Prednisolone-α-cyclodextrin-star PEG polypseudorotaxanes with controlled drug delivery properties†‡

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The reaction of α-amino-ω-methoxypoly(ethylene glycol) [M = 5000] or star α-amino-poly(ethylene glycol) [M = 20 000] with hemiesters of prednisolone dicarboxylic acids (succinic, glutaric, adipic, phthalic acid) has been used to prepare the corresponding conjugates. The rate of esterase catalyzed hydrolysis of the conjugates is controlled by the molecular mass of poly(ethylene glycol) and the length of the linker between prednisolone and poly(ethylene glycol) ($\tau_{1/2}$ ~ 5–0.5 h). The enzymatic hydrolysis proceeds most rapidly at conjugates with linkers derived from adipic and phthalic acids. The synthesized conjugates form polypseudorotaxanes with α-cyclodextrin which were characterized by 2D NOESY NMR spectra, powder X-ray diffraction patterns and in one case also by STM microscopy. In the case of the polypseudorotaxane having the linker derived from adipic acid, the enzymatic release proceeds *ca.* five times slower in comparison with the rate of prednisolone release from the corresponding conjugate. The rate of prednisolone release from the carrier can be controlled by three factors: character of the linker between the polymeric carrier and prednisolone, the molecular mass of poly(ethylene glycol) and complex formation with α-cyclodextrin. The synthesized polypseudorotaxanes represent new promising transport systems intended for targeted release of prednisolone in transplanted liver.

Introduction

Prednisolone (PS) belongs among the group of glucocorticoids which exhibit a high anti-inflammatory potential. For this feature they are extensively used for the prevention of rejection in organ transplantation. Nevertheless, the application of steroids and other immunosuppressants in these cases is often complicated due to their adverse side effects, such as diabetes, hypertension, Cushing's syndrome and osteoporosis.[1] In spite of new pharmaceutical formulas of prednisolone and methylprednisolone such as liposomal forms,[2] dextran conjugates[3,4] and also nanoparticles of prednisolone[5] have been developed recently, still minimizing of these side effects is a challenging issue. The results of therapeutic studies show that it is eligible to release the immunosuppressive agent directly in the transplanted organ to furnish the tissue with the active drug.[6] Controlled release of the therapeutically active substance can be achieved by the general principle based on the presence of specific enzymes.[7–8] In our earlier works,[9–12] we have prepared and *in vitro* tested targeted conjugates of amphotericin B and nystatin[13] with substituted poly(ethylene glycols). The last types of conjugates[12,13] are designed to be sensitive towards β-glucosidases (E.C.3.2.1.21) which are specifically present in the infected tissue.[14] Local release of PS in transplanted liver can be achieved by the action of the liver carboxyesterases (E.C.3.1.1.1), as previously used in the case of dextran–methylprednisolone conjugates.[3,4] The targeted administration to liver is accomplished by the fact that the rank order of hydrolytic activity of carboxyesterases toward ester groups is liver/kidney > small intestine > lung ≫ blood.[8]

The aim of this work was to prepare and characterize such delivery systems of prednisolone that would highly selectively release PS in transplanted liver and simultaneously allow variable control of the rate of PS release at a molecular level. As carriers we have chosen α-amino-ω-methoxypoly(ethylene glycol) [M = 5000] and star α-aminopoly(ethylene glycol) [pentaerythritol α-aminopoly(ethylene glycol)ether, M = 20 000], because their biocompatibility makes them ideal to employ in such pharmaceutical applications.[15] In the structure of the proposed conjugates was the connection between PS and poly(ethylene glycols) (PEGs) attained by different linkers from a series of dicarboxylic acids (succinic, glutaric, adipic, phthalic acid). Prednisolone was linked to one carboxylic group of the individual acid *via* an ester function that can be readily cleaved by esterase (linker), whereas the other carboxylic group of the acid was suitable for linking to amino-PEG *via* amide function (Fig. 1).

We have explored several approaches to control the PS release rate in enzyme kinetics. One of the possibilities is to modify the linker employing the above-mentioned dicarboxylic acids having different lengths and characters of chain. In addition, formation of inclusion compounds of medical drugs with cyclodextrins was previously proposed as an efficient tool for modulation of drug delivery features.[16] For example, modification with cyclodextrins was adopted[17] for slowing down the release of prednisolone as well as methylprednisolone. However, a more

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‡ Dedicated to Professor Jitka Moravcová on the occasion of her 60th birthday.

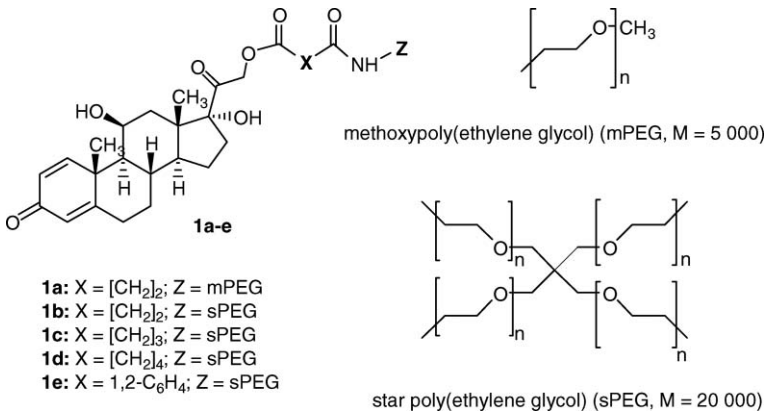


Fig. 1 Structures of conjugates **1a–e**.

recent insight into this area is represented by conjugates based on polypseudorotaxanes[18–28] (*i.e.* complexes of substituted PEGs with cyclodextrins). Polypseudorotaxanes obtained through the complex formation of substituted PEGs and α-cyclodextrin (α-CD) have proven distinct retardation of the overall excretion rate of the conjugate from organism.[18–22] Therefore, in continuing our efforts to develop potential pharmaceutical formulations of prednisolone with prolonged effects, we have prepared and characterized polypseudorotaxanes **3b** and **3d** (Fig. 2.) derived from α-CD and from conjugates **1b** and **1d**.

