Faculty of Science
CHARLES UNIVERSITY IN PRAGUE

Department of Physical and Macromolecular Chemistry

INSTITUTE OF MACROMOLECULAR CHEMISTRY
AS CR, V.V.I.

Department of Supramolecular Polymer Systems

POLYMER CARRIERS FOR NUCLEAR MEDICINE

Doctoral thesis

Mgr. Ondřej Sedláček

Supervisor: Mgr. Martin Hrubý, PhD.

PRAGUE 2014

Přírodovědecká fakulta
UNIVERZITY KARLOVY V PRAZE

Katedra fyzikální a makromolekulární chemie

ÚSTAV MAKROMOLEKULÁRNÍ CHEMIE
AV ČR, V.V.I.
Oddělení nadmolekulárních polymerních systémů

POLYMERNÍ NOSIČE PRO NUKLEÁRNÍ MEDICÍNU

Disertační práce

Mgr. Ondřej Sedláček

Školitel: Mgr. Martin Hrubý, PhD.

PRAHA 2014

Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl/a všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

Declaration:

Hereby I declare that I have worked out this doctoral thesis independently, under the guidance of Mgr. Martin Hrubý, PhD. and I have fully cited all the sources used. This work has not been used in order to earn any other academic degree.

Praha, 17.12.2014

Signature

Acknowledgement

First of all, I would like to express my thankfulness to my supervisor Mgr. Martin Hrubý, PhD. for offering me this interesting topic and his supporting me with a lot of advice and motivation, always in the friendly mood.

Then I would like to thank Dr. Petr Štěpánek, DrSc., head of the depart I currently belong to, as well as prof. Karel Ulbrich, DrSc., head of my former department, for many inspiring consultations, but also for creating us excellent working conditions.

Indeed I would like to thank my colleagues from both groups of Supramolecular Polymers and Biomedical Polymers for creating an enjoyable working atmosphere. Especially I would like to thank Mgr. Miroslav Vetrík and RNDr. Jan Kučka PhD. for a lot of advice in the field of radiochemistry.

Finally, I would like to thank my wife Mgr. Renata Sedláčková for her patience and supporting me during this work.

Abstract

In the thesis, we developed and studied a novel polymer delivery system for the DNA-intercalator bearing radioisotope iodine-125. Auger electrons emitting radioisotopes (such as iodine–125 or indium-111) are a potentially effective cancer treatment. Their use as an effective cancer therapy requires that they will be transported within close proximity of DNA, where they induce double-strand breaks leading to the cell death. This type of therapy may be even more beneficial when associated with drug delivery systems. The DNA intercalators proved to be effective carriers for the delivery of Auger electron emitters into DNA. Therefore, the new radioiodinated DNA-intercalating ellipticinium derivatives were synthesized and characterized. These compounds were linked to *N*-(2-hydroxypropyl) methacrylamide copolymer with narrow molecular weight distribution via acid-sensitive hydrazone linker. The structure of the linker plays a crucial role in the biological effectivity of the delivery system, so it was optimized to be stable at pH 7.4 (representing the pH of blood plasma), whereas in slightly acidic pH in endosomes after the cell internalization, the radioiodine-containing biologically active intercalator is rapidly released from its polymer carrier. The intercalating ability of the active compound was determined by titration with a DNA solution while its fast cell nuclear internalization was proven by confocal microscopy. Because the hydrazone conjugates showed incompatibility with the standard radioiodination conditions, the intercalator was first radiolabeled by iododestannylation procedure, followed by the polymer conjugation. In vivo experiments on mice with 4T1 murine breast cancer resulted in a statistically significant increase in the survival time of mice treated with the polymer radioconjugate. The free radioiodinated intercalator was also shown to be effective, but in a less extent than the polymer conjugate.

Keywords: Ellipticine, polymer conjugates, drug delivery systems, PHPMA, radiotherapy, Auger electrons, hydrazone bond, iodine-125

Abstrakt

V rámci této disertační práci byl vyvinut a studován nový polymerní systém pro cílený transport a řízené uvolné uvolňování DNA-interkalátoru nesoucího radionuklid jód-125. Radioizotopy emitující Augerovy elektrony (např. jód-125 nebo indium-111) jsou studovány jako nový prostředek léčby onkologických onemocnění. Pro zajištění jejich protinádorové účinnosti musí být dopraveny do těsné blízkosti DNA, kde působí její dvouřetězcové zlomy vedoucí ke smrti buňky. Tento typ terapie může být vylepšen spojením s polymerními systémy pro cílený transport léčiv. DNA interkalátory se osvědčily jako efektivní cílení Augerových zářičů do DNA. Proto byly připraveny a charakterizovány nové radiojodované deriváty DNA-interkalátoru elipticinu. Tyto sloučeniny byly navázány na kopolymer N-(2-hydroxypropyl)methakrylamidu s úzkou distribucí molárních hmotností pomocí kysele štěpitelného hydrazonového můstku. Chemická struktura spojky hraje zásadní roli pro biologickou efektivitu systému, proto byla optimalizována pro maximální stabilitu při pH 7,4 (pH krevní plazmy), zatímco v mírně kyselém prostředí endozómu po internalizaci do buňky bude radiojodovaný biologicky aktivní interkalátor uvolněn velmi rychle ze svého polymerního nosiče. Interkalační schopnost aktivní jodované látky byla potvrzena titracemi roztokem DNA, zatímco její rychlá internalizace do buněčného jádra byla potvrzena konfokální mikroskopií. Protože polymerní konjugáty obsahující hydrazonovou vazbu se ukázaly nestabilní za podmínek standardních radiojodací, elipticinový interkalátor byl nejprve radioaktivně označen pomocí jododestannylace a následně navázán na polymerní nosič hydrazonovou vazbou. In vivo experimenty na myších s 4T1 prsním nádorem prokázaly významné prodloužení délky přežívání při léčbě polymerním radiokonjugátem ve srovnání s neléčenými kontrolami. Volný radiojodovaný interkalátor byl také účinný, avšak méně než polymerní systém.

Klíčová slova: Elipticin, polymerní konjugáty, PHPMA radioterapie, Augerovy elektrony, hydrazonová vazba, jód-125.

Contents

LIST OF ABBREVIATIONS

^{125}I-UdR	5-(^{125}I)iodo-2'-deoxyuridine
AE	Auger electron
AHMA	3-(9-Acridinylamino)-5-hydroxymethylaniline
BNCT	Boron neutron capture therapy
Boc	*t*-Butyloxycarbonyl
BRT	Brachytherapy
CA	*cis*-Aconityl
CMC	Critical micelle concentration
CMC	Critical micelle concentration
DDD	Drug delivery device
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOTA	1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid
DOX	Doxorubicin
DTPA	Diethylene triamine pentaacetic acid
EBRT	External beam radiation therapy
EC	Electron capture
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EPR	Enhanced Permeability and Retention
FDA	Food and Drug Administration
GRP	Gastrin-releasing peptide
HER2	Human epidermal growth factor receptor 2
HPLC	High-performance liquid chromatography
HPMA	(*N*-(2-Hydroxypropyl)methacrylamide)
LCST	Lower critical solution temperature
LET	Linear energy transfer
MATA	*N*-Methacroyl-L-tyrosinamide
MIBG	*m*-Iodobenzylguanidine
NHL	Non-Hodgkin lymphoma
NLS	Nuclear localization signal

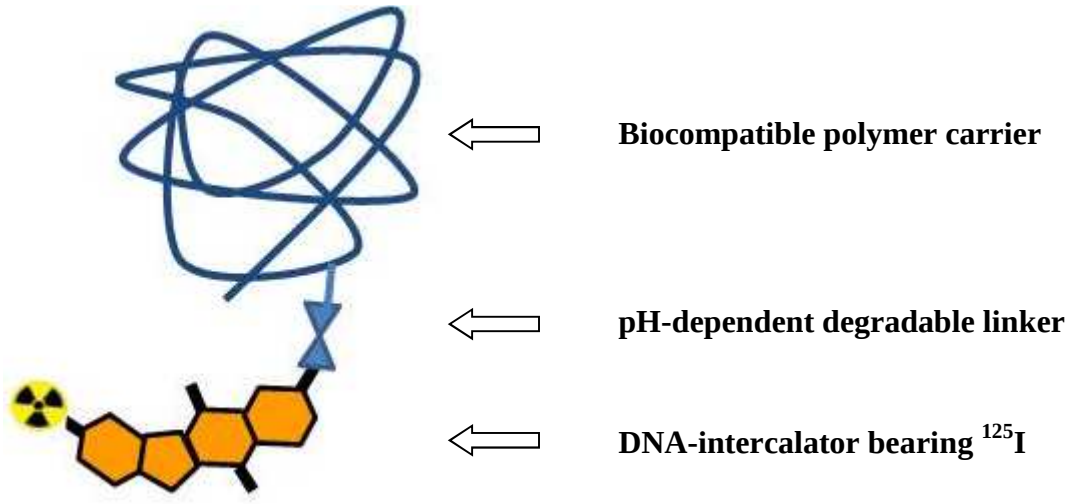
PCL	Polycaprolactone
PDI	Polydispersity index
PEO	Polyethylene oxide
PET	Positron emission tomography
PHPMA	Poly(*N*-(2-hydroxypropyl)methacrylamide)
pHPMA-MAAcap hydrazide	Poly[*N*-(2-hydroxypropyl)methacrylamide-*co*-1-*N*-(6-hydrazino-6-oxohexyl)-2-methacrylamide
PMMA	Poly(methyl methacrylate)
PNIPAAM	Poly(N-isopropylacrylamide)
POx	Poly(2-alkyl-2-oxazoline)
PRRT	Peptide Receptor Radionuclide Therapy
PTX	Paclitaxel
RAFT	Reversible Addition-Fragmentation chain Transfer
RES	Reticuloendothelial system
RIA	Radioimmunoassay
RIT	Radioimmunotherapy
ROS	Reactive oxygen species
RT	Room temperature
SEC	Size-exclusion chromatography
SPECT	Single-photon emission computed tomography
TATE	(Tyr^3)-octreotate
TFA	Trifluoroacetic acid

LIST OF SYMBOLS

A	Absorbance
Bq	Becquerel
d	Day
eV	Electronvolt
E_β	Energy of β radiation
E_γ	Energy of γ radiation
M_n	Number average molecular weight
M_w	Weight average molecular weight
n	Refractive index
R_h	Hydrodynamic radius
$t_{1/2}$	Decay half-time of radionuclide
α	Alpha radiation
β	Beta radiation
γ	Gamma radiation
λ	Wavelength

1. AIMS OF THE THESIS

The aim of this study is to synthesize and characterize conceptually new multistage-targeted polymeric delivery system intended for solid tumor therapy with Auger electron emitter ^{125}I. The structure of the system will be as follows:



The polymer carrier will assure the passive accumulation of the whole system in the tumor tissue. After internalization into endosomes in cancer cells, the DNA-intercalator bearing Auger electrons emitting radionuclide ^{125}I will be released in an active form (by pH - triggered release) and the intercalator will bring the radionuclide into nuclear DNA, where the Auger electrons will cause double-strand DNA breaks and thus the cell apoptosis.
Particular emphasis is put on the following partial tasks:

1. Fine-tuning the intercalator-polymer hydrazone linker structure to optimize the pH-dependent release profile for different intercalators (ellipticine or acridine derivatives, respectively). The release rate should be negligible at pH of in blood plasma (7.4) during transport to target tissue, but the intercalator should be released as fast as possible upon the drop of pH after cellular internalization (pH ca 5).
2. Optimizing the strategy for the radioiodination of hydrazone linker-containing drug delivery systems.
3. Radiolabeling strategy for the radioiodination of ellipticine intercalator with respect to the high specific radioactivity of the product as well as the synthesis of its polymer conjugate, followed by radiochemical and biological examination of both systems.

2. INTRODUCTION

2.1. Cancer therapy

In the last century, significant progress was achieved in the field of cancer treatment, yet its full control still remains one of the most important goals of the modern medicinal research.[1] Nowadays the cancer therapy relies mostly on the surgical excision of the tumor tissue followed by adjuvant chemotherapy or external gamma irradiation. Surgery is especially useful in the case of solid, well demarcated and readily anatomically accessible tumors, such as those of the prostate (prostatectomy) or breast (mastectomy).[2] Because cancer cells often penetrate into surrounding tissues, a margin of the seemingly healthy tissue is usually removed as well.[3] However, the surgical treatment has also its limitations. Primarily, in order to be excised, the tumor size has to be big enough to be precisely localized. Thus, surgical therapy is obviously not applicable for the treatment blood cancer (e.g., leukemias, myelomas and certain types of lymphoma) or to manage the metastatic activity of the tumor, probably the most serious complication in cancer therapy. In these cases, chemotherapy represents a powerful tool to handle the disease.

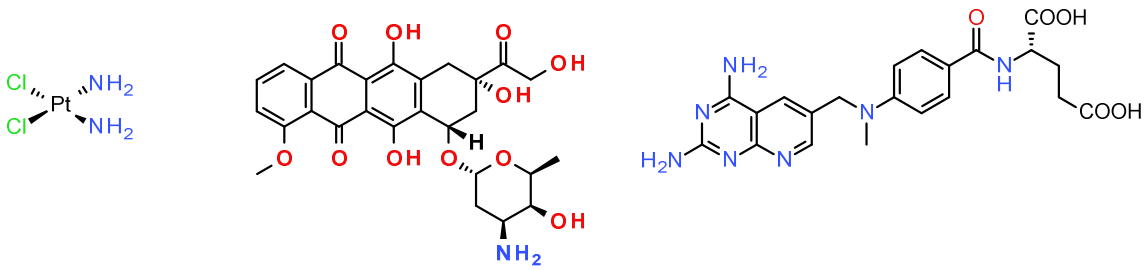
Chemotherapy with cytostatics is used most often to either suppress the growth of the tumor cells or to palliate the symptoms.[4,5] It is usually used together with other forms of cancer treatment, such as surgery, radiotherapy or immunotherapy. According to the mechanism of their action we can divide cytostatics into several categories (**Figure 1**):

I. Cytostatics interfering with DNA replication

- Alkylating agents (e.g., cisplatin, nitrogen mustards, nitrosoureas)[6] modify DNA in the position 7 of the guanine base by alkylation or platinum coordination.[7] Because the proliferation of cancer cells proceeds more rapidly and with less DNA reparation efficacy than in the case of the healthy cells, cancer cells are more sensitive to the DNA-damage including alkylation.[8,9] Alkylation obstructs the tumor DNA replication process. Unfortunately, this therapy also inhibits proliferation of other fast prolifering healthy cells, such as those in testes, ovaries or bone marrow, and often leads to secondary carcinogenicity.[10]
- Topoisomerase inhibitors (e.g., topotecan, doxorubicin, ellipticine derivatives, etoposide, amsacrine) target the DNA-chain-ligation step of the enzymes

Topoisomerase I or Topoisomerase II.[11] These therapeutics (often potent DNA intercalators) inhibit the replication DNA due to the single- or double-strand breaks. Intercalation into DNA is usually the first step required for topoisomerase inhibition.

- Antimetabolites (e.g., methotrexate, gemcitabine, 5-fluorouracil) are structural analogs of nucleotides from which DNA is biosynthesized or of factors necessary for their biosynthesis.[12] They block the metabolism of nucleic acid resulting in slower cell proliferation



Cisplatin **Doxorubicin** **Methotrexate**

Figure 1.: Structures of selected therapeutically used cytostatics.

II. Cytostatics restraining mitosis

- Vinca alkaloids (e.g., vincristine, vinblastine, podophyllotoxin) from plant *Vinca rosea*[13] block the mitosis by inhibiting of the microtubule assembly (tubulin polymerization) and thus disrupting its fragile assembly/disassembly dynamics.
- Taxane (e.g., paclitaxel, docetaxel) from *Taxus brevifolia* tree[14] possess opposite mechanism as vinca alkaloids, inhibiting the microtubule disassembly.
- Inhibitors of cyclin-dependent kinases (e.g., flavopiridol, roscovitine, olomoucine)[15] block the mitosis usually in the G1 phase.

III. Cytostatics with other mechanism of action (e.g., mitomycin, bleomycin)

Together with the surgery and chemotherapy, the most widely used methods for treatment of cancer are radiotherapy (see chapter 2.4.), immunotherapy[16] followed by hormonal therapy[17] of hormonally dependent tumors (e.g., the use of antiandrogens to treat prostate cancer) and inhibition of tumor growth factors.

2.2. Polymer systems for the drug delivery

In spite of their unreplaceable role in the modern cancer treatment, chemotherapy with cytostatics suffers from many drawbacks coming mostly from the fact that they target fast-proliferating cells in general.[18] Therefore, healthy dividing cells are also affected. This may lead to the immune system depression caused by the damage of the bone marrow (decrease in the white blood cell number),[19] gastrointestinal problems (nausea, vomiting and diarrhea),[20] infertility (reproductive system impairment)[21] or hair loss (hair follicles damage).[22] Cytostatics may also damage elimination organs in which they accumulate (e.g., nefrotoxicity of cisplatin)[23] or may have specific other side effects (cardiotoxicity of doxorubicin,[24] neurotoxicity of *Vinca* alkaloids[25]).

Many of these drawbacks could be dramatically reduced by implementing the drug delivery devices (DDD) to the particular therapeutics, which improve biodistribution increasing tumor uptake and decreasing uptake into other organs.[26,27] These devices usually consist of the low molecular weight cytostatic connected, either covalently or non-covalently, to the biocompatible carrier, which assures prolonged drug circulation half-life and preferably the enhanced accumulation of the drug in the tumor tissue with respect to the other parts of the body.[28,29] The drug carrier, usually nanoparticle, like antibody,[30] hormone,[31] polymer,[32] liposome,[33] etc., should be completely non-toxic, biocompatible and non-immunogenic. After the controlled release of the drug, the carrier should be also easily eliminated from the body.

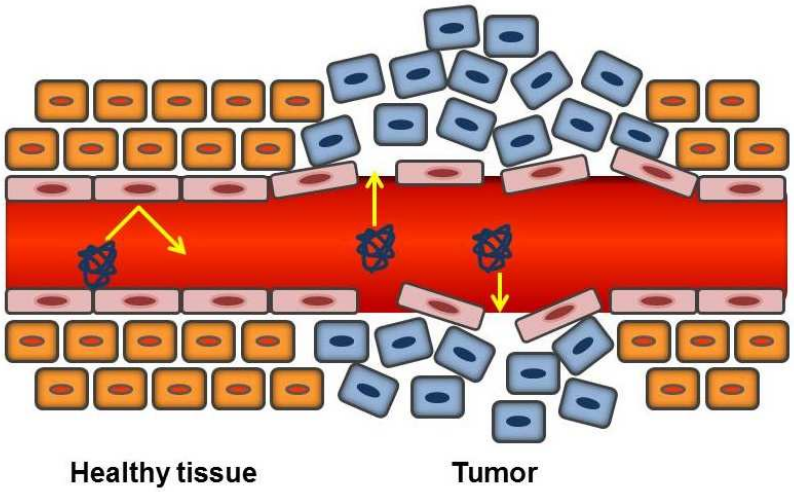


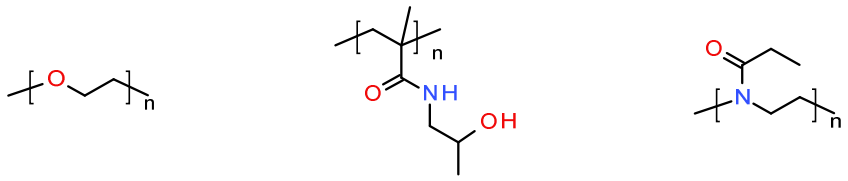
Figure 2.: Scheme of EPR effect.

