PEG functionalization is possible up to M_n = 10 000 g/mol [27], so this method would be very advantageous for hydrogel synthesis and other applications requiring relatively low molecular weight.

This paper presents the results of our investigation.

*Corresponding author, e-mail: puskas.19@osu.edu

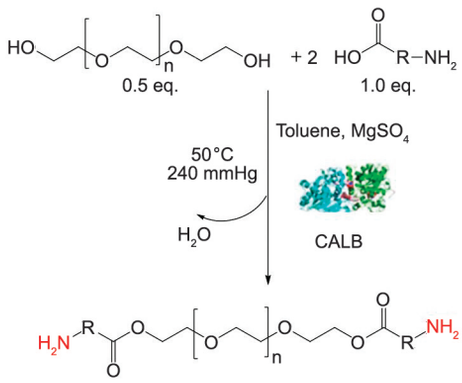

Figure 1. Reaction scheme for the CALB-catalyzed synthesis of PEG-diamine.

2. Experimental

2.1. Materials

L-Alanine (L-Ala, 98+%, Aldrich, Burlington, MA, USA), β-Alanine (β-Ala, 99+%, Aldrich, Burlington, MA, USA), Di-*tert*-butyl dicarbonate (99%, Aldrich, Burlington, MA, USA), triethylamine (TEA, ≥99.5%, Sigma-Aldrich, Burlington, MA, USA), magnesium sulfate ($MgSO_4$, anhydrous, EMD), tetraethylene glycol (TEG, 99%, Aldrich, Burlington, MA, USA), *Candida antarctica* lipase B (CALB, 33 273 Da, 20 wt% immobilized on a macroporous acrylic resin Novozyme® 435) (Aldrich, Burlington, MA, USA), poly(ethylene glycol) (M_n = 2050 g/mol, PEG_{2050}, Aldrich, Burlington, MA, USA), toluene (anhydrous, 99.8%, Sigma-Aldrich, Burlington, MA, USA), methanol (MeOH, 99.8%, Fisher Chemical, Waltham, MA, USA), tetrahydrofuran (THF, contains about 0.026% butylated hydroxytoluene as a preservative, Fisher Chemical, Waltham, MA, USA) and hexane (99%, ACS reagent, Acros Organics, New Jersey, USA) were all used as received.

2.2. Procedures

2.2.1. Protection of L-Alanine and β-Alanine

L-Ala (0.3030 g, 3.40 mmol, 1.0 eq.) or β-Ala (0.3093 g, 3.47 mmol, 1.0 eq.) and Di-*tert*-butyl dicarbonate (1.1790 g, 5.40 mmol, 1.59 eq.) were dissolved in MeOH (5 ml). TEA (0.44476 g, 4.42 mmol, 1.30 eq.) was then added dropwise to the mixture, and the contents were stirred for 60 minutes at *RT* and then refluxed for an additional 60 minutes at 65 °C (Figure 2, STEP 1). The MeOH was evaporated using a rotavap, and the reaction mixture was then dried under vacuum at *RT* to recover the products (*t*BOC-L-Ala or *t*BOC-β-Ala).

2.2.2. Synthesis of *t*BOC-L-Ala-PEG2050-L-Ala-*t*BOC and *t*BOC-β-Ala-PEG_{2050}-β-Ala-*t*BOC

PEG_{2050} (3.4766 g, 1.7 mmol, 0.5 eq.) was dried under vacuum at 65 °C and 0.2 Torr for 16 hours (Figure 2, STEP 2). The dried PEG was dissolved in toluene (3 ml), and the temperature was reduced to 50 °C. *t*BOC-L-Alanine (0.6407 g, 3.38 mmol, 1.0 eq.) or *t*BOC-β-Alanine (0.6569 g, 3.47 mmol, 1.0 eq.), CALB (0.3072 g resin at 20 wt% enzyme, $1.84 \cdot 10^{-3}$ mmol, 0.00054 eq.), and $MgSO_4$ (0.1 g) were added to the mixture and the pressure was reduced to 240 mmHg. After 24 hours, the reactor contents were diluted with 3 ml of THF and centrifuged for 30 minutes. The polymer was purified by precipitation into hexane twice, and the product was dried under vacuum at *RT* until constant weight.