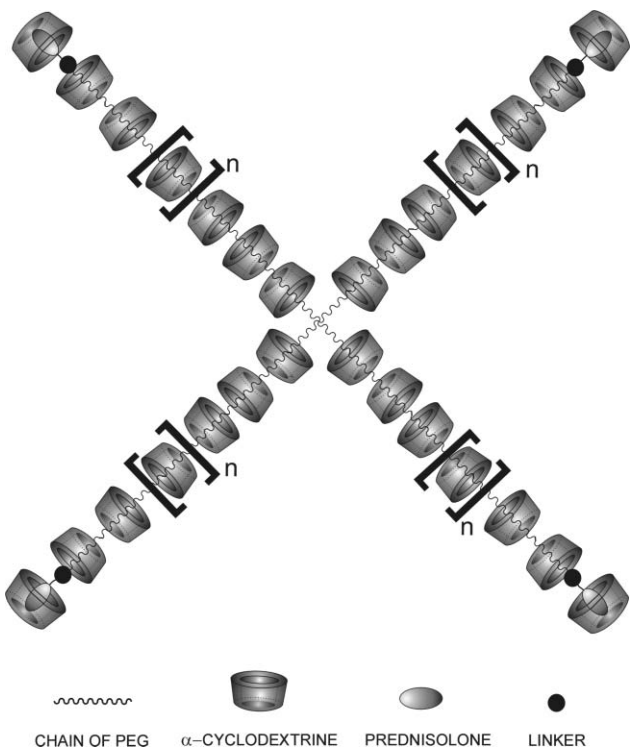


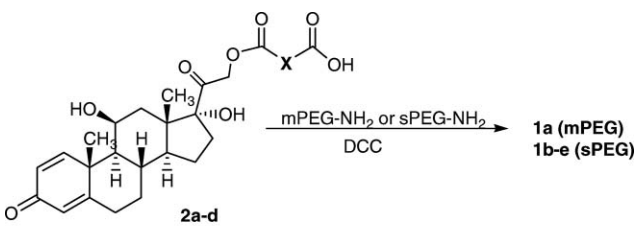
Fig. 2 Schematic structure of polypseudorotaxanes derived from α-cyclodextrin-star poly(ethylene glycol)amid-linker-ester-prednisolone; n ~ 26 for linker succinate (**3b**); n ~ 12 for linker adipate (**3d**).

Results and discussion

Synthesis and characterization of conjugates 1a–e

The new hemiesters of dicarboxylic acids and prednisolone **2b–d** were prepared in analogy to the earlier described method[29] for prednisolone 21-hemisuccinate (**2a**), *i.e.* by the reaction of corresponding anhydrides (succinic, glutaric, adipic, phthalic acid) with prednisolone in the presence of pyridine (57–67%). All the prepared hemiesters **2a–d** were characterized by melting points, microanalysis, ^{1}H, ^{13}C NMR and FT-IR spectroscopy.

After activation with dicyclohexylcarbodiimide (DCC), the hemiesters of prednisolone **2a–d** were subsequently connected to amino PEGs (mPEG, sPEG) *via* amide function (Scheme 1). The conjugates **1a–e** prepared in this way were recrystallized from propan-2-ol and characterized by ^{1}H NMR spectroscopy and GPC. The integral intensities of two signals of –CH═ in prednisolone (~7.3; ~6.0 ppm) and the signals of $-CH_2-$ in PEG (3.2–3.8 ppm) in the ^{1}H NMR spectra showed that in conjugate **1a** the molar ratio is PS : mPEG = 1 : 1. In conjugates **1b–e** the molar ratio was PS : sPEG = 4 : 1. The ^{1}H NMR spectra were also used for determination of the M_n value and the GPC analysis for determination of the M_w value, thus giving the M_w/M_n ratio for the individual conjugates **1a–e**.



Scheme 1 Synthesis of PS-PEG conjugates **1a–e**.

Synthesis and characterization of polypseudorotaxanes 3b and 3d

The formation and decomposition of polypseudorotaxanes PEG/α-CD represent an equilibrium process;[23–28] therefore the complex formation of conjugates **1b** and **1d** was performed with excess of α-CD. Polypseudorotaxanes **3b** and **3d** precipitated

within 14 days standing of a mixture of saturated aqueous solution of α-CD and saturated aqueous solution of the corresponding conjugate at room temperature; they were isolated by centrifugation, purified by repeated decantation with distilled water, again isolated by centrifugation, and dried in vacuum at room temperature. Polypseudorotaxanes **3b** (37%) and **3d** (62%) were characterized by using powder X-ray diffraction, ^{1}H and 2D-NOESY NMR spectroscopy. In the case of polypseudorotaxane **3d** the characterization was supplemented by Scanning Tunneling Microscopy (STM).

Fig. 3 shows powder X-ray diffraction patterns of the α-CD alone (Fig. 3(a)) as compared with the physical mixture of α-CD and conjugate **1b** (Fig. 3(b)), and polypseudorotaxanes **3b** (Fig. 3(c)) and **3d** (Fig. 3(d)). The diffraction pattern of polypseudorotaxanes **3b,d** is obviously different from those of the physical mixture and α-CD alone. When compared to previously published results,[26–28] it indicates that the PEG chain forms a polypseudorotaxane arrangement in which the long PEG chain is embedded in the stacked host channel. The diffraction peaks at $2\Theta = 12.9$, 19.8 and 22.6° resembled arrangement of α-CD in the order head-to-head/tail-to-tail,[30] as shown in Fig. 3(c) (d).