The nanoparticles could be passively accumulated in the tumor tissue due to the EPR ("Enhanced Permeability and Retention", **Figure 2**) effect first described by Maeda et al.[34-36] To supply the fast growing tumor with blood, the tumor angiogenesis proceeds rapidly. This causes the improper structure of the tumor blood vessels. Their leaky architecture allows permeation of the nanoparticles to the tissue from the bloodstream (not possible in the healthy tissue). Furthermore, lymphatic drainage of the tumor is usually damaged or missing, preventing the nanoparticles from leaving the tumor. As a consequence, concentration of nanoparticles in tumor tissue may be up to one to two orders of magnitude higher compared to healthy tissues.[34]

The extent of accumulation usually rises gradually with the size of nanoparticles and the cut-off size of pores in tumor vessels is 200 nm - 1.2 μm.[37] The maximum therapeutic effect was reported for the particles of 50 - 200 nm in diameter.[27] The important issue, which has to be taken into account, is the possibility of elimination of the DDDs from the organism by kidney excretion. In kidneys, the blood plasma is filtered through the molecularly porous glomerular membrane resulting in renal threshold of the size.[38] That means that nanoparticles under this size are excreted from the organism by urine, whereas the bigger ones circulate in the organism for a long time (up to weeks) until they are excreted by liver *via* hepatobiliary pathway.[39] Aside from the hydrodynamic diameter, the renal threshold for the nanoparticles is also function of conformational shiftiness, shape and charge, with overall negatively charged particles being eliminated less efficiently than neutral and positively charged ones.[40] However, some polymers with higher degree of conformational flexibility and larger hydrodynamic radius than the renal threshold can be slowly eliminated due to the so called "worm-like effect".[41]

In the case of simple non-degradable polymer carriers (**Figure 3**), their size has to be below the renal threshold (ca. 30 kDa for poly(ethylene oxide)[42] (PEO) or 45 kDa for poly[*N*-(2-hydroxypropyl)methacrylamide] (pHPMA) linear chains, respectively)[43] to avoid unnecessary accumulation in the body resulting in, e.g., the liver toxicity. The use of nanoparticles with the size above the renal threshold gives us the benefit of their higher accumulation in the tumor due to the prolonged circulation time.[42] However, after the release of the drug, the system should be degraded to units smaller than the renal threshold. This could be achieved by using e.g., biodegradable polymers like polysaccharides,[44] synthetic block copolymers interconnected with biodegradable bonds[45] or supramolecular

structures (micelles, liposomes, etc.) with the size of unimer units below renal threshold (see below).[26]



poly(ethylene oxide) **poly[*N*-(2-hydroxypropyl) methacrylamide]** **poly(2-ethyl-2-oxazoline)**

Figure 3.: Structures of the most common non-degradable polymer carriers used for DDDs.

The most exploited synthetic polymer used for DDDs is poly(ethylene oxide) (PEO).[46] Due to its hydrophilic and biocompatible nature, connection of drugs with PEO carrier (PEOylation, before also known as PEGylation) leads to the increase of their solubility in aqueous media and shielding the drug from being opsonized by serum proteins followed by their rapid excretion using the reticulo-endothelial system (RES).[47] By utilizing of high molecular weight PEO architectures a substantial EPR effect could be achieved.[47] First PEOylated compounds have been marketed over 20 years ago. Despite their extensive usage, use of PEO based DDDs has also its limitations. Mainly, the potential drug loading of PEO covalent conjugates is limited, usually only the semitelechelic/telechelic groups of the polymer could be used for the drug binding. Therefore the relative drug loading capacity is decreased with the increased length of the chain. For these design of high drug loaded devices, other biocompatible polymers prepared from the monomers capable of statistical copolymerization with (protected)functionalized monomers, such as poly[*N*-(2-hydroxypropyl)methacrylamide] (pHPMA, synthesized by radical polymerization), or poly(2-alkyl-2-oxazolines) (pOX, synthesized by cationic polymerization) are preferred. Due to extensive use of PEO in last decades, significant fraction of population in developed countries (22-25 % of population) was reported to produce anti-PEO antibodies.[48] Also, the PEO is oxidatively unstable, forming hazardous and potentially toxic peroxides when exposed to air in a long term.[49] There are however recent reports that also other types of biocompatible polymers may be oxidatively biodegradable under biologically relevant conditions.[50]

With regard to the synthesis, pHPMA, is prepared in easier way than the pOXs, especially when higher molecular weights (> 10 kDa) are desirable, mostly due to the moisture and air sensitivity of the 2-oxazolines cationic polymerization process.[43] Methods for controlled radical polymerization of HPMA into the high polymerization degrees (with M_n > 100 kDa) and low polydispersities (< 1.2) have been developed,[51] mostly utilizing the Reversible Addition-Fragmentation chain Transfer (RAFT) polymerization. Furthermore, using carefully chosen initiator / chain transfer agent system allows us having high degree of functionality on both sides of the polymer chain.[52] The post-polymerization modification of the RAFT-end group could be performed by different methods, e.g., by thiol-ene reaction or radical coupling.[53] The drug is usually attached to HPMA (co)polymer by biodegradable linker susceptible to intracellular enzymatic, hydrolytic or reductive degradation. The first pHPMA based polymer DDD in clinical studies was doxorubicin bound to pHPMA by enzymatically degradable linker, developed by Kopecek in 80`s, however its development was discontinued in the stage of clinical trials.[54] Since that time, many conjugates with different polymer architecture, drug, linker or the drug application have been prepared to achieve the maximal therapeutical effect.[55]

Apart from the linear polymer systems, the most researched area of DDDs is the family of supramolecular systems like micelles, vesicles, liposomes etc.[26] Polymeric micelles are assemblies of amphiphilic block or graft copolymers with hydrophilic shell and hydrophobic core.[56] The core is capable of encapsulating the hydrophobic drug such as paclitaxel (PTX) using hydrophobic interactions, while being externally biocompatible thanks to the hydrophilic shell.[57] The assembly/disassembly of the micelle is a thermodynamically driven process.[58] At low concentration, unimers behave as single separate molecules. After reaching certain concentration (critical micelle concentration, CMC), micelles start to appear in solution.[59] The CMC depends mainly on the structure (relative hydrophobicity) and size of the hydrophobic block as well as on the ratio of the hydrophobic/hydrophilic blocks. For charged polymers, coulombic interactions may also play important role.[60] Due to the reversibility of the process, after the administration of drug loaded micellar DDDs, their concentration in the body is gradually decreasing, resulting finally in their disassembly under CMC. The drug is then released and the unimers are subsequently eliminated by kidneys.[61] However, this release could not be performed in such a controlled way as in the case of covalently bound drug *via* degradable

linker. If the hydrophobic moiety is an anticancer drug, like in the case of hydrazone-bound doxorubicin-PEO micelles,[62] both drug release and the micelle disassembly are controlled at the same time. The premature micellar drug release could be handled by the stabilization of the core using either its further hydrophobization[45] or by its crosslinking preferably with a bioerodable linker.[63] The micelle could be destabilized in a controlled way using stimuli based on:

(1) *Change of pH* - Due to the hypoxia, the pH value in the tumor interstitium is slightly acidic (ca 6.5).[64] In addition to it, after internalization into the cells, the pH value in late endosomes drops to values below 5.[65] Using cores consisting of protonated polybases with pKa values in the range of 6 - 7, like in poly(L-histidine)-PEO micelles,[66] ensures us the stable hydrophobic (uncharged) character of the micellar core during the transport in blood plasma (pH 7.4) while destabilizing the micelle by charging the core in acidic conditions (**Figure 4A**). Another approach relies on the synthesis of the block copolymers which are composed of two hydrophilic blocks from which one is selectively hydrophobized with acid-removable hydrophobic moiety.[67] The created micelle could be disassembled in a controlled way upon the release of hydrophobic substituent resulting in hydrophilization of the whole polymer.

(2) *Change of temperature* - Using thermo-responsive micelles, only slight change of solution temperature can cause a dramatic change of polymer properties. Above all, polymers exhibiting the lower critical solution temperature (LCST) under and around the body temperature are of particular focus in area of DDDs.[68] The LCST is the critical temperature, below which the substance (e.g., polymer) is soluble at any concentration. This is usually determined as the minimum in the graph of system phase separation temperature (cloud point temperature) dependence on the concentration of the solute.[69] The most studied LCST exhibiting polymers are poly(2-isopropyl-2-oxazoline) or poly(*N*-isopropylacrylamide) (PNIPAAM).[70,71] Micelles formed from block copolymers of thermosensitive and hydrophilic block, like PNIPAAM-*block*-PEO, are able to incorporate the hydrophobic drug above the LCST (**Figure 4B**).[72] Another approach involves the micelles from copolymers of thermo-sensitive and hydrophobic blocks, like poly(methylmethacrylate) (PMMA)[73] or poly(caprolactone) (PCL).[74] If the LCST is set to around 40 °C, the micelle is carrying the anticancer drug to the tumor, where it disassembles by external heating to or slightly above LCST.

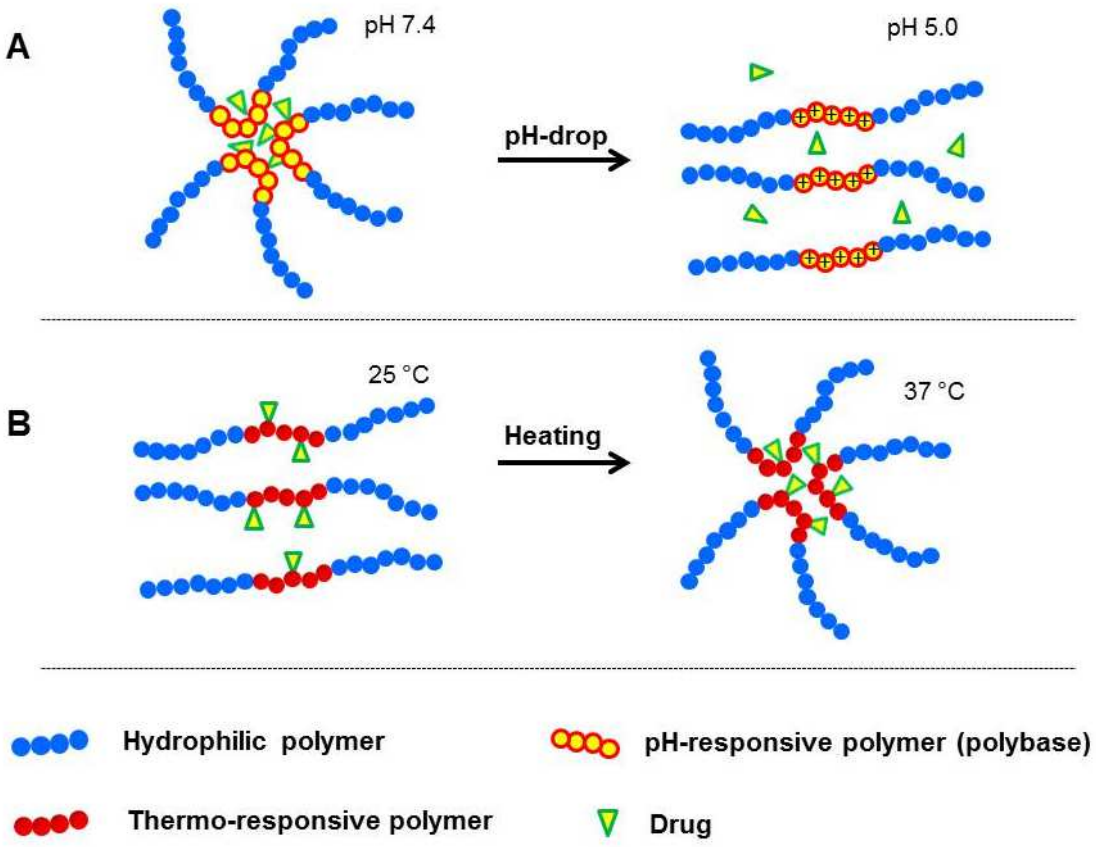


Figure 4.: Stimuli-responsive micellar DDDs. A: pH-responsive micelles; B: Temperature-responsive micelles

Aside from synthetic polymers and their micelles, there is a broad range of other different DDDs, like liposomes,[75] vesicles,[76] metal nanoparticles,[77] nanogels,[78] antibodies,[79] etc. that are useful for drug delivery.

2.3. The pH-sensitive polymer conjugates of cytostatics

Targeting to the more advanced delivery systems, development of efficient methods for the triggered release of the drug from the polymer is desirable. One of the most efficient methods is the use of pH-degradable linker between the polymer and drug. Whereas the pH in blood plasma or cytosol is ca 7.4, the pH in cancerous tissue drops to 6.5 - 7.2[64] depending on the type and size of the tumor, with inhomogeneous spatial distribution. After the cell internalization by endocytosis, the DDDs are exposed to even more acidic conditions inside late endosomes (pH 5.0 - 6.0)[65] or lysosomes (pH 4.0 - 5.0).[80] This pH gradient could be very profitable for the triggering the drug release from the polymer by incorporating the acid-sensitive linker in between.[81] The aim is then to find out the appropriate linker structure, which ensures the sufficient of the conjugate during the transport (pH 7.4), with relatively fast unload of the cytostatic cargo once reaching the acidic target.

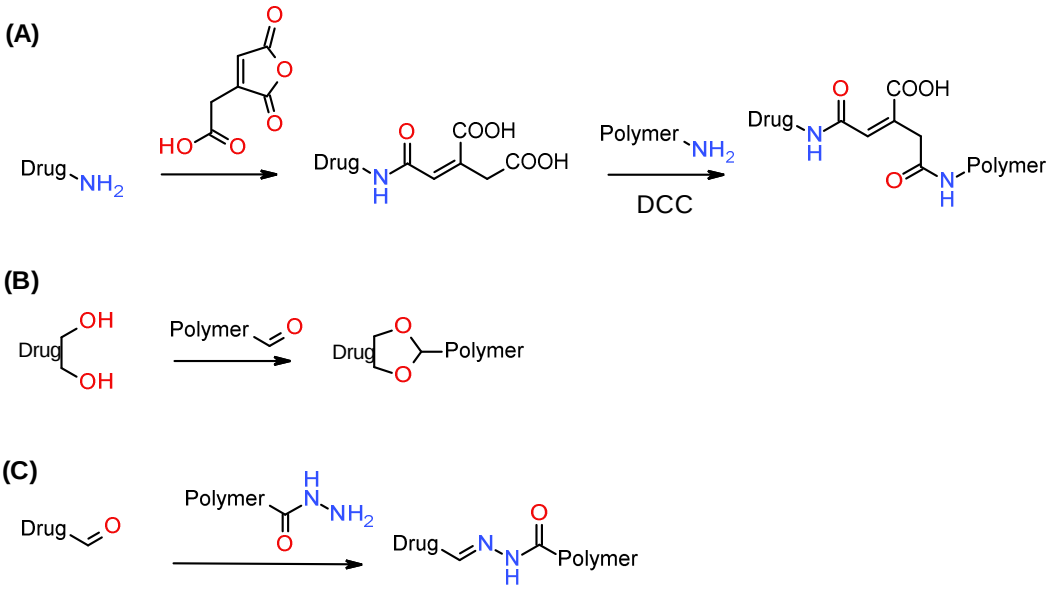


Figure 5.: Synthesis of acid-degradable conjugates.

The first synthesis of the polymer conjugate containing pH-sensitive *cis*-aconityl (CA, **Figure 5A**) linker was published in 1981 by Shen and Ryser.[82] In the first step, they functionalized daunomycin with *cis*-aconityl anhydride *via* amidic bond followed by ligation of the intermediate to poly(L-lysine). The cytotoxicity of those conjugates premixed in the buffer of pH 5 was substantially higher (showing the release from the polymer) than that of conjugate without previous hydrolysis. Similarly, Duncan et al.

prepared a conjugate of daunomycin linked to high molecular weight alginate by CA linker.[83] *In vitro* release experiments proven the conjugate stability in neutral pH 7.0 buffer (only 2 % of daunomycin was released from alginate in 48 h), whereas in the acidic buffer of 5.0, daunomycin was rapidly released (22 % in 48 h). Despite being pioneer in the area of acid sensitive linkers, the major drawback of the CA linker is in the rather slow release in acidic media and a little space for its tunability with respect to currently used acetal and hydrazone linkers.

Acetals can be a favorable linker for the attachment of hydroxy-, respectively diol-containing cytostatics (**Figure 5B**). Depending on their chemical structure, acetals can exhibit good stability at neutral pH and increasing lability with dropping pH.[84] Frechet et al. have recently synthesized a series of conjugates of semitelechelic PEOs with acetal-linked drugs podophylotoxin, 5-fluoro-2´-deoxyuridine, estradiol and 5-fluorouridine, respectively.[85] These conjugates exhibited different hydrolytic stability, with the 5-fluorouridine cyclic acetal conjugate having highest pH selectivity in the applicable pH-range (5 - 7.4).

Hydrazones are organic compounds usually formed by acid-catalyzed reaction of hydrazide (or hydrazine) with ketone or aldehyde, respectively (**Figure 5C**).[86] The pH dependent stability of hydrazones can be exploited in the delivery and controlled release of ketone-containing drugs by hydrazide-containing DDDs. In 1991, Greenfield and co-workers reported the first hydrazone-containing conjugate of cytostatic drug doxorubicin with monoclonal antibodies.[87] These conjugates showed pH-responsive behavior, which can be further optimized by altering the structure of the original hydrazide. Since that time, many hydrazone conjugates of doxorubicin with polymers,[88,89] antibodies[90] or metal nanoparticles[91] were studied. Unfortunately, doxorubicin is one of the few cytostatics possessing the ketone in this native structure. In the case of other chemotherapeutics, the ketone (oxo-) group has to be introduced by attaching suitable linker. The simplest way is to functionalize the amino- or hydroxy-group containing cytostatic with oxo-carboxylic acid (e.g., levulic acid).[92] This modification allows us to attach the drug *via* hydrazone bond. However, the modification of the original cytostatic with linker could hamper its biological activity.

