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# Synthesis, biodistribution and excretion of radiolabeled poly(2-alkyl-2-oxazoline)s

Florian C. Gaertner [a,1], Robert Luxenhofer [b,1], Birgit Blechert [a], Rainer Jordan [b,*], Markus Essler [a,*]

[a] *Nuklearmedizinische Klinik und Poliklinik, Klinikum rechts der Isar, Technische Universität München, Ismaninger Str. 22, 81675 München, Germany*
[b] *Lehrstuhl für Makromolekulare Stoffe, Department Chemie, Technische Universität München, Lichtenbergstrasse 4, 85747 Garching, Germany*

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**Abstract**

Here we report on the preparation of well defined water-soluble poly(2-methyl-2-oxazoline) and poly(2-ethyl-2-oxazoline) terminally equipped with a chelator (*N*,*N*′,*N*″,*N*‴-tetraazacylododecane-1,4,7,10-tetraacetic acid (DOTA)) for radionuclide labeling. The tissue distribution and excretion of $^{111}$In-labeled poly(2-alkyl-2-oxazoline)s were studied in mice. We found that the hydrophilic polymers do not accumulate in tissues and are rapidly cleared from the blood pool, predominantly by glomerular filtration in the kidneys. In contrast only a small fraction is excreted via the hepatobiliary tract. Only minimal amounts of poly(2-alkyl-2-oxazoline)s are taken up by the reticuloendothelial system (RES). Scintigraphic studies revealed the feasibility of *in vivo* imaging of $^{111}$In-labeled poly(2-oxazoline)s. Since additional functionalities for targeting can readily be introduced into poly(2-oxazoline)s via functional monomer units, these compounds fulfill fundamental requirements for an application as carrier molecules in radionuclide therapy.


*Keywords:* Poly(2-oxazoline); $^{111}$In; DOTA; Biodistribution; Biocompatibility

## 1. Introduction

The development of tumor-specific targeting compounds is a central aim of modern oncology. A prerequisite for specific tumor targeting is the generation of appropriate carriers for anti-tumor drugs. Already in 1975 Ringsdorf [1] suggested a polymer-based carrier system which should be chemically well defined, water soluble and biocompatible. Compared to the use of low molar mass compounds the 'polymer approach' has various advantages as specificity and avidity of the drug conjugate can be easily modified. Multi-functional polymers for conjugation with several copies of targeting molecules, drugs or radionuclides can be synthesized in a single polymerization reaction, whereas multi-functional, low molar mass compounds typically require a multi-step reaction. Moreover, it was found that polymer-conjugates of cytotoxic drugs such as doxorubicin show dramatically reduced unspecific toxicity and improved bioavailability [2]. In this context, the conjugation of drugs to the hydrophilic poly(ethylene glycol) (PEG) is the most prominent example (PEGylation) [3]. PEG is synthesized via living anionic polymerization and is therefore highly defined in terms of targeted molar mass and molar mass distribution. However, the functionalization of PEG is limited to the two chain ends and multiple functionalization is only possible with branched or dendritic PEG derivatives [4,5].

A potential alternative to PEG is poly(2-oxazoline) (POx). Just like PEG, POx with a methyl- or ethyl-side chain are highly water-soluble and some conclusive studies report a good biocompatibility of this class of polymers [6]. Studies on POx grafted liposomes and micelles [7–9] did not report any adverse

* Corresponding authors. R. Jordan is to be contacted at Lehrstuhl für Makromolekulare Stoffe, Department Chemie, Technische Universität München, Lichtenbergstrasse 4, 85747 Garching, Germany. Tel.: +49 89 289 13581; fax: +49 89 289 13562. M. Essler, Nuklearmedizinische Klinik und Poliklinik, Klinikum rechts der Isar, Technische Universität München, 81675 München, Germany. Tel.: +49 89 4140 2969; fax: +49 89 4140 4950.
*E-mail addresses:* Rainer.Jordan@tum.de (R. Jordan), Markus.Essler@gmx.de (M. Essler).
[1] Both authors contributed in same parts to this article and are to be regarded as first authors.

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effects of the polymer in animal models and suggest that POx behave similar to PEG for this type of conjugates (stealth liposomes). POx is synthesized via living cationic polymerization and can be synthesized with the same structural definition. Typically, the polydispersity (PDI) is low ($M_w/M_n \sim 1.2$) and the chain termini can be functionalized via the termination [10–16] or initiation method [10,11,17–22].

In contrast to PEG, POx can be easily equipped with side chain functionalities to modify the solubility (e.g. longer *n*-alkyl chains) or with chemical functions to allow coupling of targeting moieties such as peptidic motifs. Additional to the already existing functions such as hydroxyl- [23,24], phenyl- [25], carboxyl- [23,26], carbazol-functionalities [27,28], iodo-aryl- [29], bipyridyl- [30,31], as well as furan- and maleimide modifications [32], we introduced recently the aldehyde [33], alkyne [10] and amine [34] functionalities.

Despite their interesting characteristics and their chemical variability only one study about the biodistribution of POx *in vivo* has been published. Goddard et al. [6] reported on the synthesis and biodistribution studies of a radiolabeled poly[(2-methyl-2-oxazoline)-co-(2-(4-hydroxyphenyl)-2-oxazoline)] (PMOx-co-HphOx) polymer in mice. After injection, significant tracer accumulation was only found in samples of skin and muscle tissue. Especially, the low accumulation of the polymer in the reticuloendothelial system (RES), in liver and spleen would make POx a potential candidate for an application in radionuclide therapy.