The stoichiometry of both polypseudorotaxanes **3b** and **3d** was determined by integration of peak surface areas in ^{1}H NMR spectrum of anomeric proton of α-CD (4.84 ppm) and the ethylene oxide protons of PEG chain (~ 3.55 ppm) in dimethyl sulfoxide solution (Fig. 4). The 2D NOESY NMR spectra of polypseudorotaxanes **3b** and **3d** (Fig. 5) clearly show the crosspeaks belonging to non-bonding interaction through space, namely between the hydrogen atoms of the poly(ethylene glycol) unit [CH_2CH_2O] and the hydrogen atom 3 of the α-CD units, which also confirms the polypseudorotaxane arrangement in accordance with the literature.[26] The integral intensities show that the stoichiometries

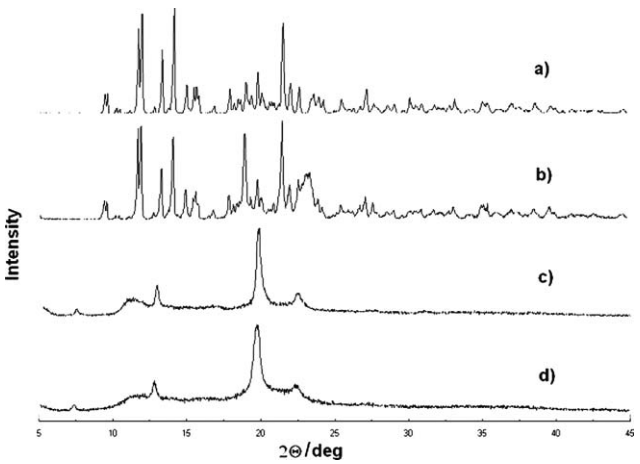


Fig. 3 Powder X-ray diffraction patterns: (a) α-CD alone; (b) physical mixture of α-CD with conjugate **1b**; (c) polypseudorotaxane **3b**; (d) polypseudorotaxane **3d**.

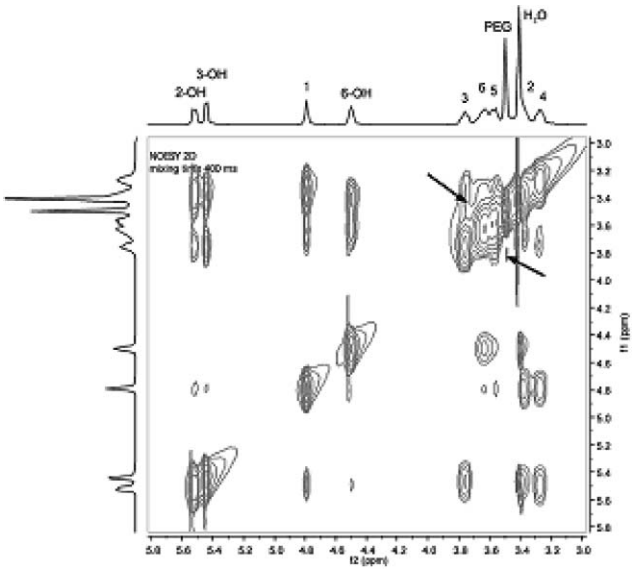


Fig. 5 2D NOESY NMR spectrum of polypseudorotaxane **3d**. Arrows point to crosspeaks belonging to non-bonding interaction through space between the hydrogen atoms in -CH_2CH_2O- of PEG and hydrogen atom 3 of α-cyclodextrin.

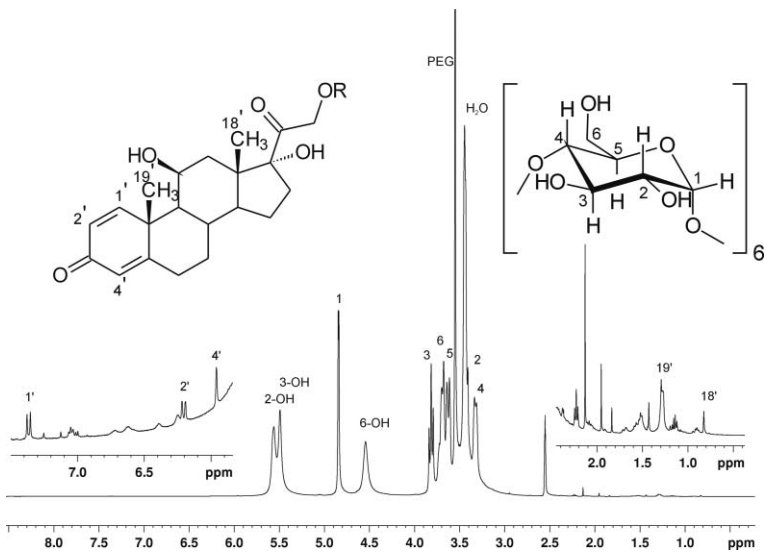


Fig. 4 ^{1}H NMR spectrum of polypseudorotaxane **3d**. Insets represent parts of the spectrum at increased intensity with the assigned signals of prednisolone.

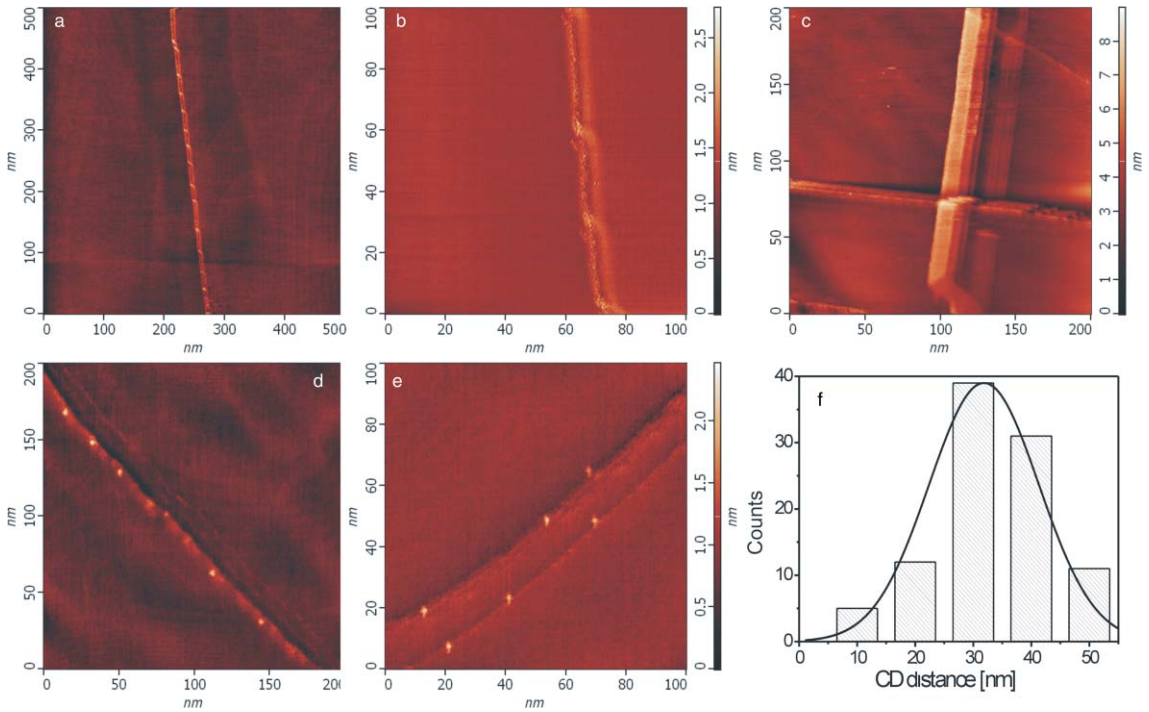


Fig. 6 STM images of hydrated **3d** (a, b, c) and dehydrated **3d** (d, e), histogram of α-CD distances (f).