2.4. Radiation therapy of cancer

Apart from the surgery and chemotherapy, therapy with radionuclides (**radiotherapy**) is one of the most efficient methods of cancer treatment.[93] It is mainly based on the radiation damage of the tumor tissue with ionizing radiation while minimizing the radiation burden of healthy tissues. Radiotherapy can follow the surgical intervention (as adjuvant therapy) to prevent recurrence of cancer after primary tumor removal or can be used as main therapeutical strategy for late-stage tumors where the surgery would be too risky. Furthermore, there is a beneficial synergy of radio- and chemotherapy frequently exploited in combinatorial therapy.[94] Based on the location of the radionuclide source with respect to the human body, there are two radiotherapeutical sub-divisions: External beam radiotherapy (EBRT or XRT, where the radiation source is localized outside the body) and internal radiotherapy (radiation source inside the body).

External beam radiotherapy (EBRT) is the most frequent form of radiotherapy.[95] It is based on the irradiation of tumor with photons (X-ray), electrons and recently also protons. The biological effect of the irradiation depends on its energy. For example, the irradiation with "kilovoltage" X-ray (the energy, defined as the maximum electric potential on linear accelerator to produce a photon beam, is in the range 50 to 200 kV) is used for treatment of skin malignancies,[96] whereas the mostly used "megavoltage" X-rays (energy 1 to 25 MV) are used for the radiosurgery of deeper laying tumors (brain, lung, prostate, etc.).[97] Apart from the linear accelerator produced X-ray beam, medically useful photon beams could be originated by decay of certain radioisotopes like ^{60}Co, which is abundantly used as radiation source for the treatment of brain tumors (Leksell gamma knife).[98] Recently, the therapy with heavy nuclear particles (mainly protons) attracted more attention.[99]

Energy delivered by gamma photons decreases exponentially along the path in the tissues delivering the highest dose to skin and a decreasing dose with increasing depth in tissue, with zero dose theoretically delivered at infinite depth.[100] In the case of electrons, the amount of energy transferred from the particle to the surrounding matter (i.e. the ionization density) is approximately linear defined by linear electron transfer (LET, loss of particle`s energy relative to its traveling distance) until the electrons stop, beyond which point the delivered dose is zero.[101] The electron is losing the energy mostly by production of secondary electrons and bremsstrahlung photons.[101] However, in the case of heavy

particles capable of massive ionization (protons and α-particles), the LET increases with the path distance from a certain energy, as the particle slows down.[102] Near the end of the particle path, the particle velocity is low, which means more time to Coulomb interactions to occur, resulting in more efficient ionization of the surrounding matter, called the Bragg peak (see **Figure 6**).[103] Beyond the Bragg peak the energy drops to zero. Therefore, the radiotherapy with heavy particles (e.g., proton therapy) enables better targeting of radiation damage by aiming the Bragg peak to the tumor tissue.[104] In the case of electrons (β^- radiation), the Bragg peak can be observed only at very low energies (< 1 keV, eV is defined as particle energy acquired when 1 unit of electrical charge 1.609×10^{-19} C is accelerated by electrical potential of 1 V in vacuum),[105] so the energy released in the Bragg peak is negligible with respect to the initial energy of the common β^- particles (typically 0.5 - 3 MeV).

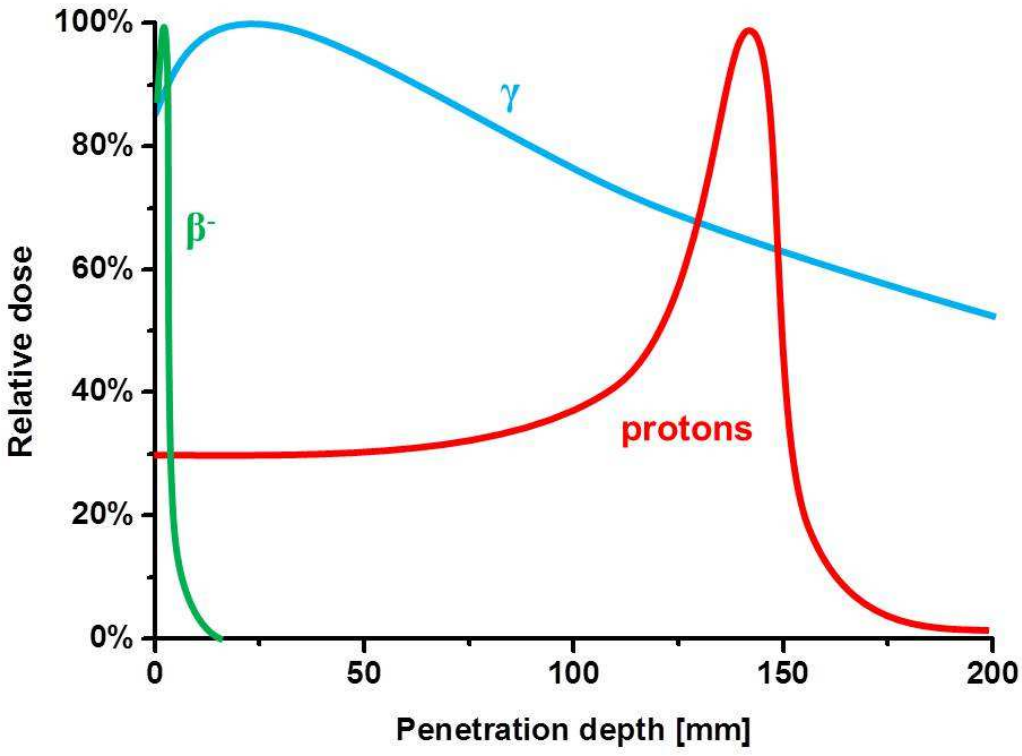


Figure 6.: Dependence of absorbed dose on the tissue penetration depth for clinically used radiation sources.

Despite being a superior method of radiotherapy for decades, EBRT also possess some serious drawbacks, mainly related to the non-specific radiation burden of the healthy tissue (irritation of the bowel and bladder, incontinence and impotence after prostate

cancer treatment, skin burns, stomal cavity and esophagus burns after radiotherapy of head and neck cancer).[106] Furthermore, the benefits of EBRT are limited to the treatment of medium-size to larger solid tumors, but fails in the therapy of disseminated cancer cells and micrometastases.[107] Also, EBRT requires everyday irradiating for several weeks.

Internal beam radiotherapy. Some drawbacks of the EBRT can be overcome by using **internal radiotherapy** (endoradiotherapy), where the source of radiation is located inside the body and preferentially inside the tumor tissue.[108] For this purpose, therapy with individual radionuclides rather than accelerated particle beam is used. **Radionuclides** used for the internal radiotherapy can be gamma (γ), beta (β^-), alpha (α) or low energy Auger electron (AE) emitters (**Table 1**).

Radiation	Examples	LET [keV/µm]	Range in cells	Use
β^-	^{131}I	0.2	50-200 cells	Therapy of solid tumors
α	^{213}Bi, ^{211}At, ^{223}Ra	80-100	4-10 cells	Treatment of single cancer cells/cell clusters
AEs	^{125}I, ^{111}In, ^{m99}Tc	4-26	< 1 cell	Treatment of single cancer cells

Table 1.: Comparison of radiation types useful for the internal therapy of cancer.

Gamma radiation is a high-energy electromagnetic radiation originating by de-excitation of nucleus after α, β^- or electron capture decay.[105] Especially in the case of electron capture decay, there are also X-rays of the daughter atom coming from reorganization of the electron shell. In the tissue, gamma radiation causes indirect ionization by producing secondary electrons from the tissues atoms mainly by Compton scattering, electron-positron pair production (dominant at higher energies above 1022 keV) and photoelectric effect (dominant at low energies).[109] The ionization properties of γ-radiation depend mainly on their energy and because photons have no charge, their range in the living tissue is the longest from all types of radiation. Low energy γ- emitters (like ^{m99}Tc) are often used for the tumor diagnostics on gamma camera.[110] Higher-energy γ-emitters can be used to irradiate tumor tissues in EBRT and sometimes in brachytherapy

(see below).[111] However, because of their long range, γ-emitters are not suitable for the targeted endoradiotherapy.[105]

In practice, β^- emitters are by far the most common radiation sources in internal radiotherapy. The β^- particles are electrons emitted from the nucleus of an atom which undergoes a decay of neutron to a proton.[105] They typically have energies between few keV to a few MeV and a mean range in the tissue between 0.2 mm (^{177}Lu) and 4 mm (^{90}Y).[112] Because of its range that represents approximately 50 - 200 cell diameters,[113] the β^- radiation covers also cancer cells without direct contact but adjacent to the decayed radionuclide (so called "cross-fire effect"), which is beneficial for the therapeutic response.[114] The ionization properties of electrons in tissue mainly consist in the ionization of oxygen molecules to form reactive oxygen species (ROS, especially hydroxyl and superoxide radicals), which subsequently attack the cancer cell structures.[109] However, after some time of rapid cancer cells proliferation and tumor growth, there is usually lack of oxygen in the tumor (hypoxia). This can make the radiotherapy of large tumors less effective.[115] Therefore, the ideal targets for the β^- radiation therapy are small to medium-size tumors. The disadvantage of the β^- therapy is the unavoidable radiation burden of the healthy tissue.[113] The β^+ decay, producing positron and neutrino, is rather employed in the area of tumor imaging than therapy.[116] The positron rapidly annihilates with electron to produce γ rays (E = 511 keV) observable by gamma camera, which is routinely exploited by the positron emission tomography (PET) imaging technique.[117]

Alpha (α) emitters are heavy atoms that emit α-particles (helium nuclei) to release the repulsive forces between protons.[105] Because of their substantial mass and charge, α-particles are highly ionizing when penetrating the tissue. It is considered as a high-LET radiation - with a mean range of 40 - 80 μm (5 - 10 cell diameters) and energy of 4 - 9 MeV.[118] The high density of the α-particle`s energy in a small volume predestine them for the therapy of single tumor cells (e.g., blood malignancies) or their small lesions, since there is almost no cross-fire effect compared to the β^- irradiation. Therapeutical effect of α-radiation is based on the direct ionization damage of DNA by double-strand breaks.[109] When brought to the proximity of the tumor cells, 1 - 4 α-decays can usually destroy the cell (compared to < 20,000 β^- decays).[119] The disadvantage of the α-radiation therapy is particularly the high systemic radiotoxicity during transport to the target tissue and worse availability of the α-emitters.[120]

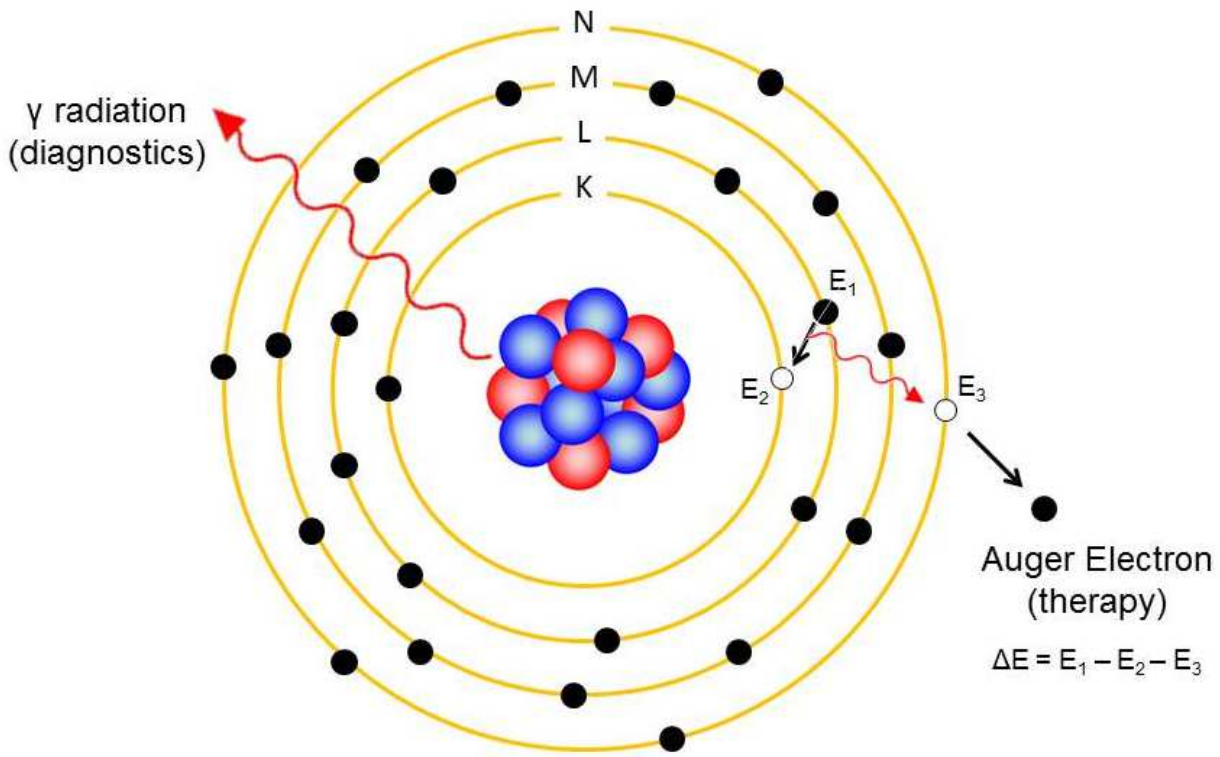


Figure 7.: Scheme of Auger electron production.

Auger electrons are low-energy (E < 30keV) electrons emitted by radionuclides decaying by electron capture (EC).[105] In EC, the proton in the nucleus captures one of the inner-shell electrons, forming a neutron ("reversed β^- process"). The formed inner-orbital vacancy can be filled with one of the higher-sphere electrons. The energy from this process can be either released as X-ray photons or can be transferred to some of the outer-shell electron resulting in their emission (**Figure 7**).[121] These secondary electrons, so-called Auger electrons (AEs), have low energy and a very low tissue range (1 - 20 nm).[122] They have enormous destructivity (high LET), but only within a small area. Therefore, to be biologically effective, they need to be internalized into the cell cytoplasm and preferentially to the cell nucleus, where they cause the DNA double-strand breaks and thus the cell death.[123] Because of their high selectivity, they are often considered as "magical bullets" for cancer therapy, as designed by Paul Ehrlich almost a century ago. Recently, the studies of AE emitters functionalized compounds gained more popularity due to the advances in nanomedicine and drug-delivery systems.[124] They (similarly to the α-emitters) are studied as a potential targeted therapy of disseminated cancer cells, micrometastases, as well as of small tumors.[125]

Internal radiotherapy may be divided according to the character of the emitter (sealed *versus* unsealed).

The internal radiotherapy with sealed emitters is called **brachytherapy** (BRT) and is used especially for treatment of prostate,[126] breast[127] or cervical cancer.[128] The radionuclide is sealed in a container (usually small seeds, needles or pellets), which is surgically implanted to the place of tumor or placed in its close proximity. A common radiation sources (radioactive fillings of metal containers) for BRT are high-energy γ-emitters (^{196}Ir, ^{60}Co, ^{137}Cs) for ambulant treatment, low-energy γ-emitters (^{125}I, ^{103}Pd) and β^--emitters (^{90}Y, ^{90}Sr) for permanent interstitial treatment.[111] The advantages over the EBRT are particularly the reduced radiation burden of the healthy tissue resulting in suppression of some side effects, and the therapy requires fewer sessions of the patients at the oncologist (usually only for introducing and removing the irradiating container) than with EBRT.[127]

Another approach is the internal radiotherapy with **unsealed radionuclides** and their chemical compounds.[129] By choosing the appropriate combination of their physical-chemical properties and their administration route (orally or parenterally), one can achieve more or less specific targeting of the radionuclide to the tumor tissue. The first effective therapy with unsealed radioisotopes was treatment of thyroid cancer with $Na^{131}I$ (β^-, $t_{1/2}$ = 8.02 d, E = 971 keV), as the thyroid glands tend to accumulate iodine avidly.[130] For the therapy of polycythemia rubra vera, a myeloproliferative blood cancer of bone marrow, therapy with ^{32}P sodium phosphate (β^-, $t_{1/2}$ = 14.3 d, E = 1.71 MeV) was successfully employed.[131] Further, inorganic derivatives (mainly chlorides) of ^{89}Sr (β^-, $t_{1/2}$ = 50.5 d, E = 1.50 MeV) and ^{153}Sm (β^-, $t_{1/2}$ = 46.3 d, E = 808 keV) are used for the palliative therapy of skeletal metastases because they are metabolized in the similar way as calcium.[132] Similarly, a solution of $^{223}RaCl_2$ (Xofigo®, α, $t_{1/2}$ = 11.4 d, E = 5.98 MeV) was approved by U.S. Food and Drug Administration (FDA) for treatment of prostate cancer with bone metastases in year 2013.[133]

Therapy with simple inorganic compounds of unsealed radionuclides is effective; however the targeting effect is limited to the properties of these simple compounds. Therefore, the better therapeutical potential and broader application of radionuclides could be often empowered by their linking to the organic molecules. Then, biological properties

(including the targeting) of the radiopharmaceutical can be precisely set by the choice of the particular organic "carrier" molecule.[134] This can be a simple organic compound, antibody, polymer, etc. Depending on their chemical nature, radioisotopes can be attached to the carrier either by a covalent bond, as in the case of radioactive isotopes of halogens (for radioiodination see chapter 2.6.),[135] or with the use of coordination complexes (chelators) for metal nuclides. The most common chelators for radiolabeling are diethylenetriammine pentaacetic acid (DTPA) and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA).[136] DOTA complexes are generally more stable; however the rate of formation is slower than those of DTPA. This can be overcome by heating of the DOTA - metal labeling mixture provided the delivery system is sufficiently heat resistant.[137]

Therapy with radiopharmaceuticals, unlike EBRT or brachytherapy, does not rely on precise localization of the tumor before treatment; the radiopharmaceuticals are targeting solely the metabolic pathways of tumor proliferation and are therefore effective for the treatment of small tumors, micrometasases or even single cancer cells.[134] This way of therapy is sometimes called biologically targeted radionuclide therapy. One of the most common radiopharmaceuticals is [^{131}I]-*m*-iodobenzylguanidine (MIBG, Iobenguane®), molecule with similar structure with norepinephrine (noradrenaline).[138] Due to this, it is used for the effective treatment of cancer cells with enhanced uptake and metabolism of noradrenaline, e.g., phaeochromocytoma and neuroblastoma.[138] Another type of endoradiotherapy is boron neutron capture therapy (BNCT), in fact the sensitivity to the neutron flow.[139] When an isotopically enriched (^{10}B) boron-containing compound is accumulated in tumor and then irradiated with epithermal neutron flux, nuclear fission reaction starts to take place leading to the production of secondary radiation biologically much more effective than the neutron flux.[140] Despite the possibility of multistage targeting, the necessity of nuclear reactor (or a spallation source), requirement for high concentration of boron in target tissue and activation of natural sodium ^{23}Na omnipresent in tissues to radioactive ^{24}Na are severe limitations of BNCT in general.[141]

Therapy with simple radiolabeled organic compounds has its limitations. It is mainly their low-specificity and high immunogenicity, which results in a rapid blood clearance and short blood-circulation time.[134] Therefore, more complex systems for the delivery of radionuclides to the tumor were developed.