2.2.3. Deprotection

*t*BOC-β-Ala-PEG_{2050}-β-Ala-*t*BOC (2.6662 g, 1.115 mmol, 1.0 eq.) was dissolved in ethyl acetate at *RT* followed by the addition of methanol (0.3 ml, 7.41 mmol, 6.65 eq.). Acetyl chloride (0.5 ml, 7 mmol, 6.28 eq.) was then added dropwise to the reaction mixture. The reaction was continued for 15 hours at room temperature. The ethyl acetate and excess methanol from the reaction mixture were then removed using a rotavap. The product was then dissolved in 3 ml THF and precipitated in hexane twice to recover the product, which was then dried in a vacuum oven at *RT* until constant weight.

2.2.4. NMR

^{1}H-NMR spectra were recorded on a Varian NMRS 500 spectrometer (Varian, Palo Alto, CA, USA) using deuterated chloroform (Chemical Isotope Laboratories, 99.8% $CDCl_3$, Tewksbury, MA, USA) as solvent. The resonance of non-deuterated chloroform at δ = 7.27 ppm was used as an internal reference.

2.2.5. MALDI-ToF

MALDI-ToF mass spectra were acquired with a Bruker UltraFlex-III time-of-flight (ToF) mass spectrometer (Bruker Daltonics, Billerica, MA) equipped with a Nd:YAG laser (355 nm), a two-stage gridless reflector, and a single-stage pulsed ion extraction source. Separate THF (anhydrous, 99.9%, Aldrich, Burlington, MA, USA) solutions of polymer (10 mg/ml), 1,8,9-anthracenetriol (dithranol,

20 mg/ml, >97%, Alfa Aesar, Haverhill, MA, USA), sodium trifluoroacetate (10 mg/ml, >98%, Aldrich) or silver trifluoroacetate (10 mg/ml, 98%, Aldrich, Burlington, MA, USA) were mixed in a ratio of 10:1:2 or 14:1:4 (matrix:cationizing salt:polymer), and 0.5 µl of the resulting mixture was introduced on to the MALDI target plate and allowed to dry. The spectra were obtained in reflection mode. The attenuation of the nitrogen laser was adjusted to minimize unwanted polymer fragmentation and to maximize the sensitivity. The calibration of the mass scale was carried out externally using a poly(methyl methacrylate) or polystyrene standard having a similar molecular weight as the sample.

3. Results and discussion

Alanine was selected as the amino acid for the studies. There are two types of alanine present in nature, α and β, so we decided to try both. α-Alanine has a methyl side group, and due to its chiral center, two stereoisomers, L and D. D-Alanine is toxic to living systems [32], so L-Alanine (L-Ala) was tested. β-Alanine is a straight-chain amino acid. Unfortunately, neither L-Alanine nor β-Alanine reacted with TEG or PEG in the presence of CALB so the direct functionalization shown in Figure 2 did not work. We thought that the possible reason was zwitterion formation that CALB could not accommodate, thus the amine groups were protected with *tert*-butyloxycarbonyl (*t*BOC) before the esterification. Figure 2 displays the ^{1}H-NMR spectra of protected β-Ala and L-Ala, but these are also available commercially.

3.1. Functionalization using L-Alanine

*t*BOC-protected L-Alanine was reacted with PEG_{2050} and Figure 2 shows the ^{1}H-NMR spectrum. The signal corresponding to the methine proton from *t*BOC-L-Alanine (f'') appears at δ = 4.25 ppm, overlapping with the signals of (b'). The $–CH_3$ protons from *t*BOC appear at δ = 1.46 ppm.