of polypseudorotaxanes **3b** and **3d** prepared repeatedly under the same conditions are substantially different. In the case of polypseudorotaxane **3b** each arm of the PEG chain is threaded into 32 α-CD units on average, and polypseudorotaxane **3b** contains in total 128 α-CD units, *i.e.* there are 3.5 [CH_2CH_2O] units per one α-CD unit. However, in the case of polypseudorotaxane **3d** the stoichiometry found is half the previous, *i.e.* there are *ca.* 6 units [CH_2CH_2O] units per one α-CD unit. These findings show that the stoichiometry ratio [CH_2CH_2O]/α-CD is significantly influenced by the character of the linker between PS and PEG. For instance, the ratio determined earlier[31] for non-functionalized sPEG [M = 7400] was 2.2 units [CH_2CH_2O] per one unit of α-CD.

The necklace like structure of α-CD units threaded onto PEG chains was directly observed by using STM for hydrated (Fig. 6(a, b)) and dehydrated (d, e) samples of **3d**. The α-CD units were detected as bright dots and PEG chains as lines connecting them. The distance between two α-CD units was analyzed (Fig. 6(f); the L value according to Koji Miyake[32]). The minimum L value found was 6.3 nm and the typical value was 30 nm. No shuttle manipulation according to Ref. 32 was observed during STM experiment due to the higher interaction to HOPG in comparison to MoS_2 substrate. Self-assembly effects show lamellar structure of **3d** (Fig. 6(c)).

The lateral dimension of α-CD units in the STM image of polypseudorotaxane **3d** was changed dramatically according to degree of hydration of the sample. The diameter of hydrated particles was found between 6–8 nm and the observed diameter of PEG reached a similar value (Fig. 7(a)). This was caused by residues of the solvating layer covering α-CD units as well as the PEG chain. After more exhaustive drying the particle diameter detected decreased to 2.5–3.5 nm, which is in better agreement with the particle size of α-CD (1.7 nm) and with previously published

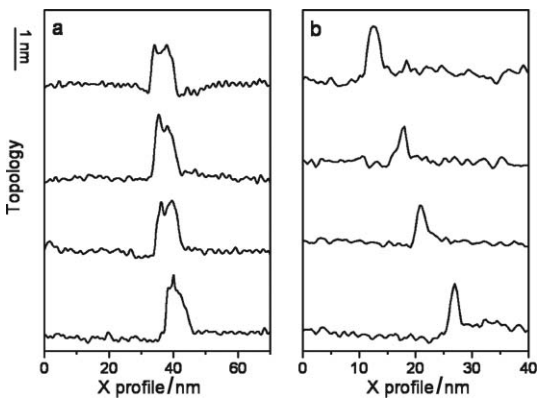


Fig. 7 Line-scans of hydrated **3d** (a) and dehydrated **3d** (b) α-CD particles.

results.[32–35] The bigger size resulted as an artefact due to tunneling current acquisition from the side of the α-CD particle as well as residues of solvating layer from inadequate drying or progressive hydration adsorbed during the experiment.

Stability of conjugates 1a–e, 3b,d in phosphate buffer solutions and hydrochloric acid solutions

Conjugates **1a–e**, **3b,d** did not exhibit any measurable increase in PS concentration at least for one week at the temperature of 37 °C in the phosphate buffers (pH: 7.4, 5.8, 1×10^{-1} mol L^{-1}) and in acetate buffer (pH 4.0, 1×10^{-1} mol L^{-1}). In the hydrochloric acid solution (1×10^{-2} mol L^{-1}; I = 1 mol L^{-1}; KCl) an observable increase in PS concentration took place only in more than 72 h.

Table 1 Rate constants (k) and half-lives ($\tau_{1/2}$) of enzymatic hydrolysis of conjugates **1a–e** and polypseudorotaxanes **3b,d** (pig liver esterase, E.C. 3.1.1.1, pH 7.4, 37 °C)

Conjugate or polypseudorotaxane	k/min^{-1}	$\tau_{1/2}$/min
1a	$3.8 \times 10^{-3} \pm 3 \times 10^{-4}$	184 ± 15
1b	$2.7 \times 10^{-3} \pm 1 \times 10^{-5}$	259 ± 10
1c	$1.3 \times 10^{-2} \pm 2 \times 10^{-3}$	52 ± 7
1d	$1.8 \times 10^{-2} \pm 6 \times 10^{-4}$	37 ± 2
1e	$1.4 \times 10^{-2} \pm 3 \times 10^{-3}$	48 ± 8
3b	$2.5 \times 10^{-3} \pm 1 \times 10^{-4}$	275 ± 11
3d	$3.8 \times 10^{-3} \pm 3 \times 10^{-4}$	184 ± 16

Enzymatic hydrolysis of conjugates 1a–e and polypseudorotaxanes 3b,d by carboxyesterase (E.C.3.1.1.1)

For the purpose of verification of release of PS from conjugates **1a–e** and polypseudorotaxanes **3b,d** the kinetics of enzymatic hydrolysis was studied by means of HPLC measuring the free PS concentration increase (Table 1).