Radioimmunotherapy (RIT) uses specific monoclonal antibodies to deliver radioactivity to the target cells.[142] Nowadays, the only FDA approved RIT agents are β^- emitting ^{90}Y-ibritumomab (Zevalin®) and ^{131}I-tositumomab (Bexxar®) for the therapy of B-cell non-Hodgkin`s lymphoma (NHL). Zevalin is the monoclonal IgG1κ monoclonal antibody connected with DTPA chelator and labeled with ^{90}Y (β^-, $t_{1/2}$ = 64 h, E = 2.28 MeV).[143] Bexxar is the murine IgG1 λ monoclonal antibody with its tyrosine residues covalently iodinated with ^{131}I (β^-, $t_{1/2}$ = 8.02 d, E = 971 keV).[144] They both have high affinity for the CD20 antigen expressed by both normal and cancer B-cells allowing the radiation from the β^- emitter to kill them.[142] Further, the antibody itself can work as an immunotherapeutic agent and kill the B-cell by triggering its opsonization by macrophages.[145] The missing healthy B-cells are then rapidly replenished from lymphoid stem cells. The therapy with Bexxar and Zevalin is usually preceded by infusion of the unlabeled antibody to deplete the B-cell level.[146] In studies with Bexxar, the overall NHL patient response was 65%, whereas only 28% of the patients responded to the chemotherapy.[147] Aside from the Bexxar and Zevalin, other radioimmunoconjugates for RIT of NHL are intensively studied, for example the ^{90}Y-DOTA labeled humanized anti-CD22 antibody epratuzumab.[148] ERBB2 protein, also known as CD340 or HER2 is an attractive target for RIT. It is a proto-oncogene human epidermal growth factor receptor; over-expressed in about 30% of aggressive breast cancers.[149] Some over-expression occurs in ovarian and stomach cancer, as well. HER2 is the target for monoclonal antibodies, e.g, trastuzumab (Herceptin®) or pertuzumab (Perjeta®), which, in their unradiolabeled form, already achieved the FDA approval for the immunotherapy of metastatic breast cancer.[150] Therefore, their radioconjugates are intensively studied as a possible RIT of breast cancer. As an example, Abbas et al used ^{177}Lu labeled trastuzumab for the treatment of ovarian cancer xenografts in mice.[151]

The epidermal growth factor receptor (EGFR) is a similar receptor responsible for the DNA synthesis and cell proliferation. It is frequently overexpressed in different epithelial cancer cells, e.g., lung cancer, anal cancer or glioblastoma.[152] Several radiolabeled anti-EGFR antibodies have been studied for RIT. The chimeric human-murine antibody cetuximab (Erbitux®) was radiolabeled with plethora of radionuclides. The ^{90}Y-labeled cetuximab proved to be effective against human squamous cell carcinoma,[153] the ^{131}I-labeled one against brain metastases from non-small cell lung cancer[154] and the ^{64}Cu-

labeled one against colon tumors.[155] The most common adverse effect of EGFR inhibitors is the papulopustular rash, found in more than 71 - 90 % patients.[156] Despite their efficiency, RIT with antibodies also suffers from several drawbacks, e.g., difficult manufacturing of antibodies, sometimes immunogenicity and side effects (however to a lesser extent than with chemotherapy).[142]

Peptide receptor radiation therapy (PRRT) is another form of targeted cancer endoradiotherapy. It is based in the site-specific accumulation of radiolabeled peptides in malignant cells overexpressing certain regulatory peptide receptors.[157] PRRT is most often applied for the treatment of gastroenteropancreatic and neuroendocrine tumors.[158] The majority of the PRRT radiopharmaceuticals are targeted to the somastatin receptor overexpressed in various tumors.[159] Those clinically available include octapeptides octreotide and lanreotide labeled with DOTA complexes of ^{90}Y and ^{177}Lu for therapy of various neuroendocrine tumors.[160] Bombesin is a 14-amino acid peptide with high affinity to the gastrin-releasing peptide (GRP) receptor. It is widely used as a tumor marker for gastric cancer, small cell lung carcinoma and neuroblastoma. Radiolabeled with ^{177}Lu or ^{166}Ho, bombesin proved to be promising candidate for therapy of breast and prostate cancer.[161]

Nowadays, the vast majority of targeted radiopharmaceuticals employ high- or low-β^- emitters, which is useful for the treatment of the larger clusters of tumor cells to small tumors. However, they are not effective for the therapy of larger tumors, due to their lack of oxygen (hypoxia) as the main physical target of the β^- particles resulting in their poor radiosensitivity.[162] Furthermore, as the main benefit of β^- emitters consists in the crossfire effect (non-specific additive ionization damage of surrounding cells), they are not effective either in the therapy of single cancer cells, small cancer clusters or micrometastases, because the radiation dose from the distant separate tumor cell is low.[114] Also, in the case of separate tumor cells, the radiation damage is mostly absorbed by the surrounding healthy tissue. Therefore, high LET radionuclides, such are α and AE emitters are suitable for the targeted therapy of single cancer cells because of their low range and concentrated ionization density.[113,120,163] Radiotherapy with α-emitters is now widely studied, practically all the above mentioned target devices were attempted to be radiolabeled with α-emitters, mainly ^{213}Bi, ^{225}Ac and ^{211}At. Some of the α-radiolabeled antibodies and peptides are in different stages of preclinical and clinical research.[120] Because of their range (4-10 cell

diameters), α-radiopharmaceuticals do not need to by internalized inside the cell, the sufficient cell-lethal dose is achieved by decay in their proximity (e.g., at cell membrane). Because of their high LET, therapy with directly ionizing α-radiopharmaceuticals is not dependent on the oxygen level and is thus suitable for the therapy of hypoxic solid tumors.[105] The effectivity of α-radiopharmaceuticals is usually overall better than that of their β^- counterparts. However, because of their high toxicity, the α-radiopharmaceuticals have to be precisely targeted to the tumor to avoid damage of the healthy tissues. Furthermore, the low availability of α-radioisotopes and their difficult detection often limit their application.[164]

2.5 Auger electron (AE) radiotherapy

2.5.1. Introduction to AE emitters

Endoradioherapy with emitters of Auger electrons (used as a collective name for low-energy Auger, Coster-Kronig and super Coster-Kronig electrons), such as ^{125}I and ^{111}In, represent an appealing alternative to the therapy with β^- and α emitters.[165,166] Because of their extremely low range, they are sufficiently biologically effective only when internalized to the cell cytoplasm, or better, to the cell nucleus, where they cause unrepairable double-strand breaks of DNA (**Figure 8**). Their decay outside the cell or on its surface does not have any substantial biologic effect (unlike β^- and α emitters) and there is no non-specific radiation burden of the healthy tissues during the transport. Because of their low energy, the overall radiation dose from the therapy is far smaller than from other sources of radiation.[123] However, the maximal tumor targeting has to be achieved so the AE emitters-functionalized radiopharmaceutical is accumulated and internalized largely to the tumor cells. Therefore, systems for tumor delivery of AEs have been intensively studied in last decades.[167] Their biggest advantage consists in high effectivity against single disseminated cancer cell, where other conventional methods suitable for bigger tumors (surgery, EBRT, radiotherapy with β, γ emitters) fail. Further, their selective radiotoxicity can minimize the burden of bone marrow stem cells.[168]

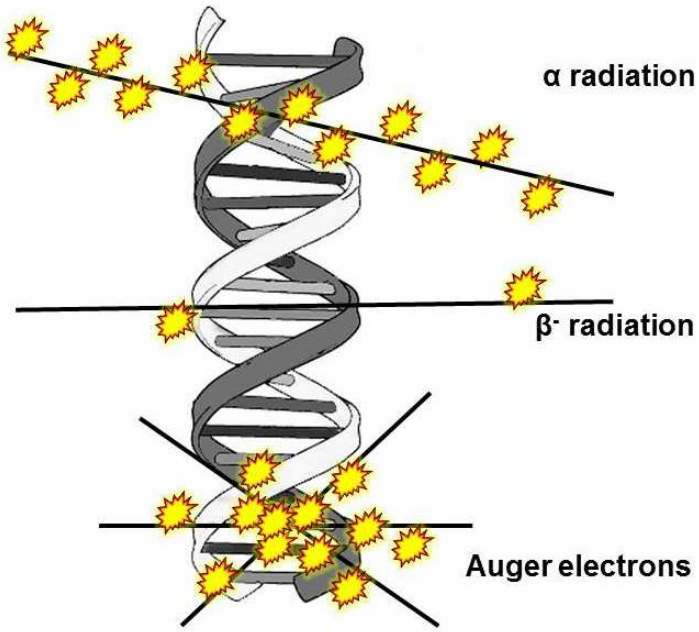


Figure 8.: Effect of ionizing radiation from α, β^- and AE sources.

The most widely studied isotopes for AE therapy are ^{125}I, ^{111}In.[169] Their nuclear properties have been calculated by Monte Carlo method and are summarized in **Table 2**.[170] ^{125}I has an overall higher yield of Auger electrons, and is substantially cheaper than ^{111}In. On the other hand, ^{111}In has more suitable half-life and its intensive γ radiation is an outstanding tool for the simultaneous tumor diagnostic by gamma-camera. However, this accompanying low-LET radiation causes some radiation burden of the healthy tissue and thus lower selectivity in targeted tumor cells. Radiopharmaceuticals bearing either of these isotopes possess some minor metabolic drawbacks. Those labeled with ^{125}I exhibit deiodination followed by the accumulation of radioactive iodine in thyroid gland.[171] DTPA complexes of indium are known to be trapped by kidneys.[172] In conclusion, one cannot favor any of these two radioisotopes; their choice depends on the specific application. Other isotopes, like ^{123}I of ^{m99}Tc were used, as well, but they have very low yield of AEs per decay and low half-lives, so their suitability for targeted therapy is dubious.[170]

Features	**^{125}I**	**^{111}In**
Half-life	60.5 d (++)	**2.1 d (+++)**
AEs per decay	**~ 20 (+++)**	~ 8 (++)
Energy of AEs per decay	12.2 keV (++)	2.0 keV (++)
% Decay energy in AEs	**20 % (+++)**	0.52 % (+)
Tumor imaging	(+)	**(+++)**
Accessibility (price)	**(+++)**	(+)
Labeling	Covalent bond	Chelation

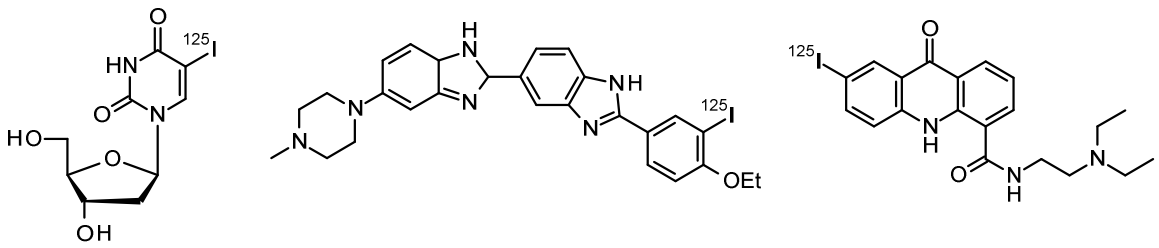
Table 2. Comparison of ^{125}I and ^{111}In, the most studied AE emitters.

2.5.2. Nuclear delivery of AEs

To be biologically effective, AEs have to be localized in the proximity of the DNA.[123] The first and up-to-date most widely studied radiopharmaceutical exploiting the effect of AEs was ^{125}I-iododeoxyuridine (^{125}I-UdR, **Figure 9**), a radioiodinated nucleoside analog that is incorporated directly to the DNA during its replication by resembling natural thymidine.[173] *In vitro* studies confirmed the massive radiotoxicity and double-strand breaks when ^{125}I-UdR was incorporated into DNA.[174] However, the first *in vivo* studies demonstrated three serious drawbacks of cell replication cycle-dependent radiohalogenated pharmaceuticals. The first is their instability due to the rapid degradation in liver.[175] The second serious drawback is the unwanted uptake to all fast-proliferating healthy tissues (mainly gut and bone marrow) resulting in systemic toxicity and severe side effects (resembling those of chemotherapy).[176] The third drawback is the slow rate of DNA incorporation, since ^{125}I-UdR is incorporated into the DNA only in the S-phase of cell growth.[177] Furthermore, ^{125}I-UdR competes with the natural nucleoside thymidine, which lowers the rate of its DNA-uptake.[178] The combination of slow ^{125}I-UdR DNA incorporation with its fast catabolism decreases its therapeutical effectiveness. However some studies tried to somehow overcome these drawbacks. For example, the loco-regional (intratumoral) infusions of ^{125}I-UdR to rats bearing intracerebral 9L gliosarcoma (3 - 7 mm) significantly prolonged their survival time with complete healing of 10 - 20 % animals.[179] The competing uptake of thymidine can be reduced by simultaneous administration of thymidine synthase (TS) inhibitors, favoring the DNA incorporation of ^{125}I-UdR.[178] Thus, Kassis et al. have shown that the administration of TS inhibitor methotrexate prior to loco-regional injection of ^{125}I-UdR significantly improves the survival time of rats with TE-671 human rhabdomyosarcoma.[180] In spite of this, the therapy with ^{125}I-UdR did not gain the status of an effective method of AE radiotherapy.

Later, various studies showed that the AE emitters do not have to be covalently bound to the DNA to cause its double-strand to break. In fact, the simple proximity of radionuclide (up to several nm) can deliver sufficient radiation dose causing cell apoptosis.[168] Therefore, molecules with high affinity to DNA, e.g., DNA intercalators[181] or groove binders,[182] prove to be efficient vehicles for the delivery of AEs. To assess the effect of DNA-AE emitter distance to biological activity, Kassis et al. synthesized a series of Hoechst 33342 DNA groove binder analogues (**Figure 9**) labeled with ^{125}I isotope *via*

linkers differing in length. These compounds were subsequently incubated *in vitro* with DNA and a yield of DNA double-strand breaks was determined. Not surprisingly, the number of DNA double-strand breaks decreased with increased distance of the ^{125}I. The critical distance from the axis of DNA was determined to be 1.2 nm. Beyond this the number of double-strand breaks rapidly drops. Furthermore, experiments with DMSO, a scavenger of hydroxyl radical, have shown, that below the 1.2 nm distance, the damage of DNA is caused solely by direct ionization, whereas beyond this distance only the low-effective indirect ionization *via* reactive oxygen species occur.[183] Aside from DNA groove binders, outstanding DNA-targeting effect is exhibited by DNA intercalators. Most of them, when labeled with ^{125}I, exhibited distinctive cellular toxicity associated with DNA double-strand breaks.[184] Gedda et al. synthesized ^{125}I radiolabeled anthracycline daunomycine which inhibited the growth of SK-BR-3 human breast adenocarcinoma cells.[185] Gardette et al. described ^{125}I iodinated acridine derivative ^{125}I-ICF01035 (**Figure 9**) which shown significant *in vitro* radiotoxicity to B16F0 melanoma cells.[186] In subsequent *in vivo* experiments, the same radiopharmaceutical was used to significantly prolong the survival time of mice inoculated with abovementioned tumor cells.[187] DNA intercalators can be a promising tool for nuclear delivery of AEs. However, they have to be targeted directly to the tumor tissue to prevent the systemic toxicity.



^{125}I-UdR **^{125}I-Hoechst 33342** **^{125}I-ICF01035**

Figure 9.: Examples of AE emitter - nuclear delivery systems.

Effective nuclear targeting can be also achieved by attaching a nuclear localization sequence (NLS) to the structure of radiopharmaceutical. NLS is a cation-rich peptide sequence which is recognized by the importin α/β carrier proteins. These ensure the transport of the cargo to the nucleus. Since the importins are intracellular proteins, the radiopharmaceutical has to be already internalized inside the cell to be transported towards the nucleus.[124,188]

2.5.3. Tumor delivery of AE emitters

Because of their high toxicity when internalized to both healthy and malignant cells, the precise tumor targeting of AE emitter-labeled radiopharmaceuticals plays a crucial role for their potential application in radiotherapy.[168] Essentially, all principles involving targeting and tumor delivery of non-radioactive cancerostatics are applicable. Most of the studies with actively targeted systems have been performed with ^{111}In, because its radioconjugates have been successfully used for the tumor imaging with single photon emission computer tomography technique (SPECT) for a long time. As an example ^{111}In labeled somatostatin targeted peptide octreotide (OctreoScan®) is used for imaging of somatostatine receptor positive tumors (mainly those of neuroendocrine system).[189] *In vitro*, ^{111}In-DTPA-octreotide showed high cytotoxicity against somatostatin positive cells.[190] Further, Forssell-Aronsson et al. observed extremely high tumor to blood ration of ^{111}In-DTPA-octreotide, up to 1500, in patients with endocrine pancreatic tumor.[191] In clinical trials, 40 patients with different somatostatin expressing tumors were treated with cumulative doses 20 - 160 GBq of ^{111}In-DTPA-octreotide. Therapeutic effect was seen in 21 patients, mainly the stabilization effect of previously progressive tumors. However, at doses higher than 100 GBq, 3 of the 6 patients developed myelodysplastic syndrome or leukemia. There was also poor effect on tumor regression, resulting presumably from the low nuclear uptake of ^{111}In-DTPA-octreotide.[192] Therefore, methods of nuclear delivery of radiolabeled somatostatin analogues were intensively studied.[193] Unfortunately, the derivatization of ^{111}In-DOTA-octreotide with nuclear localization sequence lead to liver toxicity of the system.[124] Another somatostatin analogue TATE ((Tyr^3)-octreotate), decorated with ^{125}I labeled groove binder Hoechst showed promising targeting to the human tumor xenografts in mice, whereas the same system without Hoechst (^{125}I-TATE) was localized predominantly in kidneys.[194]

Several studies focusing on ^{111}In labeled antibodies have been done, predominantly targeting the human epidermal growth factor receptor 2 (HER2) and the epidermal growth factor receptor (EGFR), frequently overexpressed in different tumors. For example, anti-EGFR ^{111}DTPA-nimotuzumab substantially reduced the clonogenic survival of MDA-MB-468 breast cancer cells. Furthermore, the attachment of nuclear localization sequence (NLS) lead to the sevenfold increase in cytotoxicity, but also to the diminished tumor uptake *in vivo*.[195] Similarly, treatment of HER2-positive cancer xenografts and mice with

anti-HER2 antibody trastuzumab decorated with ^{111}In and the NLS lead to the substantial increase in the survival time of mice than of those treated with unlabeled antibody.[196] As shown in many studies, incorporation of NLS frequently leads to the increased nuclear uptake, but can also alter the *in vivo* biodistribution, mainly the increased liver and kidney uptake, respectively their rapid scavenging by immune system resulting in their rapid blood clearance.[197] Recently, other systems based solely on the tumor-specific receptor targeting were described; however, most of them proved to be less efficient mostly because of the low nuclear or tumor uptake causing unwished toxicity against healthy cells.[198,199] Furthermore, their application is always limited to the tumors containing over-expressing specific receptors.