When the integral value is set to 18, the main chain proton integral (d) at 200.76 translates to 50 repeat units instead of the theoretical 44, which indicates less than full conversion. MALDI-ToF verified the presence of H_2N-L-Ala-PEG_{2050}-L-Ala-NH_2 at m/z = 2186.710, together with some monosubstituted product at m/z = 2186.710.

3.2. Functionalization using β-Alanine

*t*BOC-β-Ala was reacted with PEG_{2050} in the presence of CALB. Figure 3 displays the ^{1}H-NMR spectrum of *t*BOC-β-Ala-PEG_{2050}-β-Ala-*t*BOC. The $–CH_2–$ protons from *t*BOC-β-Ala moved downfield from δ = 2.40 ppm to δ = 2.55 ppm after esterification (f''). The relative integrals of (f''):(j) are in the ratio of 4.12:18.00. The sidebands of the PEG_{2050} backbone signal (d) overlap with the signal of the $–CH_2–$ protons next to the amide groups (g') at 3.4 ppm, hence the integral of signal (g') is 5.22, higher than the expected value of 4. A new peak appeared at δ = 4.25 ppm (b'), which belongs to the $–CH_2–$ protons next to the newly formed ester group. The relative ratios of the end group signals (j) and (f'') to the main chain protons of PEG (d) indicate 44 repeat units as expected from the M_n = 2050 g/mol of the PEG.

Figure 4 shows the MALDI mass spectrum of *t*BOC-β-Ala-PEG_{2050}-β-Ala-*t*BOC. It has a single distribution, indicating the formation of very pure disubstituted PEG_{2050} with no traces of monosubstituted or unreacted PEG_{2050}. The signals were at a distance of 44 Da from each other, that corresponds to a repeat unit. As an example, the signal marked at m/z 2144.332 g represents the Na complex of a 38-mer unit of *t*BOC-β-Ala-PEG_{2050}-β-Ala-*t*BOC. The theoretical m/z for this signal is = 2144.332

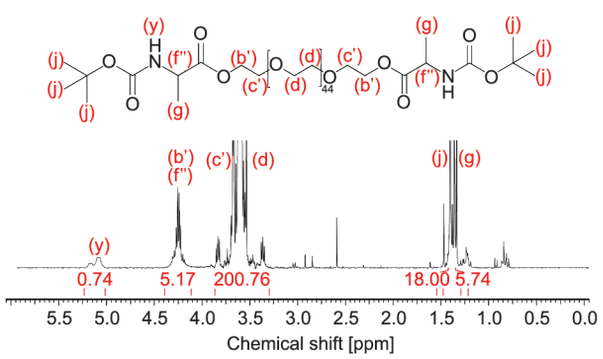

Figure 2. ^{1}H-NMR spectrum of *t*BOC-L-Ala-PEG_{2050}-L-Ala-*t*BOC.

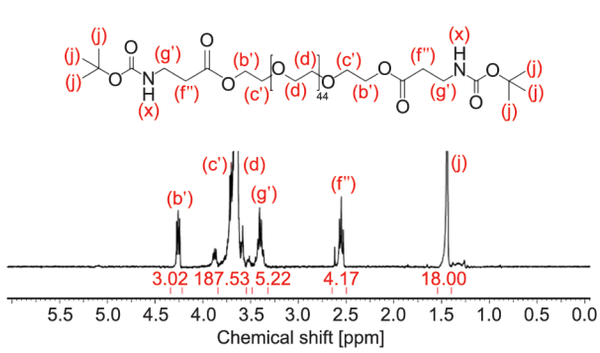

Figure 3. ^{1}H-NMR spectrum of *t*BOC-β-Ala-PEG_{2050}-β-Ala-*t*BOC.