Table 1 presents rate constants and half-lives of enzymatic hydrolysis for individual conjugates **1a–e** and for polypseudorotaxanes **3b,d**. The values given in the table show the influence of three parameters upon the rate of PS release from the carrier. The effect of the first parameter, *viz*. molecular mass of the PEGs, results from the comparison of hydrolysis rates of conjugates **1a** and **1b** which contain the same linker (succinic acid) but differ in molecular mass and character of polymeric carrier. The conjugate with lower molecular mass **1a** (M_{PEG} = 5000) undergoes the enzymatic release of PS *ca.* 1.5 times faster than conjugate **1b** (M_{PEG} = 20 000), which is in agreement with earlier findings.[10,36] The second tool for influencing the enzymatic hydrolysis rate is seen from the comparison of PS release rates of conjugates **1b–d** which differ only in the length of chain in the used homologous series of linkers derived from dicarboxylic acids and the conjugate **1e** derived from phthalic acid. The enzymatic hydrolysis rate increases with increasing length of the hydrophobic chain linked to poly(ethylene glycol) chain. Behavior of these systems exhibits a similar order as the compared enzymatic hydrolysis of butyryl, propionyl, acetyl esters and esters of aromatic acids.[37]

Furthermore, we investigated the PS release rates from polypseudorotaxanes **3b,d** which were compared with the PS release rates of the corresponding conjugates **1b,d**. The overall PS release rate from polypseudorotaxanes **3b,d** involves two kinetic processes: **3b,d** → **1b,d** → PS, *i.e.* dethreading of α-CD units (**3b,d** → **1b,d**) and enzymatic hydrolysis of the ester bond. The overall rate of PS release from the polypseudorotaxane is controlled by the slowest process, *i.e.* the rate-limiting step. In the case of conjugate **3b** the decomposition half-lives of **3b** and **1b** are comparable (Table 1), hence the rate-limiting step is enzymatic hydrolysis **1b** → PS, which means that dethreading of α-CD units must be fast enough that it is kinetically insignificant. From Fig. 8 follows that the enzymatic hydrolysis **1d** → PS obeys the pseudo-first-order kinetics (analogous kinetic dependencies were also obtained in the case of conjugates **1a–e** and polypseudorotaxane **3b**). In the case of hydrolysis **3d** → **1d** → PS the kinetic dependence is significantly affected by the rate of the first process **3d** → **1d**, and the experimental dependence begins to differ from pseudo-first-order kinetics (Fig. 8). The PS release rate of polypseudorotaxane

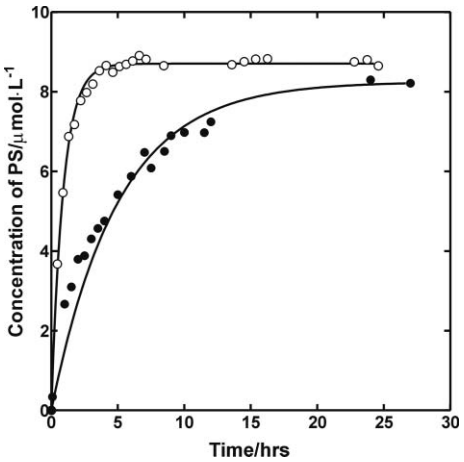


Fig. 8 Concentration-time dependence (μmol L^{-1} *versus* hours) of prednisolone release in a solution of pig liver esterase (E.C.3.1.1.1; 0.1 mg; 17 units g^{-1}, phosphate buffer pH 7.4; 1 × 10^{-1} mol L^{-1}; 1 mL; 37 °C) for conjugate **1d** (○) and polypseudorotaxane **3d**(●). The points were determined experimentally using HPLC at 245 nm; the curves correspond to pseudo-first-order rate equation.

3d was more than five times slower than that of conjugate **1d** (Table 1). The described results show that the PS release rates from the carrier can be controlled in a modular way by three factors: the linker between the polymeric carrier and PS, the molecular mass of PEGs, and complex formation with α-CD.

Conclusions

The polypseudorotaxanes derived from α-CD and PS-PEG conjugates were prepared with the aim to accomplish high selectivity of prednisolone release in transplanted liver. The selectivity of targeted release is ensured by the presence of ester linkage which is sensitive to liver esterase (E.C. 3.1.1.1). The rates of release of PS from the carrier can be controlled in a modular way by three factors: linker between polymeric carrier and PS, molecular mass of the PEGs, and complex formation with α-CD. The enzyme catalyzed PS release can be slowed down by shortening the aliphatic chain of the linker and increasing the molecular mass of the polymeric carrier. The character of the linker at the end of the PEG chain significantly affects also the stoichiometry of complex formation of PEG with α-CD, which is even reflected in the course of dethreading of α-CD units. All the conjugates and polypseudorotaxanes are relatively stable in acid medium of hydrochloric acid (1 × 10^{-2} mol L^{-1}), which is a precondition of the possibility of peroral administration that is considerate of the patient.

Experimental

General

Unless otherwise stated pentaerythritol poly(ethylene glycol) ether (M = 20 000) was obtained from JenKem Technology USA. Prednisolone and other chemicals and solvents were obtained from Fluka or Aldrich and used without further purification.

The ^{1}H NMR spectra were calibrated with respect to the middle signal in the multiplet of solvent (δ = 2.55;

DMSO-d_6) or ($\delta = 7.24$; $CDCl_3$). The ^{1}H NMR spectra of polymer conjugates were measured[38,39] with a relaxation delay of 6 s and an acquisition time of 4 s. The ^{13}C NMR spectra were measured in a standard way using broad-band proton decoupling and/or pulse sequence APT. The ^{13}C NMR spectra were calibrated with respect to the middle signal in the multiplet of solvent ($\delta = 39.6$; DMSO-d_6) or ($\delta = 77.0$; $CDCl_3$). 2D NOESY measurements were performed using the standard Bruker pulse sequence library with the experimental conditions as follows: spectral width of 4807 Hz, 128 transients per increment for the 128 increments, with 400 ms mixing time duration and 2048 data points in the F2 domain. The spectra were processed with a sine-bell squared in both dimensions. The IR spectra were measured in solid state on an FT-IR-Perkin Elmer Spectrum BX instrument with horizontal ATR modul with ZnSe crystal in the range from 650 to 4000 cm^{-1}. The HPLC analysis was performed using a Shimadzu (Kyoto, Japan) HPLC system consisting of two Model LC-10ADvp pumps, a Model SPD-M10Avp UV/vis spectrophotometric detector, a DGU-14A degasser, a CTO-10ASvp column oven and a SCL-10Avp system controller at 25 °C. Separation was performed using a guard column Security Guard, 4 mm × 3 mm, C18 (Phenomenex, Torrance, CA, USA). Elution was carried out with a gradient of methanol: water at a flow rate of 1 mL min^{-1}. The mobile phase was filtered through a 0.45 µm Hydrophilic Polypropylene Membrane Filter (Pall Corporation, Ann Arbor, Michigan, USA). The injection volume was set at 20 µL. The detection wavelength was 245 nm. The purity of conjugates **1a–e** was estimated by means of HPLC using LiChroCART® 125 mm × 4 mm column packed with LiChrospher® 100 RP-18e 5 µm (Merck, Darmstadt, Germany) and eluted with a mobile phase of acetonitrile with 20 mM chelaton II. Gel permeation chromatography was used for the estimation of M_w of conjugates **1a–e**. The HPLC device was identical with that used for purity determination but for the following parameters: HEMA-BIO columns (hydrophilic modification HEMAGel, particle size 10 µm, porosity 40/100/300/1000) at 25 °C using an RI detector and UV/vis detector. Redistilled water (pH 7.1) was used as the eluent. The columns were calibrated with a series of standard PEGs (Merck) and standard sPEGs (JenKem Technology USA) of various molecular masses. The microanalyses were performed on an apparatus of FISONS.