2.5.4. Polymer radionuclide delivery systems

Nanoparticles and polymers are passively accumulated in the tumor tissue due to the EPR effect (see chapter 2.2.) originating from the leaky tumor vasculature and missing lymphatic system. Based on the characteristics of the nanoparticle and the tumor, one can reach the accumulation in the tumor up to 100 times higher than in the surrounding tissue.[34-36] This effect may be further amplified by attaching an active targeting group, providing the suitable receptor is overexpressed. Polymer nanoparticles (including micelles and liposomes) play therefore a key role in the architecture of many passive-accumulated drug delivery systems. The majority of radiolabeled polymer nanoparticles were intended as a tool for the following of nanoparticle fate *in vivo*, i.e. the particular radionuclide was a tool to characterize biological trace properties of the material usually intended for another use.[200] Thus, labeling of nanoparticles with low-energy γ-emitters (e.g., ^{99m}Tc, ^{111}In, ^{67}Ga) or β^+-emitters (e.g., ^{18}F, ^{64}Cu), respectively, enables their *in vivo* imaging by SPECT, respectively PET technique.[201,202] The organ biodistribution of nanoparticles can be precisely determined after autopsy, when the system is labeled with a longer half-life isotope (e.g., ^{125}I).[203] Although not so common, polymer nanoparticle systems were also used to passively target the radiopharmaceutical into the solid tumor. A diblock micellar system composed of hydrophobic poly(lactic acid) and hydrophilic poly(sarcosine) block was decorated with β^- emitter ^{131}I and administered in the 4T1 mammary carcinoma mice. This resulted in the tumor growth suppression, which was further improved by simultaneous injection of ethanol percutaneously to the tumor region.[204] Sofou et al.,

proposed liposomes prepared from PEOylated lipids, 1,2-dinonadecanoyl-glycero-3-phosphocholine and cholesterol, for the tumor delivery of DOTA-attached α-emitter ^{225}Ac.[205,206] When attached to mouse antihuman PSMA J591 antibody, liposomes showed enhanced cellular uptake and cytotoxicity towards prostate-specific membrane antigen expressing human cell lines LNCaP and HUVEC.[207]

Due to their short range and high efficiency when internalized to the nucleus, Auger electron (AE) emitters represent an appealing tool for targeted radiotherapy of cancer. Aside from the aforementioned systems based solely on the active targeting, more universal passive targeting approach was utilized, as well.[124] Indeed this can be possibly improved by attaching of suitable active targeting group. Allen et al., proposed ^{111}In-containing block copolymer micelles consisting of PEO and poly(ε-caprolactone) (PCL) with or without the human epidermal growth factor targeting for therapy of EGFR overexpressing tumors. The micelles (with hydrodynamic radius of 15 nm) showed the increased receptor-mediated uptake and cytotoxicity by EGFR-overexpressing MDA-MB-468 breast cancer cells. However, only 1.9 % of the radioactivity was localized in the nucleus.[208] The MDA-MB-361 cell nuclear uptake of similar micellar system bearing specific antibody trastazumab was improved approximately 5-fold by attaching the nuclear localizing sequence peptide. Furthermore, 4.8 wt. % of cancerostatic drug and radiosensitizing agent methotrexate was incorporated into the micellar hydrophobic core to further increase the cytotoxicity.[208]

An effective way of nuclear targeting of AEs is their attachment to the DNA intercalator. Gedda et al., synthesized the ^{125}I-labeled daunomycin derivative, which was subsequently entrapped to the PEOylated liposome (called “nuclisome”) bearing epidermal growth factor (EGF) protein. This ensured the uptake to EGF-receptor containing U-343MGaCl2:6 cells, whereas the uptake to white blood cells was negligible.[209] Autoradiography showed the co-localization of the radiopharmaceutical with the cell nucleus thanks to the daunomycin intercalator. The system was also five-times more cytotoxic than the same liposomes loaded with doxorubicin. The same radiopharmaceutical was entrapped in nuclisome containing human epidermal growth factor 2 (HER2) ligand F5. This system was used for the therapy of mice bearing human ovarian adenocarcinoma SKOV-3. The F5-targeted nuclisome possessed better therapeutical activity than the non-targeted one, with the best effectivity at the dose 2 MBq per mouse, where 70 % of mice

survived. However, it is worth mentioning, that the radiotherapeutical system was applied directly after the tumor cell injection.[210]

In this thesis, we describe the first polymer system for controlled delivery and release of DNA-intercalator bearing AE emitter ^{125}I containing stimuli-responsive covalent linker between the polymer carrier and the intercalator.

2.6. Methods of radioiodination

From about 30 known isotopes of iodine, only one stable isotope, iodine-127, is found in nature. Six of the radioisotopes have been used in biomedical applications (**Table 3**).[135]

Nuclide	$t_{1/2}$	Mode of decay	E_γ (MeV)	E_β (MeV)	Application
^{120g}I	1.35 h	β^+, EC	0.601	4.0	PET
^{122}I	3.6 min	β^+, EC	0.564-3.45	1.18-3.1	PET
^{123}I	13.2 h	EC	0.159	--	SPECT
^{124}I	4.2 d	β^+, EC	0.603-2.74	0.79-2.13	PET
^{125}I	59.4 d	EC	0.035	Auger electrons	RIA, Auger electron therapy
^{127}I	stable	--	--	--	stable isotope
^{131}I	8.1 d	β^-	0.80; 0.60	0.364; 0.637	Therapy

Table 3.: Nuclear properties and application areas of some iodine nuclides.

In practice, **iodine-125** (^{125}I, $t_{1/2}$ = 59.4 d, E_γ = 35 keV, production ^{124}Xe (n,γ)→ ^{125m}Xe(57s)→^{125}I, respectively ^{124}Xe (n,γ)→ ^{125g}Xe(19.9h)→^{125}I) is used in both *in vitro* and *in vivo* biological assays and in several therapeutic procedures, e.g., brachytherapy. **Iodine-131** (^{131}I, $t_{1/2}$ = 8.1 d, $E_{\beta(max)}$ = 606 keV) is a common radioactivity fission product produced in large amounts inside the nuclear reactors. Because of its volatility and short half-life, it represents one of the main sources of radioactive contamination within the first week after a nuclear power plant accident. However, its β^- radiation is often used in the internal radiotherapy of tumor tissues, mostly in case of the thyroid cancer. In the nuclear medicine imaging, **iodine-123** (^{123}I, $t_{1/2}$ = 13.2 h, E_γ = 159 keV) is a suitable isotope for

diagnosis by scintigraphy and SPECT, whereas three positron-emitting isotopes (^{124}I, ^{122}I, ^{120}I) are promising candidates for the diagnosis using PET.[105]

In general, classical organic iodination procedures can be adapted to the radiochemistry, as well (**Figure 10**). As the aliphatic carbon - iodine bond is weak and the corresponding compounds are prone to the *in viv*o deiodination, only compounds with iodine firmly attached to aromatic or vinylic carbon are usually synthesized. Therefore, the radioiodination *via* nucleophilic or electrophilic aromatic substitution is mostly implemented.[211] The **nucleophilic aromatic substitution** reactions are facilitated by the activation of the aromatic ring with electron-withdrawing substituents, e.g., carbonyl- or cyano-group.[212] The simplest nucleophilic radioiodination is the isotopic exchange, where the non-radioactive iodine atom is replaced by radioiodine. However, relatively drastic reaction conditions (temperature, pressure) are required to obtain at least moderate radiolabeling yields.[213] Because the majority of iodine atoms in the radiolabeling product are ^{127}I, the isotopic exchange is not suitable for the synthesis of compounds with high specific radioactivity (the radioactive product cannot be separated from the starting compound, as both differ only in the isotope). Apart from the iodine, good leaving groups for the nucleophilic radioiodination are other halides (especially Br^-) and sulphonates, e.g., tosylates, triflates, mesylates or nosylates (however not so common in aromatic substrates). In these cases, the radioiodinated compounds can be separated from the starting compounds by chromatographic techniques (e.g., HPLC).[135] When the non-activated aromatic compounds are used, the nucleophilic iodination must catalyzed by transition metal compounds (e.g., Cu^+, Pd^{2+}).[214] Another approach is the nucleophilic substitution of diazonium salts with radioiodide. For the in-situ synthesis of diazonium salt followed by radioiodination, classical Sandmeyer-type reaction is not applicable. For this case, Wallach variant is used, where the diazotized amine is trapped by secondary amine forming triazene. This can be substituted by radioiodine in high yield.[215]

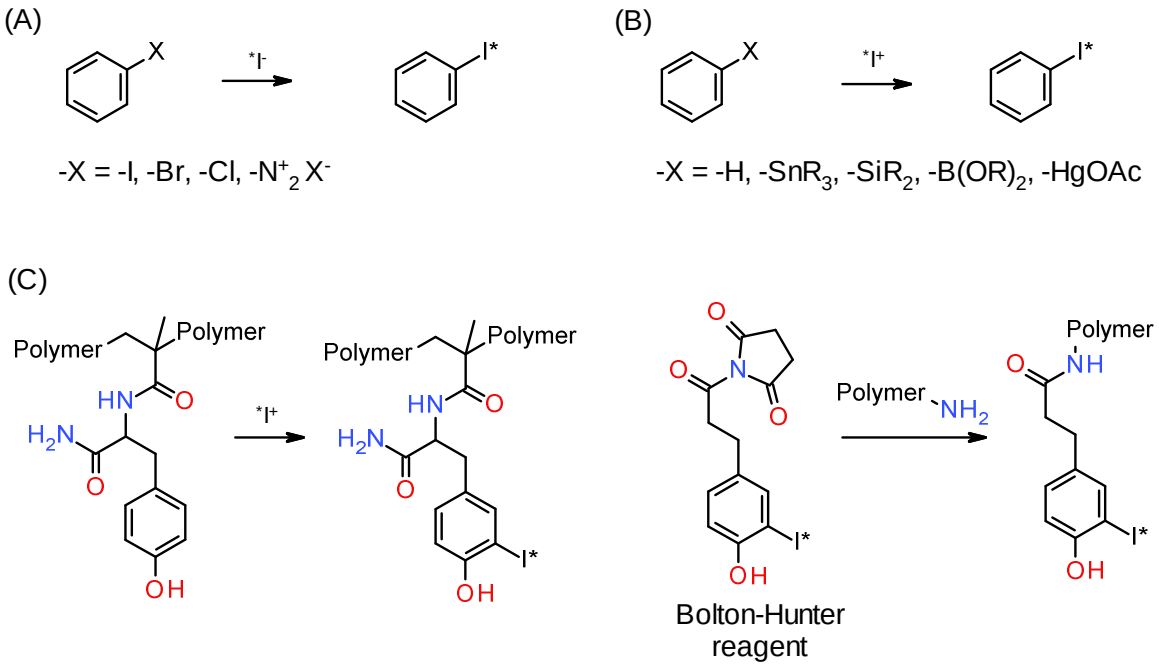


Figure 10.: Methods of radioiodination: (A) Nucleophilic iodination; (B) Electrophilic iodination; (C) Radioiodination of polymers.

The electrophilic iodination is process, where the positively charged iodine atom (I^+) attacks the aromatic ring or double bond. Contrary to the nucleophilic substitution, the high electron density of the aromatic ring enhances the rate of the electrophilic substitution. This can be achieved by activation of the ring by electron-donating groups, e.g., hydroxy-group of phenolic moieties. This activation is necessary, when the leaving group is hydrogen cation (i.e. halogenation of aromatic core). Second option is the substitution of organometallic (e.g., trialkylstannyl), respectively organometalliod (e.g., trialkylsillyl or boronic acid derivatives) compounds, where the non-activated aromatic compounds are radioiodinated in high yield, as well. The electrophilic radioiodine species can be generated from the sodium radioiodide by numerous oxidizing agents. The most frequently used oxidizers are peracetic acid, chloramine-T, Iodogen® and *N*-halosuccinimides.[135]

Radiolabeled polymers are frequently prepared to either follow the *in vivo* polymer properties or to deliver the therapeutic doses of radioactivity to the targeted tissue by the polymer carrier.[216,217] Methods of polymer radioiodination were mostly adapted from the

strategies developed for radiolabeling of proteins. These must be rapid, mild and offer high radiochemical yields. The fundamental labeling method is the direct electrophilic iodination of tyrosine residues (**Figure 10C**). For the labeling of polymers, small amount of tyrosine-containing monomer can be added to the polymerization mixture.[218] For example, *N*-methacroyl-L-tyrosinamide (MATA) monomer is often used to synthesize methacrylamide-type polymers eligible for radioiodination.[219] Alternatively, the polymer can be decorated with tyrosine moieties after the polymerization. This direct method is not applicable, when functional groups interfering with the radiolabeling mixture, mostly groups destroyed by oxidizing agent, are present in the polymer structure. When the tyrosine-iodination protocol is destructive to the macromolecule, the two-step indirect radioconjugation procedure can be used. This involves the reaction of the polymer with already iodinated small organic compounds. These agents should contain functional groups that can be used for efficient conjugation to the amino- (e.g., active esters, imidate esters, aldehydes, isothiocyanates) or thio- (e.g., maleimides, activated halides) group of the polymer.[218] The most commonly used radioiodinated conjugation agent is Bolton-Hunter reagent, i.e. radioiodo-labeled *N*-succinimidyl-3-(4-hydroxyphenyl)propionate.[220]

3. LIST OF PUBLICATIONS INCLUDED IN THE THESIS

Publication D1

Sedláček O., Hrubý M., Studenovský M., Kučka J., Větvicka D., Kovář L., Ulbrich K.: Ellipticine-aimed polymer-conjugated Auger electron emitter: multistage organelle targeting approach.

*Bioconjug. Chem.***2011**, 22(6), 1194-1201

Publication D2

Sedláček, O., Studenovský, M., Větvička, D., Ulbrich, K., Hrubý, M.:

Fine tuning of the pH-dependent drug release rate from polyHPMA-ellipticinium conjugates.

Bioorg. Med. Chem. **2013**, 21 (18), 5669-5672

Publication D3

Sedláček, O., Hrubý, M., Studenovský, M., Větvička, D., Svoboda, J., Kaňková, D., Kovář, J., Ulbrich, K.:

Polymer conjugates of acridine-type anticancer drugs with pH-controlled activation.

Bioorg. Med. Chem. **2012**, 20 (13), 4056-4063

Publication D4

Sedláček, O., Kučka, J., Hrubý, M.:

Optimized protocol for the radioiodination of hydrazone-type polymer drug delivery systems.

Appl. Radiat. Isot. **2015**, 95, 129-134

Publication D5

Sedláček, O., Kučka, J., Mattová, J., Pařízek, M.,Studenovský, M., Zadinová, M., Poučková, P., Hrubý, M.:

Multistage-targeted pH-responsive polymer conjugate of Auger electron emitter: optimized design and *in vivo* activity.

Eur. J. Pharm. Sci. **2014**, 63, 216-225

4. SUMMARY OF RESULTS

This chapter summarizes the approaches and results included in publications D1-D5. According to the aims of the thesis, these results are divided into four main research topics. Auger electron (AE) emitters (e.g., ^{125}I, ^{111}In) are extremely cytotoxic when coupled with DNA-intercalators. The nuclear decay in the proximity of DNA causes its double-strand breaks and thus the cell apoptosis without any undesirable mutations. Because of the side effects resulting from their inconvenient biodistribution, as well as their short blood half-life (both features often observed at low-molecular weight cytostatics), the targeting of AE emitter-intercalators with polymer drug delivery systems would be desirable.

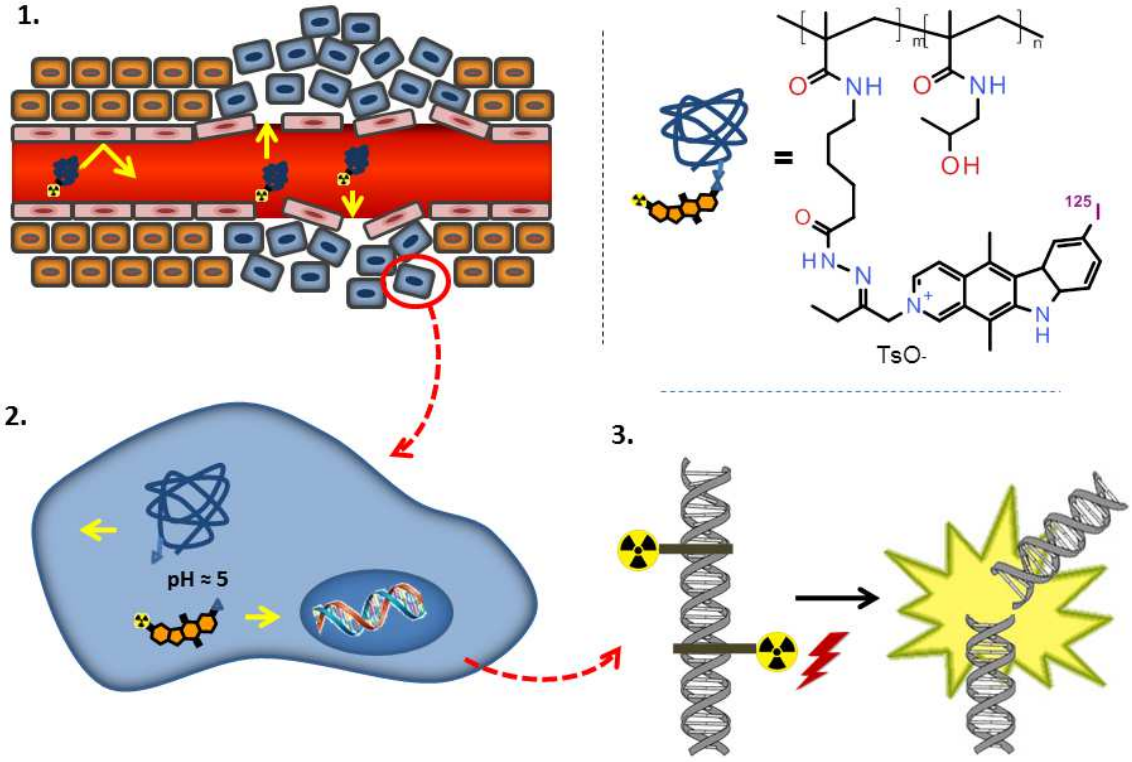


Figure D1.: Mechanism of triple-targeting approach

4.1. Synthesis and evaluation of the first polymer conjugate of ^{125}I bearing intercalator ellipticine (publication D1)

In Article D1, the first generation polymer system for the tumor delivery of AE emitter bearing intercalator was developed and studied. This system comprised of AE emitter ^{125}I covalently bound to intercalator ellipticine; this radiopharmaceutical was linked by acid degradable hydrazone bond to the biocompatible *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer carrier. This system involves triple-targeting approach (**Figure D1**) minimizing the radiation burden of healthy tissues:

- Passive accumulation of the polymer-based system in tumor tissue due to the EPR effect
- pH-triggered release of intercalator-bound Auger electron emitter in slightly acidic interstitial space in solid tumor tissue or in endosome after internalization.
- Targeting the AE emitter ^{125}I into DNA by intercalation of the ellipticine derivative on which it is bound.