However, the defined synthesis of the PMOx-co-HphOx copolymer seemed to be difficult and polymers with broad molar mass distributions were obtained, hence post polymerization fractionation had to be used to reduce the polydispersity of the polymer for biodistribution studies. This might be attributed to the 4-hydroxyphenyl-2-oxazoline comonomer which interferes with the living cationic polymerization (i.e. cross-linking, termination reaction). Thus, a more defined synthesis of radiolabeled hydrophilic POx is needed in order to investigate the biodistribution of this promising polymer class.

A first step to develop tumor-targeting devices with POx carriers could be the defined synthesis of POx-polymers conjugated with *N*,*N*′,*N*″,*N*‴-tetraazacylododecane-1,4,7,10-tetraacetic acid (DOTA), a common chelator for various diagnostically or therapeutically used radionuclides such as $^{111}$In and $^{90}$Y [35–39].

Here we report the synthesis of well defined water-soluble POx, its conjugation with DOTA at the chain end and labeling of these constructs with $^{111}$In. The *in vivo* biodistribution of this construct as well as its excretion was investigated in mice.

## 2. Materials and methods

### 2.1. Chemicals and methods

All substances were purchased from Sigma-Aldrich (Munich, Germany) and were used as received unless otherwise stated. Methyl trifluoromethylsulfonate (MeOTf), 2-methyl-2-oxazoline (MOx), 2-ethyl-2-oxazoline (EOx), acetonitrile (ACN) for polymer preparation and other solvents were dried by refluxing over $CaH_2$ under a dry nitrogen atmosphere and subsequent distillation prior to use. 2-(4-Isothiocyanatobenzyl)-*N*,*N*′,*N*″,*N*‴-tetraazacylododecane-1,4,7,10-tetraacetic acid × 3.5 HCl (*p*-SCN-Bn-DOTA) was purchased from Macrocyclics Inc. (Dallas, TX, US) and used as received.

NMR spectra were recorded on a Bruker ARX 300 ($^1$H: 300.13 MHz) or a Bruker AC 250 ($^1$H: 250.13 MHz) at room temperature. The spectra were calibrated using the solvent signals ($CDCl_3$ 7.26 ppm, $D_2O$ 4.67 ppm). Gel permeation chromatography (GPC) was performed on a Waters system (pump mod. 510, RI-detector mod. 410, precolumn PLgel and two PL Resipore columns (3 μm, 300 × 7.5 mm)) with *N*,*N*-dimethyl acetamide (DMAc) (75 mmol/L LiBr, $T$ = 80 °C, 1 mL/min) as eluent and calibrated against polystyrene standards. HPLC was performed on a HP1100 series with water (0.1% TFA, solvent A) and ACN (0.1% TFA, solvent B) as the mobile and Nucleosil 100 RP-18 as the stationary phase.

MALDI-TOF mass spectrometry was performed on a Bruker-Daltonic, Ultraflex TOF/TOF system using a dithranol matrix. The samples were prepared from a chloroform solution ($c$ = 0.5 mg/mL). Detection was made in linear as well as reflection mode. Otherwise, the sample preparation and data accumulation was performed according to [11].

### 2.2. Animals

Female 7–8 week old CD1 mice (Charles River, Germany) were used for *in vivo* studies. The animals were given access to food and water ad libitum. Animal studies were conducted according to the guidelines of the ethical board of the TU München.

### 2.3. Preparation of amine terminated poly(2-oxazoline)s

#### 2.3.1. Poly(2-methyl-2-oxazoline)-N-tert.-butyloxycarbonylpiperazine, $PMOx_{48}$BocPip

$PMOx_{48}$BocPip was prepared by living cationic polymerization of the monomer 2-methyl-2-oxazoline (1.56 g, 18.3 mmol, 48 eq) and with 63 mg of the initiator methyl trifluoromethylsulfonate (methyl triflate, MeOTf) (0.38 mmol, 1 eq) dissolved in 10 mL acetonitrile (ACN) at 0 °C. After polymerization for 3 days at 85 °C, the reaction mixture was again cooled to 0 °C (ice bath) and 188 mg *N-tert*.-butyloxycarbonylpiperazine (*N*-Boc-piperazine) (1.0 mmol, 2.7 eq) dissolved in 750 μL ACN were added for termination of the polymerization. After stirring at room temperature (rt) for 4 h, a spatula's tip of finely ground anhydrous potassium carbonate was added. After 3 days stirring at rt the solvent was removed under reduced pressure and the residue was dissolved in 20 mL of 3:1 (v/v) mixture of chloroform and methanol (MeOH). After filtration, the polymer product was precipitated in 300 mL cold diethylether. By lyophylization, the product was obtained as a colorless solid (1.37 g, 84%). $M_n$(theo.) = 4285 g/mol. $^1$H NMR ($D_2O$, 300 MHz): $\delta$ = 3.45 (br, 240H, N–C$H_2$–C$H_2$–N), 2.98/2.84 (br, 3H, C$H_3$–N), 2.00 (br, 180H, N–CO–C$H_3$), 1.37 ppm (s, 9H, C(C$H_3$)$_3$). GPC: $M_n$ = 6580 g/mol, PDI = 1.16. HPLC: 10%B → 100%B (30 min): $t_r$ = 10.2 min.