X-Ray diffraction

Powder X-ray diffraction data (Cu-Kα, λ = 1.5418 Å) were collected on a D8 Advance diffractometer (Bruker AXS, Germany) with Bragg-Brentano Θ-Θ goniometer (radius 217.5 mm) equipped with a secondary beam curved graphite monochromator and Na(Tl)I scintillation detector. The generator was operated at 40 kV and 30 mA. The scan was performed at room temperature from 2 to 65° (2 Θ) in 0.02° step with a counting time of 10 s per step.

Scanning tunneling microscopy (STM)

STM was performed for characterization of particle topology/location. An aqueous solution of **3d** (0.5 wt.%) was dropped onto a fresh, highly ordered pyrolytic graphite (HOPG) surface at room temperature, and then the samples were dried at a temperature of 60 °C for 2 h (hydrated samples) or for 72 h in vacuum (dehydrated samples). The STM experiment was performed under ambient conditions by using Solver ProM, Nt-MDT (Russia) with a Pt/Ir tip with calibration based on atomic scale resolution. The measurement was carried out in constant current mode with a sample bias voltage of +400 mV, a tunneling current of 2.10 nA with resolution 256 × 256 pxs^2.

Study of stability of conjugates 1a–e in phosphate buffer solutions and hydrochloric acid solution

A methanolic solution of studied conjugates **1a–e** (20 µL; 30 mg mL^{-1}) was injected into solutions (1 mL) of buffers with pH values: 7.4, 5.8 (phosphate), 4.0 (acetate), 1×10^{-1} mol L^{-1} and into solution of hydrochloric acid (1×10^{-2} mol L^{-1}; $I = 1$ mol L^{-1}; KCl). The increases of PS concentration were monitored by means of HPLC at 37 °C.

Study of hydrolysis of conjugates 1a–e by esterase

The kinetic measurements were carried out at 37 °C. A methanolic solution of the conjugate studied (20 µL; 30 mg mL^{-1}) was injected into a solution of commercial pig liver esterase (E.C.3.1.1.1; Sigma; 0.1 mg; 17 units g^{-1}) in phosphate buffer (pH 7.4; 1×10^{-1} mol L^{-1}; 1 mL). The solution obtained was kept at constant temperature and samples were taken at definite time intervals for determination of PS concentration in the course of time by using HPLC. The concentration-time dependence was optimized to obtain the pseudo-first-order rate constants ($k = \ln 2/\tau_{1/2}$) and half-lives ($\tau_{1/2}$) of enzymatic hydrolysis.

General procedure for synthesis of prednisolone hemiesters (2a–d)

A solution of prednisolone (1 g; 2.77 mmol) and the corresponding anhydride (12.55 mmol) in pyridine (8 mL) was stirred at room temperature. After 24 h, the reaction mixture was poured onto a mixture of ice (25 g), water (25 mL) and conc. HCl (10 mL). The separated crystals were collected by filtration, washed with water, dried, recrystallized from toluene and dried again.

Prednisolone 21-hemisuccinate (2a). Yield: 0.8 g (63%), mp 172–174 °C (lit.,[29] 176 °C). ^{1}H NMR (500.13 MHz, DMSO-d_6): δ_H: 0.82 (s, 3H); 0.92 (m, 1H); 1.06 (m, 1H); 1.33 (m, 1H); 1.42 (s, 3H); 1.46 (m, 1H); 1.58 (m, 1H); 1.68 (m, 2H); 1.91 (m, 1H); 2.06 (m, 2H); 2.33 (dd, $J = 13$ Hz, $J = 3$ Hz, 1H); 2.52 (m, 4H); 2.65 (m, 2H); 4.11 (d, $J = 19$ Hz, 1H); 4.32 (s, 1H); 4,53 (d, $J = 19$ Hz, 1H); 4.80 (d, $J = 17$ Hz, 1H); 5.12 (d, $J = 17$ Hz, 1H); 5.95 (s, 1H); 6.19 (d, $J = 10$ Hz, 1H); 7.37 (d, $J = 10$ Hz, 1H). ^{13}C NMR (DMSO-d_6): δ_C: 16.6, 20.9, 23.6, 28.5, 28.7, 31.0, 31.4, 33.0, 34.0, 43.9, 46.6, 47.1, 51.1, 55.5, 66.0, 67.7, 68.4, 77,2, 88.6, 121.6, 127.10, 156.9, 170.7, 171.7, 173.3, 185.3, 205.2. FT-IR: ν_{max}/cm^{-1} 3407, 2938, 2855, 1721, 1708, 1654 and 1611. Anal. calcd for $C_{25}H_{32}O_8$ (460) (%): C, 65.24; H, 7.00. Found: C, 64.89; H, 7.21.