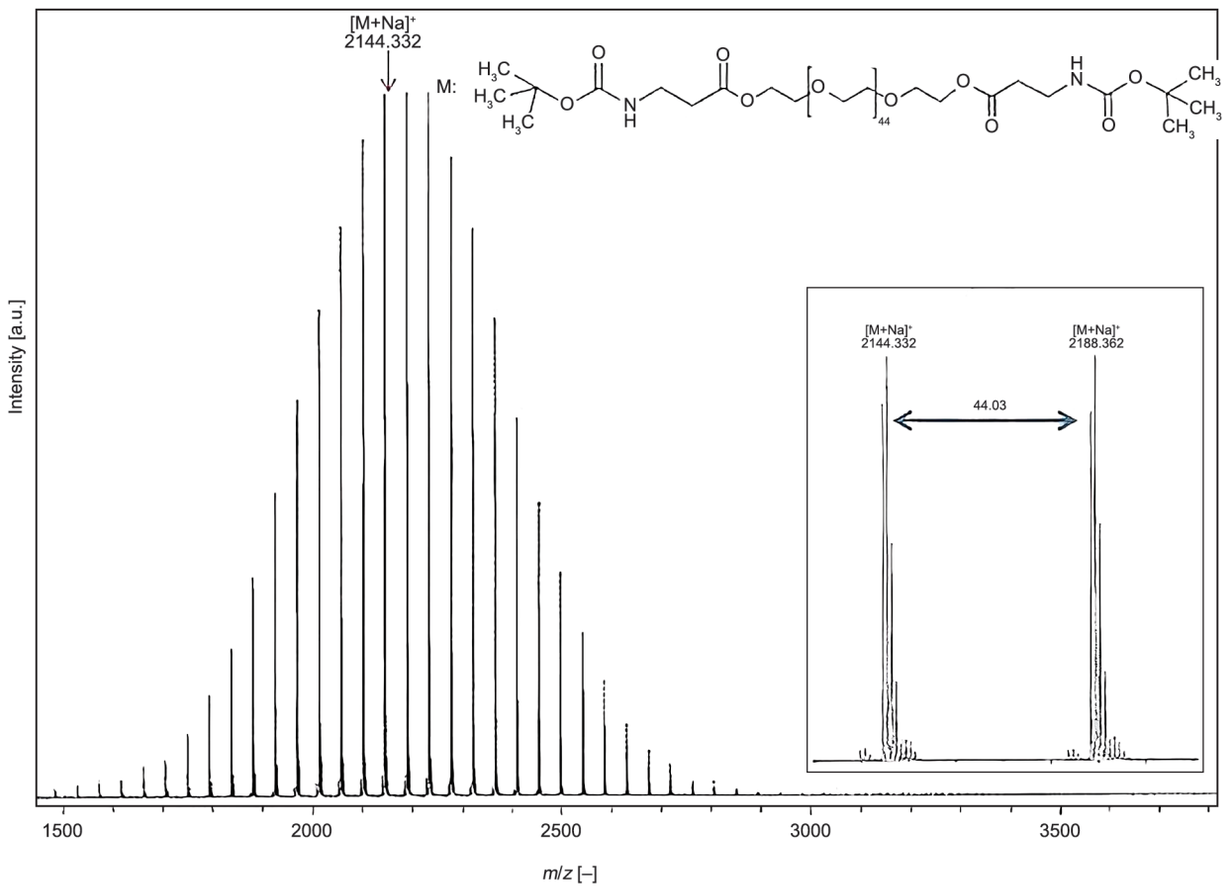

Figure 4. MALDI mass spectrum of *t*BOC-β-Ala-PEG_{2050}-β-Ala-*t*BOC. Inset: spectrum of 38- to 39-mer fractions, 44 *m*/*z* = PEG repeat unit.