First of all, an ellipticine analogue with stable natural iodine (which is monoisotopic ^{127}I) was synthesized to determine the main physico-chemical characteristics of the iodinated ellipticine derivative, such as the *in vitro* drug intercalation, internalization, and the release rate of the drug from the conjugate, all of which are largely independent of the isotope of the iodine used. 9-Iodoellipticine was prepared through the acetomercuration of ellipticine followed by the iodination of the mercuro-intermediate (**Figure D2**). Because the 9-iodoellipticine moiety does not contain an oxo-group, which is necessary for the formation of hydrazone bond, it was quaternized with 1-bromobutan-2-one to form the *N*-(2-oxobutyl)-9-iodoellipticinium bromide (**1**). This compound was used as the non-radioactive analogue of the radioactive drug described below. The high DNA binding ability of the iodinated intercalator is essential for efficacy of the whole system. This affinity was determined from its fluorescence emission intensity increase upon the titration with DNA solution. Furthermore, the compound readily penetrates into the cell nuclei, as proven by confocal microscopy on 4T1 breast cancer cells.

Hydrazone bond was chosen to connect ellipticine derivatives to polymer because of its susceptibility to hydrolysis under mildly acidic conditions as well as relative stability toward hydrolysis at neutral pH. *The conjugate would thus be relatively stable during the*

transport in blood plasma at pH 7.4. However, in more acidic tumor tissue (pH ca. 6.5) or especially after internalization into the tumor cell (pH in late endosomes drops to ca. 5.0) the iodinated intercalator would be rapidly released from the polymer carrier. The ketone-containing 9-iodoellipticinium derivative (**1**) was conjugated with hydrazide-containing HPMA copolymer poly[*N*-(2-hydroxypropyl)methacrylamide-*co*-1-*N*-(6-hydrazino-6-oxohexyl)-2-methacrylamide] (pHPMA-MAAcap hydrazide) by acetic acid-catalyzed condensation in analogy to conjugation of anticancer anthracycline antibiotic doxorubicin, forming conjugate (**2**). The copolymer precursor (M_w = 26.8 kDa, M_w/M_n = 1.87) was synthesized by free radical copolymerization of HPMA with 1-*N*-(6-hydrazino-6-oxohexyl)-2-methacrylamide. The drug loading was 8.5 wt. %. The system was stable in the buffer of pH 7.4 (0 % intercalator released after 24 h of incubation) while iodine-containing biologically active intercalator **1** is released upon decrease of pH (25 % intercalator released after 24 h incubation at pH 5.0 - model of pH in late endosomes).

As a next step, the ^{125}I-containing radioactive derivative was synthesized in analogy to the stable ^{127}I-containing derivatives. First, the no-carrier-added approach with direct radioiodination of ellipticine using the chloramine method was utilized. However, high amounts of ellipticine-*N*-oxide and radioiodinated ellipticine-*N*-oxide were produced as byproducts. Therefore the same approach as for the synthesis of non-radioactive **1** (i.e. acetomercuration with subsequent reaction with iodine) was implemented. Because the chromatographic purification is necessary, carrier-added iodine (radioactive iodine diluted with excess of stable iodine isotope) must be used and the specific activity of the product is relatively low (8.2 MBq/mg). The subsequent quaternization to yield **[^{125}I]-1** and conjugation with copolymer was done in analogy to the non-radiolabeled conjugate. Because of the low specific radioactivity of the products, only statistically insignificant differences between cytotoxicities of radiolabeled and non-radiolabeled analogs were obtained.

As a result, several features of the first generation system have to be improved:

- The structure of the hydrazone linker should be tuned-up so the drug release will be faster at acidic pH 5.0, while staying stable at neutral pH of blood plasma (see chapter 4.2.).
- The HPMA copolymer carrier of lower polydispersity should be used, as this will provide more reliable results of system biodistribution (see chapter 4.4.).

- The radioiodination procedure has to be optimized to achieve the high specific radioactivity of the bioactive products (see chapters 4.3. and 4.4.).

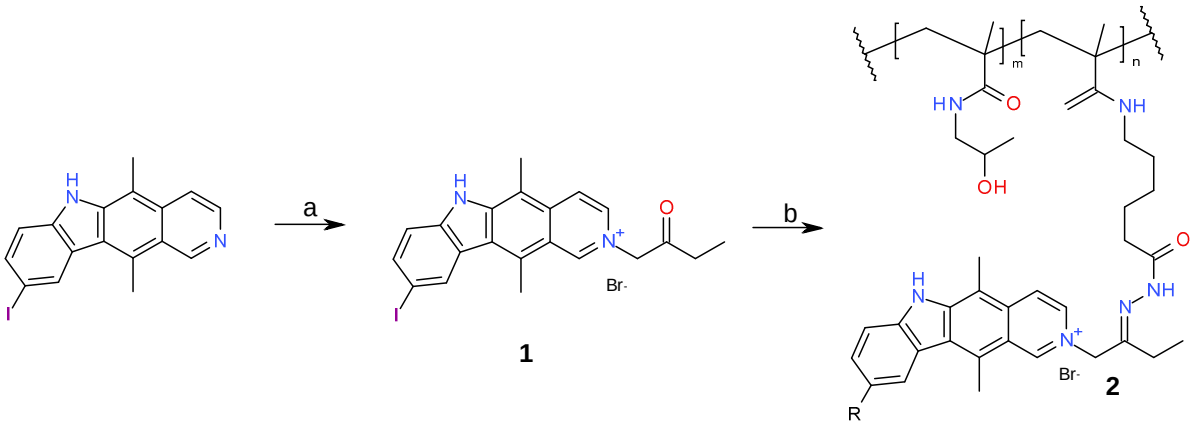


Figure D2.: Synthesis of polymer-bound 9-iodoellipticine derivative **2**. Reagents and conditions: (a) 1-bromo-2-butanone, MeOH, RT; (d) pHPMA-MAAcap hydrazide, 9:1 MeOH/AcOH, RT.

4.2. Optimization of the polymer-intercalator hydrazone linker structure (publications D2 and D3)

Because the release of the drug from the first generation conjugate was rather slow at acidic pH, the hydrazone linker structure was optimized. The aim was to achieve the fast release at pH 5.0, representing the pH in late endosomes, but the release at pH of blood plasma should be as slow as possible to prevent the drug cleavage during blood circulation before reaching the targeted tissue. As a cheap and relatively nontoxic alternative to ellipticine, yet exhibiting the same structural fragment, isoquinoline was used. Isoquinoline was quaternized with plethora bromo- or tosyloxy ketones to produce oxo-alkyl isoquinolinium salts **3a-f**. These were conjugated with hydrazide groups-containing HPMA copolymer (M_w = 24.5 kDa, M_w/M_n = 1.87) by acetic acid-catalyzed condensation, forming derivatives **4a-f** (**Figure D3**).

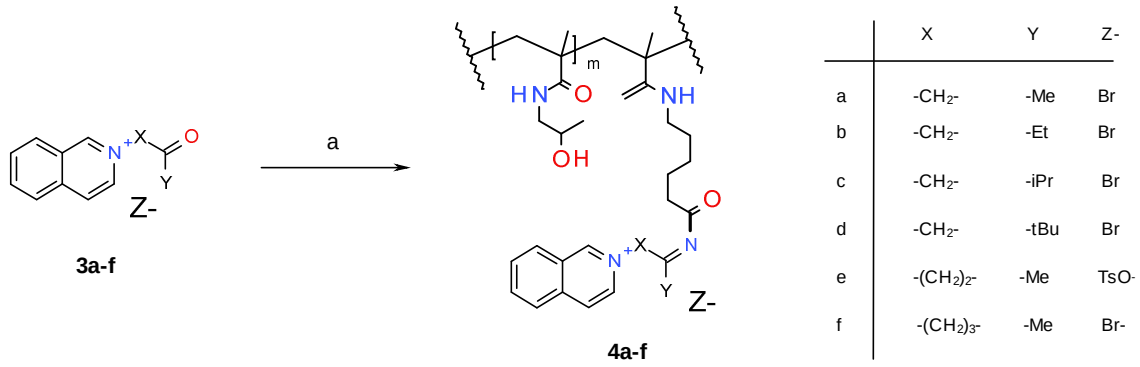


Figure D3.: Syntheses and structures of the polymer conjugates.

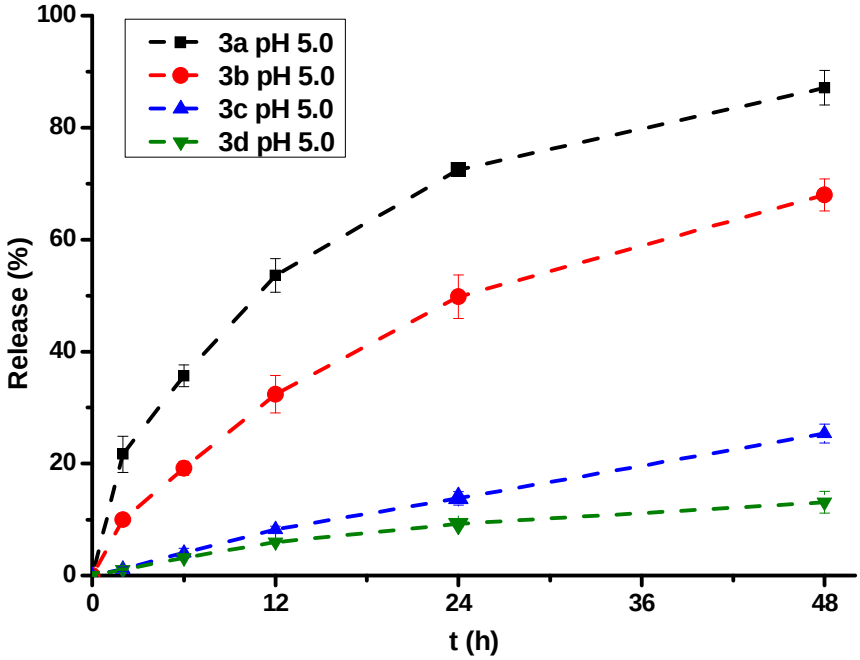


Figure D4A.: The release profile of derivatives **3a-d** from their conjugates **4a-d** in phosphate buffered media at 37°C. The release in the pH 7.4 phosphate buffer was under 2% after 24 h in all cases.

To assess the influence of steric hindrance on the acidic cleavage of hydrazone, the isoquinolinium conjugates containing methyl (**4a**), ethyl (**4b**), isopropyl (**4c**) and *t*-butyl (**4d**) groups adjacent to the ketone were synthesized, and their hydrolytic release profiles were determined. In the pH of blood plasma (pH 7.4), nearly no low molecular weight isoquinolinium model compounds were released. Sterical hindrance had a dramatic effect on the release rate at pH 5.0; the polymer conjugate of the methyl derivative **3a** had the fastest release rate, and the polymer conjugate of *t*-butyl derivative **3d** had the slowest

release rate. This slow release rate could be ascribed to the steric hindrance of the transition state, which is most likely to have hybridization close to the sp^2 state.

To determine the influence of adjacent permanent positive charge on the rate of hydrolysis, we compared the release of the aforementioned derivative **3a**, which contained a positive charge in the β-position respective to the oxo-group, with the derivatives with positive charges in the γ- (**3e**) and δ- (**3f**) positions, respectively, from their conjugates. It can be clearly observed in **Figures D4A** and **D4B** that the presence of a positive charge proximal to the original ketone substantially reduces its release rate. This decrease of release rate made the derivative with the closest charge (β-oxo-derivative **3a**) the most stable derivative, whereas the conjugate with most remote charge (δ-oxo-derivative **3f**) was the most labile conjugate, even at a pH of 7.4 (77 % of the drug released within 24 h). This behavior could be explained by the electrostatic disinclination of hydrazones with proximate positive charges towards their protonation as the first step of the hydrolysis mechanism.

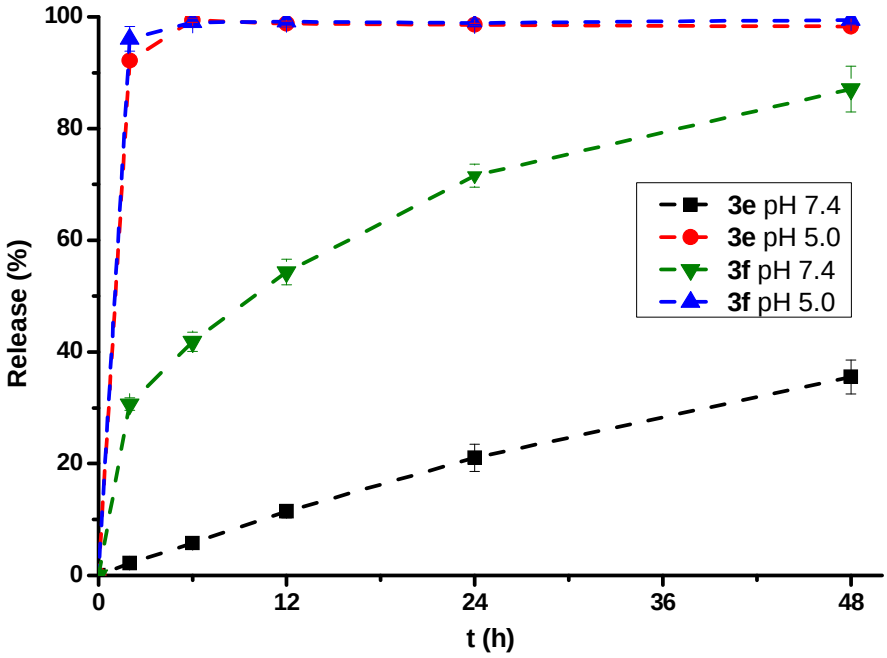


Figure D4B.: The release profile of derivatives **3e-f** from their conjugates **4e-f** in phosphate buffer media at 37°C.

Of all the linkers described above, the simplest 2-oxopropyl linker showed the best release profile for cancer applications (negligible at pH 7.4 and sufficiently fast in a

slightly acidic milieu) and was thus chosen to connect ellipticine derivative to the hydrazide-containing HPMA copolymer (see chapter 4.4)

In addition to the ellipticine-type intercalator polymer conjugates, polymer systems containing acridine intercalator were studied, as well (publication D3). The 3-(9-acridinylamino)-5-hydroxymethylaniline (AHMA) is potent DNA intercalator with strong chemotherapeutic potential. Because it contains free amino group, which can be acylated without losing biological properties, the series of AHMA derivatives (**5a-e**) functionalized with different oxo-acids were synthesized (**Figure D5**). These were conjugated to the hydrazide groups-containing HPMA copolymer (M_w = 17.5 kDa, M_w/M_n = 1.87). However, part of the acridine drug was also bound in a non-cleavable manner as the product of a side reaction. In this case, the hydrazide group of the polymer substituted the aniline group at position 9 of the acridine ring (*ii*, **Figure D5**). Therefore, for the drug release experiments, we only took into account the hydrolytically cleavable part of the bound drug. This strategy is relevant from a biological point of view because the polymer bearing the drug bound *via* a non-cleavable linkage is most likely to be biologically inert, as described for doxorubicin.[221]

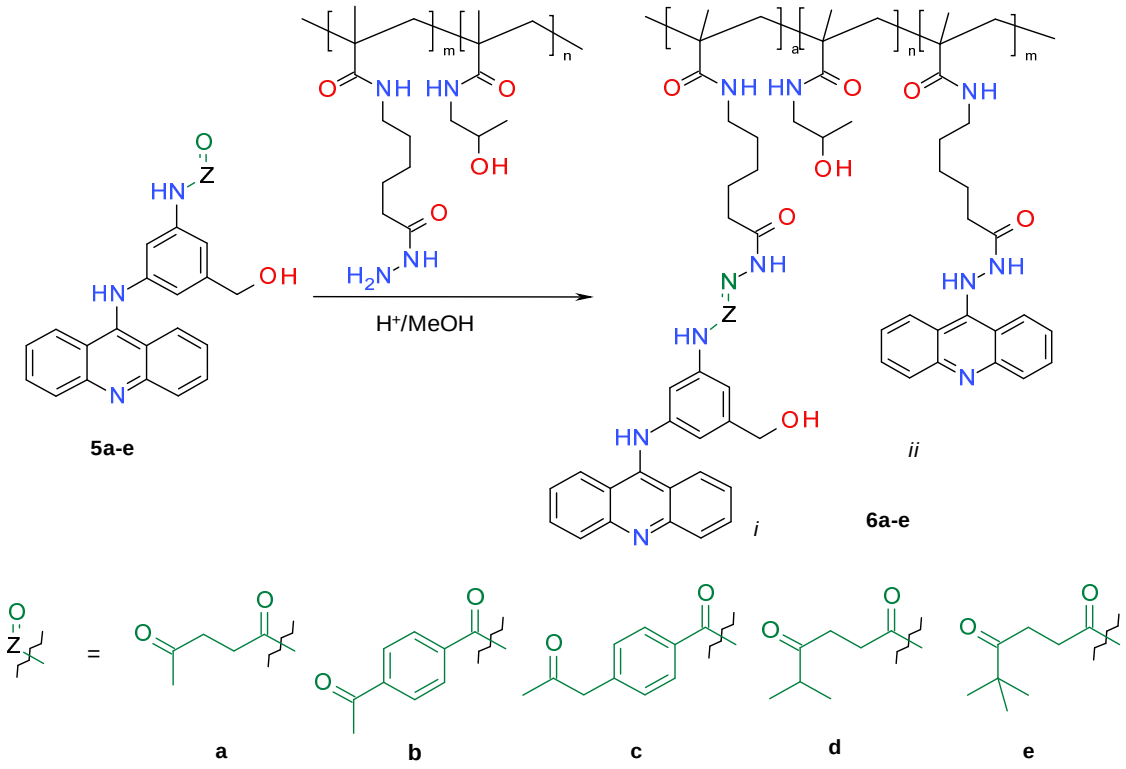


Figure D5.: Scheme of synthesis of **6a-e**.