Prednisolone 21-hemiglutarate (2b). Yield: 0.7 g (67%), mp 173–175 °C. ^{1}H NMR (500.13 MHz, DMSO-d_6): δ_H: 0.77 (s, 3H); 0.87 (dd, $J = 11$ Hz, $J = 3,4$ Hz, 1H); 0.99 (m, 1H); 1.29 (m, 1H); 1.37 (s, 3H); 1.43 (m, 1H); 1.63 (m, 3H); 1.76 (m, 2H); 1.87 (m, 1H); 2.01 (m, 2H); 2.30 (m, 4H); 2.42 (m, 2H); 2.47 (m, 1H); 4.28 (m, 1H); 4.75 (d, $J = 17.6$ Hz, 1H); 5.06 (d, $J = 17.6$ Hz, 1H); 5.91 (s,1H); 6.15 (dd, $J = 10.1$ Hz, $J = 1.8$ Hz, 1H); 7.32 (d, $J = 10.1$ Hz, 1H). ^{13}C NMR (125.76 MHz, DMSO-d_6): δ_C: 16.5,

20.0, 20.9, 23.5, 30.9, 31.4, 32.4, 32.5, 33.1, 34.0, 43.8, 47.1, 51.1, 55.4, 67.6, 68.4, 88.7, 121.6, 127.0, 156.9, 170.8, 172.1, 174.1, 185.3, 205.4. FT-IR: ν_{max}/cm^{-1} 3477, 2936, 1744, 1710, 1651, 1591 and 892. Anal. calcd for $C_{26}H_{34}O_8$ (474) (%): C, 65.81; H, 7.22. Found: C, 65.62; H, 7.56.

Prednisolone 21-hemiadipate (2c). Yield: 0.8 g (59%), mp 177–179 °C. ^{1}H NMR (500.13 MHz, DMSO-d$_6$): δ_H: 0.75 (s, 3H); 0.85 (dd, J = 11 Hz, J = 3.3 Hz, 1H); 0.98 (m, 1H); 1.26 (m, 1H); 1.35 (s, 3H); 1.41 (m, 1H); 1.53 (m, 4H); 1.62 (m, 3H); 1.84 (m, 1H); 2.00 (m, 2H); 2.21 (m, 3H); 2.26 (m, 1H); 2.36 (m, 2H); 2.45 (m, 1H); 4.27 (m, 1H); 4.74 (d, J = 17.5 Hz, 1H); 5.02 (d, J = 17.6 Hz, 1H); 5.90 (s,1H); 6,14 (dd, J = 10.1 Hz, J = 1.8 Hz, 1H); 7.2 (d, J = 10.1 Hz, 1H). ^{13}C NMR (125.76 MHz, DMSO-d$_6$): δ_C: 16.7, 21.1, 23.7, 24.1, 24.2, 31.1, 31.6, 32.4, 33.2, 33.3, 33.6, 33.2, 44.1, 47.3, 51.3, 55.6, 67.8, 68.6, 88.9, 121.7, 127.2, 157.4, 171.3, 172.6, 174.7, 185.8, 205.7. FT-IR: ν_{max}/cm^{-1} 3480, 2936, 1713, 1651 and 1593. Anal. calcd for $C_{27}H_{36}O_8$ (489) (%): C, 66.38; H, 7.43. Found: C, 66.24; H, 7.52.

Prednisolone 21-hemiphthalate (2d). Yield: 0.8 g (57%), mp 182–184 °C. ^{1}H NMR (500,13 MHz, DMSO-d$_6$): δ_H: 0.79 (s, 3H); 0.87 (dd, J = 11.2 Hz, J = 3.1 Hz, 1H); 0.98 (m, 1H); 1.25 (m, 1H); 1.36 (s, 3H); 1.47 (m, 1H); 1.65 (m, 2H); 1.90 (m, 1H); 2.00 (m, 2H); 2.27 (m, 1H); 2.47 (m, 2H); 4.28 (m, 1H); 4.93 (d, J = 17.3 Hz, 1H); 5.26 (d, J = 17.3 Hz, 1H); 5.89 (s,1H); 6.13 (d, J = 10.1 Hz, 1H); 7.32 (d, J = 10.1 Hz, 1H); 7.63 (m, 2H); 7.73 (m, 2H). ^{13}C NMR (125.76 MHz, DMSO-d$_6$): δ_C: 16.6, 20.8, 23.5, 30.9, 31.3, 31.4, 33.1, 33.9, 43.9, 47.1, 51.1, 55.4, 68.2, 68.4, 88.7, 121.5, 127.9, 127.2, 128.3, 128.6, 128.8, 131.6, 132.3, 156.8, 167.9, 170.6, 185.2, 205.0. FT-IR: ν_{max}/cm^{-1} 3368, 2939, 1770, 1740, 1651, 1303 and 1014. Anal. calcd for $C_{29}H_{32}O_8$ (509) (%): C, 68.49; H, 6.34. Found: C, 68.38; H, 6.47.

General procedure for synthesis of conjugates 1a–e

A solution of 4-dimethylaminopyridine (31 mg; 0.25 mmol) with the corresponding hemiester **2a–2d** (0.5 mmol) in CH_2Cl_2 (5 mL) was added to a solution of α-amino-ω-methoxypoly(ethylene glycol) (mPEG-NH$_2$) (500 mg; 0.1 mmol; M_w = 5 000) or star α-amino poly(ethylene glycol) (sPEG-NH$_2$) (500 mg; 0.025 mmol; M_w = 20 000) and dicyclohexylcarbodiimide (DCC) (103 mg; 0.5 mmol) in CH_2Cl_2 (10 mL), and the reaction mixture was stirred at room temperature. After 2 days, the mixture was filtered and poured into diethyl ether (250 mL). The crystals formed were collected by filtration, and the crude product was recrystallized twice from propan-2-ol.

Methoxypoly(ethylene glycol)amid-succinate-ester-prednisolone (1a). Yield: 450 mg (82%). ^{1}H NMR (500.13 MHz, $CDCl_3$): δ_H: 0.88 (s, 3H); 0.99 (m, 1H); 1.19 (m, 2H); 1.41 (s, 3H); 1.49 (m, 1H); 1.68 (m, 3H); 2.03 (m, 3H); 2.29 (m, 1H); 2.63 (m, 4H); 2.84 (m, 2H); 3.33 (s, 3H); 3.41–3.74 (m, 446H); 4.41 (m, 1H); 4.88 (m, 1H); 5.00 (m, 1H); 5.97 (s, 1H); 6.22 (d, J = 10 Hz, 1H); 6.72 (d, J = 7 Hz, 1H); 7.30 (m, 1H); 8.10 (t, J = 6.8 Hz, 1H). M_w/M_n = 1.18.