[38×44.03 (C_2H_4O repeat unit) + 216.26 ($(CH_3)_3CHOCONHC_2H_4COOC_2H_4$ end-group) + 232.26 ($(CH_3)_3CHOCONHC_2H_4COOC_2H_4O$ end-group) + 22.99 (Na+)]. Thus MALDI-ToF confirms that each chain in the *t*BOC-β-Ala-PEG_{2050}-β-Ala-*t*BOC carries two *t*BOC-β-Alanine end groups, demonstrating the efficiency of CALB-catalyzed polymer functionalization.

The next step was deprotection of the product to obtain PEG-diamine. Many deprotection methods are available to yield diamine-functionalized PEG (H_2N-PEG-NH_2) [33–38]. Lin *et al*. [36] reported the deprotection by using concentrated H_2SO_4 in *tert*-butyl acetate or $MeSO_3H$ in *tert*-butyl acetate/dichloromethane. The yields ranged from 70 to 100% for a variety of amino acid and dipeptide substrates. Selective deprotection of the *t*BOC group of various amino acids and peptides was achieved by Han *et al*. [37] by using hydrogen chloride (4 M) in an anhydrous dioxane solution for 30 min at room temperature. We tried the method using hydrogen chloride generated in situ by a reaction of acetyl chloride and methanol, which is claimed to be a mild method [33]. Figure 5 shows the ^{1}H-NMR spectrum of H_2N-β-Ala-PEG_{2050}-β-Ala-NH_2. The disappearance of the peak (j) at δ = 1.49 ppm indicates the formation of the product. The conjugation is indicated by the shift in peak (g') from δ = 4.30 ppm to δ = 2.95 ppm. The relative integrals of (b'):(d) are in the ratio 3.61:180 demonstrating successful deprotection. MALDI-ToF verified the H_2N-β-Ala-PEG_{2050}-β-Ala-NH_2 product at *m*/*z* = 2186.717.

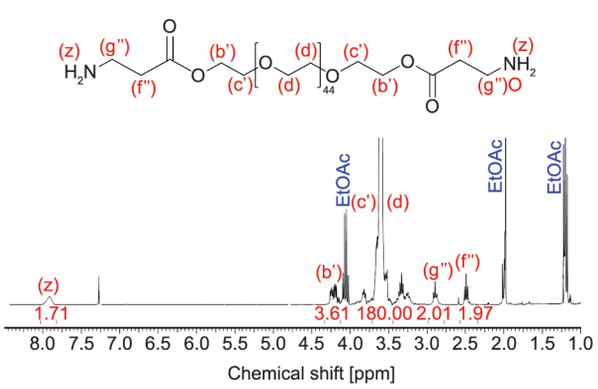

Figure 5. ^{1}H-NMR spectrum of H_2N-β-Ala-PEG_{2050}-β-Ala-NH_2 (Residual ethyl acetate signals appear at δ = 4.12, 2.05 ppm, and 1.26 ppm).

In summary, CALB-catalyzed reactions of protected alanines with PEG yielded close to 100% conversion into disubstituted products with a yield of over 90%, with β-Ala yielding an especially pure product. Deprotection yielded the desired diamine.

4. Conclusions

A new method for the synthesis of PEG-diamines using *Candida antarctica* lipase B (CALB) enzyme in the spirit of 'green chemistry' is presented in this paper. Unprotected β-Alanine or L-Alanine did not react with PEG in the presence of CALB – probably due to zwitterion formation-, but *tert*-butyloxy carbonyl (*t*BOC)-protected alanines readily reacted. The reaction of PEG with protected β-Alanine showed full conversion according to NMR, and MALDI-ToF demonstrated the purity of the product. Deprotection yielded the desired PEG-diamine. Protected L-Alanine had less than a full conversion, likely due to the steric hindrance of its methyl side group.

Acknowledgementsv

The authors would like to thank The Ohio State University and the Breast Cancer Innovation Foundation for financial support. The authors also gratefully acknowledge funding from USDA-NIFA to Hatch project number OHO01417.