In the drug release experiments, a relatively fast rate was observed at pH 5.0, and a slower to negligible rate was observed at pH 7.4 (**Figure D6**). The conjugate **6b** contains a hydrazone bond conjugated with the phenyl aromatic ring and released the drug slowly even at pH 5.0 (17 % after 24 h), and it did not release any drug at pH 7.4. The most plausible explanation is that the conjugation stabilizes the hydrazone bond by delocalization of its electrons into the aromatic ring, which dramatically slows down the rate of hydrolysis.

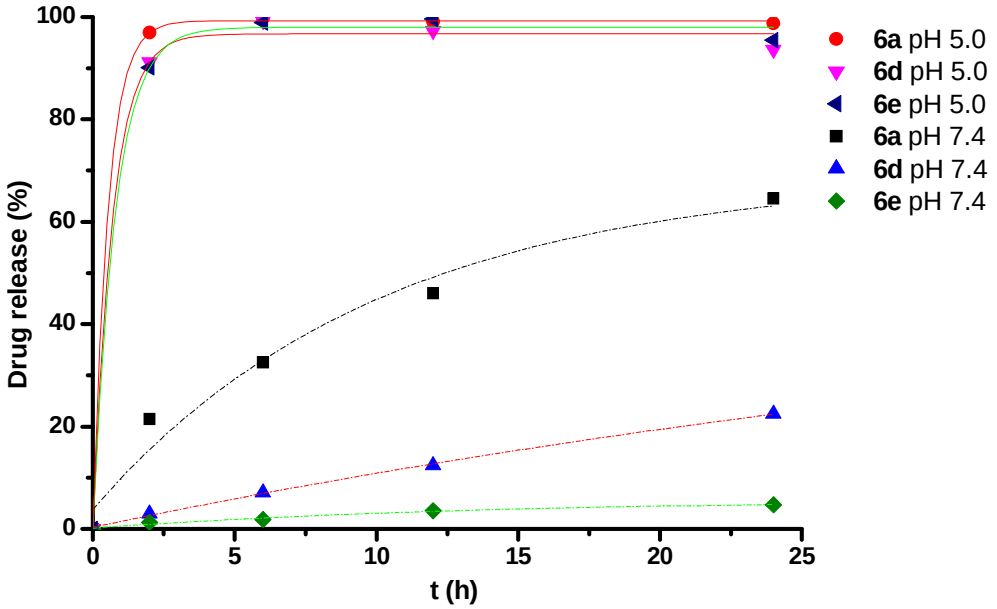


Figure D6.: The release of 9-anilinoacridine derivatives **5a** and **5d-e** from conjugates **6a** and, **6d-e** at different pH (calculated as hydrolytically cleavable amount of drug = 100 %).

The effect of steric hindrance next to the hydrazone group on its release rate was studied using conjugates differing only by their substituents adjacent to the original oxo-group, which were methyl (**6a**), *i*-propyl (**6d**) and *t*-butyl (**6e**), respectively. Steric hindrance of the hydrazone increases in the order **6a** < **6d** < **6e**. One can clearly see from Figure D6 that steric hindrance does not influence the drug release rate at pH 5.0 (it is nearly quantitative within the initial 2 h in all cases, caused by the absence of adjacent positive charge) but has a dramatic effect at pH 7.4. A greater steric hindrance induces a slower release rate at pH 7.4. The *t*-butyl group-containing conjugate **6e** has optimal release rate in this study. Unfortunately, the steric hindrance leads to the slow rate of the hydrazone formation and thus the major part of the acridine is bound *via* the hydrazone bond. This problem was overcome by synthesizing the hydrazone-containing

methacroylated monomer first, followed by the radical copolymerization. Because of this, the system did not prove to be worth following for the delivery of radiolabeled drugs (there will be too many steps involving radioactive compounds). However, this linker optimization study will be useful in the design of pH-responsive delivery systems containing other amine-bearing drugs.

4.3. Optimization of the radioiodination of hydrazone-containing conjugates (publication D4)

Because the radiolabeling is usually performed as a final step in the reaction sequence, the scopes of the direct radioiodination of hydrazone-containing polymer conjugates were studied. In our studies (depicted in article D4), the most widely studied hydrazone conjugate, i.e. the HPMA copolymer hydrazone conjugate of cytostatic doxorubicin, was utilized. The radioiodination of methacrylamide polymers is usually enabled by incorporating of *N*-methacroyl-L-tyrosinamide (MATA) comonomer unit to the polymer backbone. This unit can be smoothly electrophilically iodinated in high yield. Unfortunately, the free hydrazides are readily oxidized by chloramine T, iodine monochloride or elemental iodine which in turn could compromise the radiochemical yields. Also, the DOX itself can be iodinated.[222]

Because the radioiodination of tyrosine derivatives proceeds very rapidly, the first logical approach was to radioiodinate the tyrosine-containing polymer with already bound DOX to determine whether the radioiodination of the polymer occurred significantly faster than the aforementioned potential side-reactions (hydrazide oxidation and DOX iodination). For this purpose, a copolymer conjugate of HPMA with hydrazone-bound DOX, containing 1 mol % of MATA, was synthesized and subsequently radiolabeled with ^{125}ICl formed *in situ* by the oxidation of Na^{125}I with either chloramine T or Iodogen®, respectively. However, the radiolabeling yields were very low; only 5 %, respectively 3 % of the radioactivity was bound to the polymer (**Figure D7 (B)**). This could be explained by rapid decomposition of reactive intermediate ^{125}ICl by free hydrazides.

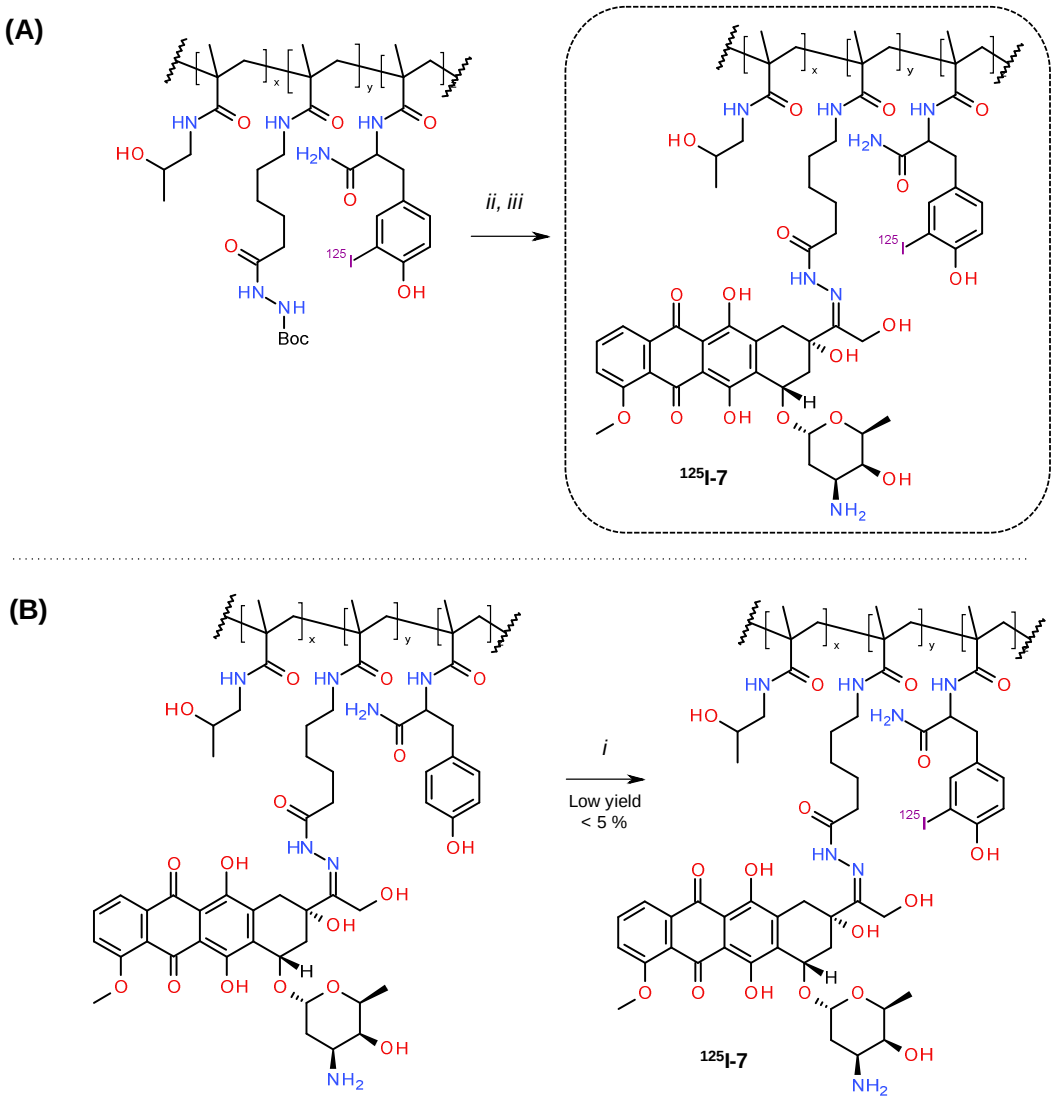


Figure D7.: Synthesis of radioiodinated polymeric conjugate **^{125}I-7**. Reaction conditions: (*i*) Na^{125}I, chloramine-T, ascorbic acid/phosphate buffer, room temperature, 15 min; *(ii)* TFA, triisopropylsilane, room temperature, 2 min; (*iii*) DOX.HCl, AcOH/MeOH, room temperature, 48 h.

Therefore, we chose another approach. We found that when the hydrazide functional groups are protected with a *t*-butyloxycarbonyl (Boc) group, they can be iodinated directly *via* the chloramine T method. Therefore, we moved the radiolabeling step before the Boc deprotection and DOX conjugation steps. Thus, the tyrosine residues of the Boc-protected polymer were radioiodinated to result in a good radiochemical yield of **^{125}I-7** (79 %, M_w = 24.4 kDa, M_w/M_n = 1.21) without any polymer crosslinking. After acidic Boc-deprotection and DOX binding, the conjugate **^{125}I-7** containing 8.7 wt. % of

DOX (M_w = 26.9 kDa, M_w/M_n = 1.25) was obtained (**Figure D7 (A)**). The profile of *in vitro* DOX release from conjugate **^{125}I-7** at body temperature (37 °C) is very similar to that of other water-soluble DOX hydrazone conjugates reported elsewhere;[88] it was released rapidly in buffer with a pH of 5.0 (mimicking the pH in late endosomes after internalization, where the drug should be released, with 85 % DOX released within 24 h) and very slowly in buffer with a pH of 7.4 (the pH of blood plasma during transport to a solid tumor; here the conjugate should be stable and 8 % DOX was released within 24 h). This release profile is ideal for the chemoradiotherapy of solid tumors, where the chemotherapeutic effect is synergistically supported by radiation if a higher dose of radionuclide such as, e.g., ^{131}I is used. Furthermore, the use of a radiotracer at lower activities enables us to follow the fate of the polymer carrier *in vivo*.

4.4. Synthesis and evaluation of the optimized system for nuclear delivery of ^{125}I (publication D5)

Based on the abovementioned research, the optimized system for the tumor delivery of ^{125}I-bearing intercalator was developed. The stable iodine-containing 9-iodoellipticine was quaternized with the simplest 2-oxopropyl-*p*-toluenesulfonate linker to form the *N*-(2-oxopropyl)-9-iodoellipticinium *p*-toluenesulfonate (**8**). This linker was used instead of the more accessible 2-oxopropylbromide, because the bromide counteranion, could be partly oxidized to bromine by hydrogen peroxide during the subsequent radioiodination (see below) and would therefore interfere in the iododestannylation process to produce 9-bromoderivative, which is hard to separate from the 9-iododerivative.

The drug-DNA intercalation constant was determined by direct titration of the iodoellipticinium **8** with a solution of calf thymus DNA, followed by measurement of the fluorescence emission spectra. From the dependence of the fluorescence intensity at maxima (534 nm) on the amount of added DNA, the intercalation constant of **8** was determined to be $K_i = 4.3 \pm 0.32 \times 10^6\ M^{-1}$ (bp), proving its effective DNA intercalation. Also, the cell internalization of ellipticine derivatives can be directly observed due to their inherent fluorescence properties. The ellipticinium **8** readily internalizes into the cell nuclei, as proven by confocal microscopy using Hoechst # 33342 as nuclear stain standard. This is a crucial requirement for the therapeutic effect of the studied system. The non-

radioactive iodoellipticinium **8** was linked to the hydrazide group containing HPMA copolymer (M_w = 25.4 kDa, M_w/M_n = 1.21) to form the polymer conjugate **9** (M_w = 26.8 kDa, M_w/M_n = 1.24). The hydrazide group containing HPMA copolymer precursor pHPMA-MAAcap hydrazide was synthesized by reversible addition and fragmentation (RAFT) copolymerization of *N*-(2-hydroxypropyl)methylacrylamide with *N*-Boc-protected *N*-(6-hydrazino-6-oxohexyl)-2-methylacrylamide using azobis(isobutyronitrile) as an initiator and 2-cyano-2-propyl benzodithioate as chain transfer agent, followed by deprotection according to the reference.[223] The release profile of drug **8** from the HPMA copolymer **9** at 37 °C was determined (**Figure D8**). At pH 7.4 (mimicking the pH of the blood plasma), the linker was stable and almost no drug was released from the polymer (0.11% in 24 h). However, at pH 5.0 (mimicking the pH in late endosomes) rapid release of drug **8** was observed (47% of the drug was released within 24 h). The release in the pH 5.0 buffer is approximately twice as fast as in our first generation system and the conjugate remained intact at pH 7.4. This represents a significant improvement of the system with respect to the second targeting step (pH-responsive drug release). Also, the confocal microscopy proved the fast cell internalization of conjugate **9**. However, the staining of the cell nuclei occurs more slowly for the polymer conjugate **9** than for the free drug **8**, because the free drug must be released from the conjugate before it acts as an intercalator.

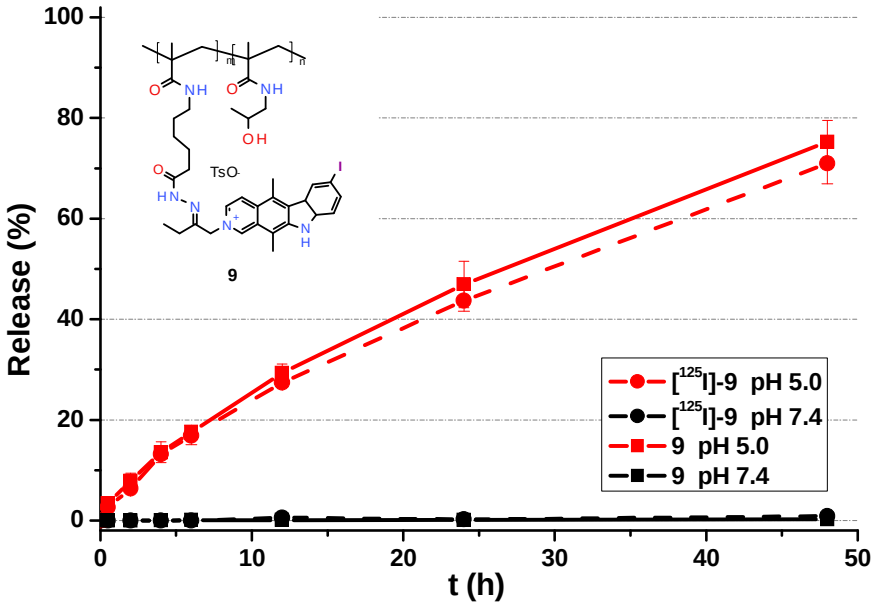


Figure D8.: The release profiles of derivatives **8** (solid line) and **[^{125}I]-8** (dashed line) from their conjugates **9**, **[^{125}I]-9**, in buffered media at 37 °C.

After observing satisfactory *in vitro* results using the non-radioactive system, the same system was developed using the Auger emitting radioisotope ^{125}I in place of the naturally occurring non-radioactive isotope ^{127}I. Because the high specific activity is desirable, the no-carrier iodine was used and the iododestannylation approach was utilized instead of previously used direct radioiodination. As the iodination of hydrazone conjugates could be problematic (see chapter 4.3.), the radioactive intercalator was synthesized first, followed by its conjugation to polymer. Therefore, trimethylstannyl group-containing precursor (**Figure D9**), suitable for radiolabeling, was synthesized by palladium complex catalyzed stannylation reaction followed by quaternization with 2-oxopropyl-*p*-toluenesulfonate. The precursor was radioiodinated with $^{125}I_2$, formed *in situ* from $Na^{125}I$, hydrogen peroxide and acetic acid and the product **[^{125}I]-8** was separated using an HPLC system. The specific radioactivity was determined to be 63.2 GBq/mg, which is four orders of magnitude higher than in our first generation system. Then, the **[^{125}I]-8** was attached to the hydrazide-containing HPMA copolymer analogous to the non-radioactive copolymer, yielding conjugate **[^{125}I]-9**. As expected, the pH-responsive release profile of the radiolabeled drug from its conjugate (negligible release in pH 7.4 buffer, 44% release within 24 h in pH 5.0 buffer) did not substantially differ from the release profile of its non-radioactive analogue **9.**

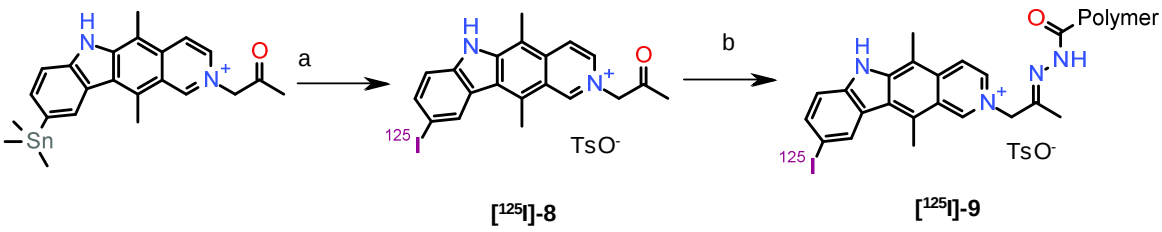


Figure D9: Synthesis of polymer-bound 9-iodoellipticine conjugate **[^{125}I]-9**. Reagents and conditions: (a) $Na^{125}I$, H_2O_2, acetic acid, RT, 15 min; (b) pHPMA-MAAcap hydrazide, 9:1 MeOH/AcOH, RT.