Star poly(ethylene glycol)amid-succinate-ester-prednisolone (1b). Yield: 500 mg (91%). ^{1}H NMR (500.13 MHz, $CDCl_3$): δ_H: 0.92 (s, 3H); 1.05 (m, 1H); 1.25 (m, 2H); 1.46 (s, 3H); 1.55 (m, 1H); 1.72 (m, 3H); 2.10 (m, 3H); 2.33 (m, 1H); 2.68 (m, 4H); 2.87 (m, 2H); 3.39–3.78 (m, 460H); 4.45 (m, 1H); 4.89 (d, J = 17 Hz, 1H); 5.03 (d, J = 17 Hz, 1H); 6.02 (s, 1H); 6.27 (d, J = 9,9 Hz, 1H); 6.73 (d, J = 7.2 Hz, 1H); 7.33 (d, J = 9.5 Hz, 1H); 8.13 (m, 1H). M_w/M_n = 1.08.

Star poly(ethylene glycol)amid-glutarate-ester-prednisolone (1c). Yield: 450 mg (82%).^{1}H NMR (500.13 MHz, $CDCl_3$): δ_H: 0.91 (s, 3H); 1.03 (m, 1H); 1.17 (m, 2H); 1.42 (s, 3H); 1.70 (m, 5H); 2.01 (m, 3H); 2.31 (m, 1H); 2.40 (m, 4H); 2.65 (m, 3H); 3.20–3.90 (m, 473H); 4.42 (m, 1H); 4.88 (m, 1H); 4.98 (m, 1H); 5.99 (s, 1H); 6.24 (d, J = 11 Hz, 1H); 7.30 (m, 1H); 7.80 (m, 1H). M_w/M_n = 1.06.

Star poly(ethylene glycol)amid-adipate-ester-prednisolone (1d). Yield: 450 mg (82%).^{1}H NMR (500.13 MHz, $CDCl_3$): δ_H: 0.90 (s, 3H); 1.05 (m, 1H); 1.15 (m, 2H); 1.42 (s, 3H); 1.60–1.75 (m, 7H); 2.05 (m, 4H); 2.29 (m, 3H); 2.41 (m, 2H); 2.53 (m, 1H); 2.65 (m, 1H); 3.35–3.85 (m, 481H); 4.24 (m, 1H); 4.88 (m, 1H); 4.94 (m, 1H); 5.96 (s, 1H); 6.22 (d, J = 9.8 Hz, 1H); 7.28 (m, 1H); 7.85 (m, 1H). M_w/M_n = 1.05.

Star poly(ethylene glycol)amid-phthalate-ester-prednisolone (1e). Yield: 500 mg (90%).^{1}H NMR (500.13 MHz, $CDCl_3$): δ_H: 0.89 (s, 3H); 0.96 (m, 1H); 1.16 (m, 2H); 1.40 (s, 3H); 1.60–1.80 (m, 4H); 2.03 (m, 3H); 2.26 (m, 1H); 2.56 (m, 2H); 3.20–3.80 (m, 505H); 4.40 (m, 1H); 5.05 (d, J = 17 Hz, 1H); 5.26 (d, J = 17 Hz, 1H); 5.97 (s, 1H); 6.22 (d, J = 10.7 Hz, 1H); 6.70 (d, J = 7 Hz, 1H); 7.34 (d, J = 9.5 Hz, 1H); 7.49 (m, 1H); 7.72 (m, 1H); 7.77 (m, 1H); 8.09 (m, 1H). M_w/M_n = 1.12.

Preparation of polypseudorotaxanes 3b and 3d

A mixture of aqueous solutions of α-cyclodextrin (889 mg; 0.91 mmol) (8 mL) and conjugate **1a** or **1d** (100 mg; 4.6 μmol) (3.5 mL) was stirred in a closed vial at room temperature. The stirring was finished after 5 h. A turbidity began to appear after 24 h, and the conjugate separated after 14 days standing was centrifuged, washed with small amount of water, centrifuged again and dried in vacuum at room temperature.

Polypseudorotaxane of α-cyclodextrin-star poly(ethylene glycol)-amid-succinate-ester-prednisolone (3b). Yield: 250 mg (37%). ^{1}H NMR (500.13 MHz, DMSO-d$_6$): δ_H: 0.83 (s, 3H); 1.28 (m, 3H); 1.43 (s, 3H); 1.6–1.7 (m, 3H); 1.95 (s, 2H); 2.13 (s, 3H); 2.38 (m, 2H); 2.71 (m, 4H); 3.32 (m, 192H); 3.43 (m, the signal is partially overlapped by the water signal); 3.55 (m, 366H); 3.60–3.64 (m, 192H); 3.67–3.72 (m, 384H); 3.82 (t, J = 9.4 Hz, 192H); 4.54 (t, J = 5.7 Hz, 192H); 4.84 (d, J = 3.2 Hz, 192H); 5.49 (d, J = 2.7 Hz, 192H); 5.57 (d, J = 7.1 Hz, 192H); 5.96 (s, 1H); 6.20 (d, J = 10 Hz, 1H); 7.36 (d, J = 10 Hz, 1H); 8.05 (m, 1H).

Polypseudorotaxane of α-cyclodextrin-star poly(ethylene glycol)-amid-adipate-ester-prednisolone (3d). Yield: 300 mg (62%). ^{1}H NMR (500.13 MHz, DMSO-d$_6$): δ_H: 0.82 (s, 3H); 1.1–1.2 (m, 3H); 1.29 (s, 3H); 1.43 (m, 1H); 1.5–1.6 (m, 3H); 1.84 (s, 1H); 1.95 (s, 2H); 2.13 (s, 4H); 2.23 (t, J = 7.4 Hz, 2H); 3.32 (d, J = 8.4 Hz, 108H); 3.41 (m, the signal is partially overlapped by the water signal); 3.55 (m, 366H); 3.60–3.72 (m, 216H); 3.81 (t, J = 9 Hz, 108H); 4.54 (brs, 108H); 4.84 (d, J = 2.8 Hz, 108H); 5.49 (brs, 108H); 5.57 (brs, 108H) 5.96 (s, 1H); 6.20 (d, J = 10 Hz, 1H); 7.37 (d, J = 10 Hz, 1H); 7.90 (m, 1H).

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