References

[1] Rocha-García D., Guerra-Contreras A., Reyes-Hernández J., Palestino G.: Thermal and kinetic evaluation of biodegradable thermo-sensitive gelatin/poly(ethylene glycol) diamine crosslinked citric acid hydrogels for controlled release of tramadol. European Polymer Journal, **89**, 42–56 (2017). https://doi.org/10.1016/j.eurpolymj.2017.02.007

[2] Peppas N. A., Keys K. B., Torres-Lugo M., Lowman A. M.: Poly(ethylene glycol)-containing hydrogels in drug delivery. Journal of Controlled Release, **62**, 81–87 (1999). https://doi.org/10.1016/S0168-3659(99)00027-9

[3] Ferretti M., Marra K. G., Kobayashi K., Defail A. J., Chu C. R.: Controlled *in vivo* degradation of genipin crosslinked polyethylene glycol hydrogels within osteochondral defects. Tissue Engineering, **12**, 2657–2663 (2006). https://doi.org/10.1089/ten.2006.12.2657

[4] Renil M., Ferreras M., Delaisse J. M., Foged N. T., Meldal M.: PEGA supports for combinatorial peptide synthesis and solid-phase enzymatic library assays. Journal of Peptide Science, **4**, 195–210 (1998). https://doi.org/10.1002/(SICI)1099-1387(199805)4:3<195::AID-PSC141>3.0.CO;2-R

[5] Liang Y., Gao W., Peng X., Deng X., Sun C., Wu H., He B.: Near infrared light responsive hybrid nanoparticles for synergistic therapy. Biomaterials, **100**, 76–90 (2016). https://doi.org/10.1016/j.biomaterials.2016.05.023

[6] Liu M., Xie C., Xu W., Lu W.: Separation of polyethylene glycols and their amino-substituted derivatives by high-performance gel filtration chromatography at low ionic strength with refractive index detection. Journal of Chromatography A, **1046**, 121–126 (2004). https://doi.org/10.1016/j.chroma.2004.06.005

[7] Sakai T., Matsunaga T., Yamamoto Y., Ito C., Yoshida R., Suzuki S., Sasaki N., Shibayama M., Chung U-I.: Design and fabrication of a high-strength hydrogel with ideally homogeneous network structure from tetrahedron-like macromonomers. Macromolecules, **41**, 5379–5384 (2008). https://doi.org/10.1021/ma800476x

[8] Wang L., Wang S., Bei J. Z.: Synthesis and characterization of macroinitiator-amino terminated PEG and poly(γ-benzyl-L-glutamate)-PEO-poly(γ-benzyl-L-glutamate) triblock copolymer. Polymers for Advanced Technologies, **15**, 617–621 (2004). https://doi.org/10.1002/pat.510

[9] Geckeler K., Bayer E.: Functionalization of soluble polymers. Polymer Bulletin, **3**, 431–434 (1980). https://doi.org/10.1007/BF00255094

[10] Hai T. T., Markoski L. J., Pereira D. E., Nordhaus M.: Process for the preparation of polyethylene glycol bias mine. Word Patent WO2004035657 (2004).

[11] Aronov O., Horowitz A. T., Gabizon A., Gibson D.: Folate-targeted PEG as a potential carrier for carboplatin analogs. Synthesis and *in vitro* studies. Bioconjugate Chemistry, **14**, 563–574 (2003). https://doi.org/10.1021/bc025642l

[12] Davis F. F., van Es T., Palczuk N. C.: Non-immunogenic polypeptides. U.S. Patent 4179337A (1977).

[13] Buückmann A. F., Morr M., Kula M-R.: Preparation of technical grade polyethylene glycol (PEG) (M_r 20,000)-N^6-(2-aminoethyl-NADH by a procedure adaptable to large-scale synthsis. Biotechnology and Applied Biochemistry, **9**, 258–268 (1987). https://doi.org/10.1111/j.1470-8744.1987.tb00407.x

[14] Wiss K. T., Kessler D., Wendorff T. J., Theato P.: Versatile responsive surfaces *via* hybrid polymers containing acetal side groups. Macromolecular Chemistry and Physics, **210**, 1201–1209 (2009). https://doi.org/10.1002/macp.200900156

[15] Mongondry P., Bonnans-Plaisance C., Jean M., Tassin J. F.: Mild synthesis of amino-poly(ethylene glycol)s. Application to steric stabilization of clays. Macromolecular Rapid Communications, **24**, 681–685 (2003). https://doi.org/10.1002/marc.200350012

[16] Neal J. C., Stolnik S., Schacht E., Kenawy E. R., Garnett M. C., Davis S. S., Illum L.: *in vitro* displacement by rat serum of adsorbed radiolabeled poloxamer and poloxamine copolymers from model and biodegradable nanospheres. Journal of Pharmaceutical Sciences, **87**, 1242–1248 (1998). https://doi.org/10.1021/js970462j

[17] Ewert K. K., Kotamraju V. R., Majzoub R. N., Steffes V. M., Wonder E. A., Teesalu T., Ruoslahti E., Safinya C. R.: Synthesis of linear and cyclic peptide–PEG–lipids for stabilization and targeting of cationic liposome–DNA complexes. Bioorganic and Medicinal Chemistry Letters, **26**, 1618–1623 (2016). https://doi.org/10.1016/j.bmcl.2016.01.079

[18] Furukawa S., Katayama N., Iizuka T., Urabe I., Okada H.: Preparation of polyethylene glycol-bound NAD and its application in a model enzyme reactor. FEBS Letters, **121**, 239–242 (1980). https://doi.org/10.1016/0014-5793(80)80351-6

[19] Shi C., Guo X., Qu Q., Tang Z., Wang Y., Zhou S.: Actively targeted delivery of anticancer drug to tumor cells by redox-responsive star-shaped micelles. Biomaterials, **35**, 8711–8722 (2014). https://doi.org/10.1016/j.biomaterials.2014.06.036

[20] Mutter M.: Soluble polymers in organic synthesis: I. Preparation of polymer reagents using polyethylene glycol with terminal amino groups as polymeric component. Tetrahedron Letters, **19**, 2839–2842 (1978). https://doi.org/10.1016/S0040-4039(01)94878-6

[21] Gouveia Z., Perinpanayagam H., Zhu J.: Development of multifunctional Si-Ca-PEG-nAg sol–gel implant coatings from calcium-2-ethoxyethoxide. Journal of Coatings Technology and Research, **18**, 1177–1189 (2021). https://doi.org/10.1007/s11998-021-00477-x

[22] Jankoa K., Kops J.: ^{1}H-NMR investigation of quantitative functionalization of poly(ethylene glycol)s. Journal of Applied Polymer Science, **54**, 1027–1032 (1994). https://doi.org/10.1002/app.1994.070540804

[23] Ranucci E., Ferruti P.: A new synthetic method for amino-terminated poly(ethyleneglycol) derivatives. Synthetic Communications, **20**, 2951–2957 (1990). https://doi.org/10.1080/00397919008051511

[24] Singh P., Gupta U., Asthana A., Jain N. K.: Folate and folate–PEG–PAMAM dendrimers: Synthesis, characterization, and targeted anticancer drug delivery potential in tumor bearing mice. Bioconjugate Chemistry, **19**, 2239–2252 (2008). https://doi.org/10.1021/bc800125u

[25] Pawar G. M., Koenigs M., Fahimi Z., Cox M., Voets I. K., Wyss H. M., Sijbesma R. P.: Injectable hydrogels from segmented PEG-bisurea copolymers. Biomacromolecules, **13**, 3966–3976 (2012). https://doi.org/10.1021/bm301242v

[26] Fu X., Shen Y., Ma Y., Fu W., Li Z.: Tunable supramolecular hydrogels from polypeptide-PEG-polypeptide triblock copolymers. Science China Chemistry, **58**, 1005–1012 (2015). https://doi.org/10.1007/s11426-014-5297-2

[27] Puskas J. E., Sen M. Y., Kasper J. R.: Green polymer chemistry: Telechelic poly(ethylene glycol)s *via* enzymatic catalysis. Journal of Polymer Science Part A: Polymer Chemistry, **46**, 3024–3028 (2008). https://doi.org/10.1002/pola.22640

[28] Sen S., Puskas J.: Green polymer chemistry: Enzyme catalysis for polymer functionalization. Molecules, **20**, 9358–9379 (2015). https://doi.org/10.3390/molecules20059358

[29] Puskas J. E., Sen M. Y.: Process of preparing functionalized polymers *via* enzymatic catalysis. U.S. Patent 8710156, USA (2014).

[30] Puskas J. E., Sen M. Y.: Process of preparing functionalized polymers *via* enzymatic catalysis. U.S. Patent 9885070, USA (2018).

[31] Albarran A. A., Rosenthal-Kim E. Q., Kantor J., Liu L., Nikolov Z., Puskas J. E.: Stimuli-responsive antifouling polyisobutylene-based biomaterials *via* modular surface functionalization. Journal of Polymer Science Part A: Polymer Chemistry, **55**, 1742–1749 (2017). https://doi.org/10.1002/pola.28540

[32] Bardaweel S. K., Abu-Dahab R., Almomani N. F.: An *in vitro* based investigation into the cytotoxic effects of D-amino acids. Acta Pharmaceutica, **63**, 467–478 (2013). https://doi.org/10.2478/acph-2013-0032

[33] Routier S., Saugé L., Ayerbe N., Coudert G., Mérour J-Y.: A mild and selective method for *N*-Boc deprotection. Tetrahedron Letters, **43**, 589–591 (2002). https://doi.org/10.1016/S0040-4039(01)02225-0

[34] Eiselt P., Lee K. Y., Mooney D. J.: Rigidity of two-component hydrogels prepared from alginate and poly(ethylene glycol)–diamines. Macromolecules, **32**, 5561–5566 (1999). https://doi.org/10.1021/ma990514m

[35] Karmakar A., Basha M., Venkatesh Babu G. T., Botlagunta M., Malik N. A., Rampulla R., Mathur A., Gupta A. K.: Tertiary-butoxycarbonyl (Boc) – A strategic group for *N*-protection/deprotection in the synthesis of various natural/unnatural *N*-unprotected aminoacid cyanomethyl esters. Tetrahedron Letters, 59, 4267–4271 (2018). https://doi.org/10.1016/j.tetlet.2018.10.041

[36] Lin L. S., Lanza T., de Laszlo S. E., Truong Q., Kamenecka T., Hagmann W. K.: Deprotection of *N-tert*-butoxycarbonyl (Boc) groups in the presence of *tert*-butyl esters. Tetrahedron Letters, **41**, 7013–7016 (2000). https://doi.org/10.1016/S0040-4039(00)01203-X

[37] Han G., Tamaki M., Hruby V. J.: Fast, efficient and selective deprotection of the *tert*-butoxycarbonyl (Boc) group using HCl/dioxane (4 M). The Journal of Peptide Research, **58**, 338–341 (2001). https://doi.org/10.1034/j.1399-3011.2001.00935.x

[38] Nudelman A., Bechor Y., Falb E., Fischer B., Wexler B. A., Nudelman A.: Acetyl chloride-methanol as a convenient reagent for: A) Quantitative formation of amine hydrochlorides B) Carboxylate ester formation C) Mild removal of *N-t*-Boc-protective group. Synthetic Communications, **28**, 471–474 (1998). https://doi.org/10.1080/00397919808005101