In the *in vivo* experiment with Balb/C mice inoculated with 4T1 mammary carcinoma, it was a priority to monitor the survival of the mice treated with radioactive drugs **[^{125}I]-8** and **[^{125}I]-9**, respectively (**Figure D10)**. The treated groups had longer survival times compared to the untreated controls (27.1 ±6.1 days). The average survival

time in the group treated with the low molecular weight drug **[^{125}I]-8** (2 MBq per mouse), was 36.0 ± 9.9 days after drug administration, and in the group treated with **[^{125}I]-9** (also 2 MBq per mouse), the survival time was 45.5 ±11.5 days. Therefore, the therapy with the intercalator-targeted Auger electron emitter ^{125}I is effective compared to controls and the therapeutic effect is even more pronounced when the ellipticinium is bound to a polymer, as in the polymer conjugate **[^{125}I]-9**.

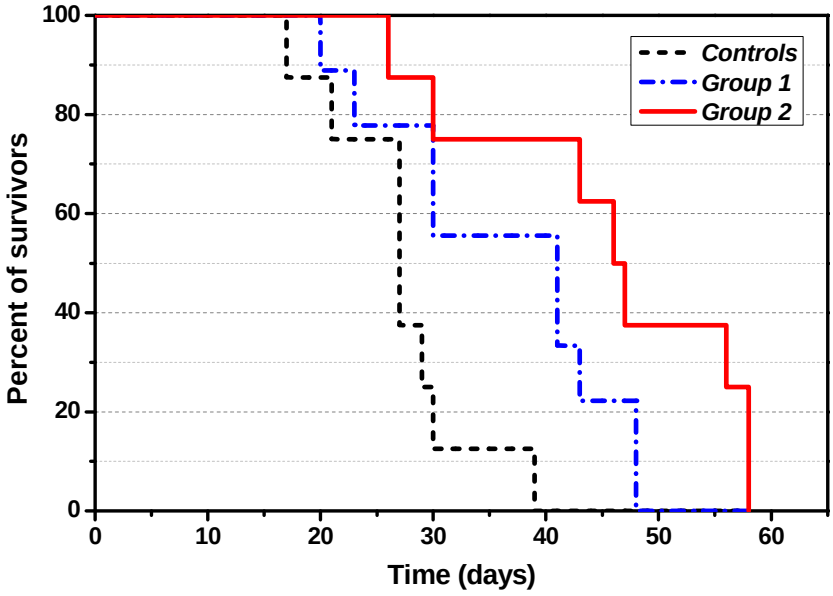


Figure D10.: Survival of mice with 4T1 mammary carcinoma; *Group 1*: [^{125}I]-2-oxopropyl-9-iodoellipticinium **[^{125}I]-8**, 2 MBq/mouse; *Group 2*: [^{125}I]-2-oxopropyl-9-iodoellipticinium linked to the HPMA copolymer **[^{125}I]-9**, 2 MBq/mouse; *Controls*: untreated controls.

5. CONCLUSIONS

1. We have successfully developed a therapeutically active drug composed of DNA-intercalator ellipticine and Auger electron emitter iodine-125. This system readily penetrates to the cell nuclei, as proven by confocal microscope, and intercalates into the DNA, as proven by DNA-titration studies. The high specific radioactivity of the drug (63.2 GBq/mg) was achieved by the radioiododestannylation method.
2. This drug was attached to the optimized biocompatible pHPMA carrier for its prolonged blood circulation, enhanced tumor accumulation and diminished immunity response. The structure of the drug-polymer hydrazone linker was optimized for the maximal drug release in the acidic tumor environment while staying intact at neutral pH during blood transport. This structure optimization is useful for the synthesis of other pH-responsive drug delivery systems.
3. Therapy with the abovementioned polymer conjugate substantially prolongs life of 4T1 mammary carcinoma bearing mice, with respect to its low-molecular weight analogue.
4. Polymer conjugates containing traces of free hydrazide groups are not suitable for the conventional radioiodination. Therefore, a new protocol was developed. The polymers with protected hydrazides were successfully iodinated, followed by the deprotection and drug (doxorubicin) binding.
5. The system is promising for the future studies.

6. REFERENCES

(1) Ferrari, M. *Nature Reviews Cancer* **2005**, *5*, 161.

(2) Bilimoria, K. Y.; Phillips, J. D.; Rock, C. E.; Hayman, A.; Prystowsky, J. B.; Bentrem, D. J. *Annals of surgical oncology* **2009**, *16*, 1799.

(3) Leo, E.; Belli, F.; Miceli, R.; Mariani, L.; Gallino, G.; Battaglia, L.; Vannelli, A.; Andreola, S. *International journal of colorectal disease* **2009**, *24*, 317.

(4) Eckhardt, S. *Current medicinal chemistry-anti-cancer agents* **2002**, *2*, 419.

(5) Foley, K. M. *Palliative Care: Transforming the Care of Serious Illness* **2011**, *33*, 251.

(6) Brookes, P.; Lawley, P. *Biochemical Journal* **1961**, *80*, 496.

(7) Ho, Y. P.; Au-Yeung, S. C.; To, K. K. *Medicinal research reviews* **2003**, *23*, 633.

(8) Sherr, C. J. *Science* **1996**, *274*, 1672.

(9) Cohen, S. M.; Ellwein, L. B. *Science* **1990**, *249*, 1007.

(10) Blagosklonny, M. *Leukemia* **2005**, *20*, 385.

(11) Pommier, Y. *Nature Reviews Cancer* **2006**, *6*, 789.

(12) Danysz, A.; Rauf Hamid, M.; Wierzba, K. *Side Effects of Drugs Annual* **1979**, *3*, 361.

(13) Jordan, M. A.; Thrower, D.; Wilson, L. *Cancer research* **1991**, *51*, 2212.

(14) Calderoni, A.; Cerny, T. *Critical reviews in oncology/hematology* **2001**, *38*, 105.

(15) Knockaert, M.; Greengard, P.; Meijer, L. *Trends in pharmacological sciences* **2002**, *23*, 417.

(16) Rosenberg, S. A.; Yang, J. C.; Restifo, N. P. *Nature medicine* **2004**, *10*, 909.

(17) CTSU, R. I. *Lancet* **2005**, *365*, 1687.

(18) Maeda, H.; Seymour, L. W.; Miyamoto, Y. *Bioconjugate chemistry* **1992**, *3*, 351.

(19) Testa, N. G.; Hendry, J. H.; Molineux, G. *Anticancer research* **1984**, *5*, 101.

(20) Hunter, T.; Bjelland, J. *American Journal of Roentgenology* **1984**, *142*, 513.

(21) Pont, J.; Albrecht, W. *Fertility and sterility* **1997**, *68*, 1.

(22) Tosti, A.; Misciali, C.; Piraccini, B. M.; Peluso, A. M.; Bardazzi, F. *Drug Safety* **1994**, *10*, 310.

(23) Arany, I.; Safirstein, R. L. In *Seminars in nephrology*; Elsevier: 2003; Vol. 23, p 460.

(24) Olson, R. D.; Mushlin, P. S. *The FASEB journal* **1990**, *4*, 3076.

(25) Todd, G.; Griffing, W.; Gibson, W.; Morton, D. *Cancer treatment reports* **1979**, *63*, 35.

(26) Cho, K.; Wang, X.; Nie, S.; Shin, D. M. *Clinical cancer research* **2008**, *14*, 1310.

(27) Allen, T. M.; Cullis, P. R. *Science* **2004**, *303*, 1818.

(28) Arap, W.; Pasqualini, R.; Ruoslahti, E. *Science* **1998**, *279*, 377.

(29) Brannon-Peppas, L.; Blanchette, J. O. *Advanced drug delivery reviews* **2012**, *64*, 206.

(30) Alley, S. C.; Okeley, N. M.; Senter, P. D. *Current opinion in chemical biology* **2010**, *14*, 529.

(31) Janaky, T.; Juhasz, A.; Bajusz, S.; Csernus, V.; Srkalovic, G.; Bokser, L.; Milovanovic, S.; Redding, T.; Rekasi, Z.; Nagy, A. *Proceedings of the National Academy of Sciences* **1992**, *89*, 972.

(32) Khandare, J.; Minko, T. *Progress in Polymer Science* **2006**, *31*, 359.

(33) Gabizon, A.; Shmeeda, H.; Horowitz, A. T.; Zalipsky, S. *Advanced drug delivery reviews* **2004**, *56*, 1177.

(34) Maeda, H.; Bharate, G.; Daruwalla, J. *European Journal of Pharmaceutics and Biopharmaceutics* **2009**, *71*, 409.

(35) Maeda, H.; Sawa, T.; Konno, T. *Journal of Controlled Release* **2001**, *74*, 47.

(36) Maeda, H.; Wu, J.; Sawa, T.; Matsumura, Y.; Hori, K. *Journal of Controlled Release* **2000**, *65*, 271.

(37) Hyung Park, J.; Kwon, S.; Lee, M.; Chung, H.; Kim, J.-H.; Kim, Y.-S.; Park, R.-W.; Kim, I.-S.; Bong Seo, S.; Kwon, I. C. *Biomaterials* **2006**, *27*, 119.

(38) Haag, R.; Kratz, F. *Angewandte Chemie International Edition* **2006**, *45*, 1198.

(39) Noguchi, Y.; Wu, J.; Duncan, R.; Strohalm, J.; Ulbrich, K.; Akaike, T.; Maeda, H. *Cancer Science* **1998**, *89*, 307.

(40) Longmire, M.; Choyke, P. L.; Kobayashi, H. **2008**.

(41) Venkataraman, S.; Hedrick, J. L.; Ong, Z. Y.; Yang, C.; Ee, P. L. R.; Hammond, P. T.; Yang, Y. Y. *Advanced drug delivery reviews* **2011**, *63*, 1228.

(42) Mosqueira, V. C. F.; Legrand, P.; Morgat, J.-L.; Vert, M.; Mysiakine, E.; Gref, R.; Devissaguet, J.-P.; Barratt, G. *Pharmaceutical research* **2001**, *18*, 1411.

(43) Sedlacek, O.; Monnery, B. D.; Filippov, S. K.; Hoogenboom, R.; Hruby, M. *Macromolecular rapid communications* **2012**, *33*, 1648.

(44) Liu, Z.; Jiao, Y.; Wang, Y.; Zhou, C.; Zhang, Z. *Advanced drug delivery reviews* **2008**, *60*, 1650.

(45) Kataoka, K.; Harada, A.; Nagasaki, Y. *Advanced drug delivery reviews* **2001**, *47*, 113.

(46) Greenwald, R. B.; Choe, Y. H.; McGuire, J.; Conover, C. D. *Advanced drug delivery reviews* **2003**, *55*, 217.

(47) Veronese, F. M.; Pasut, G. *Drug discovery today* **2005**, *10*, 1451.

(48) Garay, R. P.; El-Gewely, R.; Armstrong, J. K.; Garratty, G.; Richette, P. *Expert opinion on drug delivery* **2012**, *9*, 1319.

(49) Knop, K.; Hoogenboom, R.; Fischer, D.; Schubert, U. S. *Angewandte Chemie International Edition* **2010**, *49*, 6288.

(50) Ulbricht, J.; Jordan, R.; Luxenhofer, R. *Biomaterials* **2014**, *35*, 4848.

(51) Scales, C. W.; Vasilieva, Y. A.; Convertine, A. J.; Lowe, A. B.; McCormick, C. L. *Biomacromolecules* **2005**, *6*, 1846.

(52) Subr, V.; Kostka, L.; Strohalm, J.; Etrych, T.; Ulbrich, K. *Macromolecules* **2013**, *46*, 2100.

(53) Willcock, H.; O'Reilly, R. K. *Polymer Chemistry* **2010**, *1*, 149.

(54) Kopeček, J.; Kopečková, P.; Minko, T.; Lu, Z.-R. *European Journal of Pharmaceutics and Biopharmaceutics* **2000**, *50*, 61.

(55) Ulbrich, K.; Šubr, V. *Advanced drug delivery reviews* **2010**, *62*, 150.

(56) Masayuki, Y.; Mizue, M.; Noriko, Y.; Teruo, O.; Yasuhisa, S.; Kazunori, K.; Shohei, I. *Journal of Controlled Release* **1990**, *11*, 269.

(57) Kim, T.-Y.; Kim, D.-W.; Chung, J.-Y.; Shin, S. G.; Kim, S.-C.; Heo, D. S.; Kim, N. K.; Bang, Y.-J. *Clinical cancer research* **2004**, *10*, 3708.

(58) Robinson, B. H.; Bucak, S.; Fontana, A. *Langmuir* **2000**, *16*, 8231.

(59) Israelachvili, J. N.; Mitchell, D. J.; Ninham, B. W. *Journal of the Chemical Society, Faraday Transactions 2: Molecular and Chemical Physics* **1976**, *72*, 1525.

(60) Guillemet, F.; Piculell, L. *The Journal of Physical Chemistry* **1995**, *99*, 9201.

(61) Rösler, A.; Vandermeulen, G. W.; Klok, H.-A. *Advanced drug delivery reviews* **2012**, *64*, 270.

(62) Hrubý, M.; Koňák, Č.; Ulbrich, K. *Journal of Controlled Release* **2005**, *103*, 137.

(63) Bronich, T. K.; Keifer, P. A.; Shlyakhtenko, L. S.; Kabanov, A. V. *Journal of the American Chemical Society* **2005**, *127*, 8236.

(64) Mordon, S.; Devoisselle, J. M.; Maunoury, V. *Photochemistry and photobiology* **1994**, *60*, 274.

(65) Kneipp, J.; Kneipp, H.; Wittig, B.; Kneipp, K. *The Journal of Physical Chemistry C* **2010**, *114*, 7421.

(66) Lee, E. S.; Shin, H. J.; Na, K.; Bae, Y. H. *Journal of Controlled Release* **2003**, *90*, 363.

(67) Chytil, P.; Etrych, T.; Kostka, L.; Ulbrich, K. *Macromolecular Chemistry and Physics* **2012**, *213*, 858.

(68) Ward, M. A.; Georgiou, T. K. *Polymers* **2011**, *3*, 1215.

(69) Vlassopoulos, D. *Rheologica acta* **1996**, *35*, 556.

(70) Lutz, J.-F.; Akdemir, Ö.; Hoth, A. *Journal of the American Chemical Society* **2006**, *128*, 13046.

(71) Luxenhofer, R.; Han, Y.; Schulz, A.; Tong, J.; He, Z.; Kabanov, A. V.; Jordan, R. *Macromolecular rapid communications* **2012**, *33*, 1613.

(72) Zhang, W.; Shi, L.; Wu, K.; An, Y. *Macromolecules* **2005**, *38*, 5743.

(73) Wei, H.; Zhang, X.; Cheng, C.; Cheng, S.-X.; Zhuo, R.-X. *Biomaterials* **2007**, *28*, 99.

(74) Choi, C.; Chae, S. Y.; Nah, J.-W. *Polymer* **2006**, *47*, 4571.

(75) Sharma, A.; Sharma, U. S. *International journal of pharmaceutics* **1997**, *154*, 123.

(76) Uchegbu, I. F.; Vyas, S. P. *International journal of pharmaceutics* **1998**, *172*, 33.

(77) Liong, M.; Lu, J.; Kovochich, M.; Xia, T.; Ruehm, S. G.; Nel, A. E.; Tamanoi, F.; Zink, J. I. *ACS nano* **2008**, *2*, 889.

(78) Oh, J. K.; Drumright, R.; Siegwart, D. J.; Matyjaszewski, K. *Progress in Polymer Science* **2008**, *33*, 448.

(79) Cannon, J. B.; Hui, H.-W.; Gupta, P. K. In *Encyclopedia of Pharmaceutical Technology, Third Edition*; Taylor & Francis: 2007, p 1132.

(80) Reijngoud, D.; Tager, J. *Biochimica et Biophysica Acta (BBA)-General Subjects* **1973**, *297*, 174.

(81) Schmaljohann, D. *Advanced drug delivery reviews* **2006**, *58*, 1655.

(82) Shen, W.-C.; Ryser, H. J.-P. *Biochemical and Biophysical Research Communications* **1981**, *102*, 1048.

(83) Al-Shamkhani, A.; Duncan, R. *International journal of pharmaceutics* **1995**, *122*, 107.

(84) Greene, T. W.; Wuts, P. G. *Protective groups in organic synthesis*; Wiley New York, 1991; Vol. 403.

(85) Gillies, E. R.; Goodwin, A. P.; Fréchet, J. M. *Bioconjugate chemistry* **2004**, *15*, 1254.

(86) Kalia, J.; Raines, R. T. *Angewandte Chemie* **2008**, *120*, 7633.

(87) Kaneko, T.; Willner, D.; Monkovic, I.; Knipe, J. O.; Braslawsky, G. R.; Greenfield, R. S.; Vyas, D. M. *Bioconjugate chemistry* **1991**, *2*, 133.

(88) Etrych, T.; Chytil, P.; Jelínková, M.; Říhová, B.; Ulbrich, K. *Macromolecular bioscience* **2002**, *2*, 43.

(89) Ulbrich, K.; Etrych, T.; Chytil, P.; Jelınková, M.; Říhová, B. *Journal of Controlled Release* **2003**, *87*, 33.

(90) King, H. D.; Yurgaitis, D.; Willner, D.; Firestone, R. A.; Yang, M. B.; Lasch, S. J.; Hellström, K. E.; Trail, P. A. *Bioconjugate chemistry* **1999**, *10*, 279.

(91) Prabaharan, M.; Grailer, J. J.; Pilla, S.; Steeber, D. A.; Gong, S. *Biomaterials* **2009**, *30*, 6065.

(92) Krakovičová, H.; Etrych, T.; Ulbrich, K. *European Journal of Pharmaceutical Sciences* **2009**, *37*, 405.

(93) Fletcher, G. H. *Textbook of radiotherapy*; Lea & Febiger, 1973.

(94) Ma, N.; Xu, H.; An, L.; Li, J.; Sun, Z.; Zhang, X. *Langmuir* **2011**, *27*, 5874.

(95) Pollack, A.; Zagars, G. K. *International Journal of Radiation Oncology* Biology* Physics* **1997**, *39*, 1011.

(96) Kurup, R.; Glasgow, G. *Medical dosimetry: official journal of the American Association of Medical Dosimetrists* **1992**, *18*, 179.

(97) Malone, S.; O'Sullivan, B.; Catton, C.; Bell, R.; Fornasier, V.; Davis, A. *International Journal of Radiation Oncology* Biology* Physics* **1995**, *33*, 689.

(98) Wu, A.; Lindner, G.; Maitz, A.; Kalend, A.; Lunsford, L.; Flickinger, J.; Bloomer, W. *International Journal of Radiation Oncology* Biology* Physics* **1990**, *18*, 941.

(99) Shioyama, Y.; Tokuuye, K.; Okumura, T.; Kagei, K.; Sugahara, S.; Ohara, K.; Akine, Y.; Ishikawa, S.; Satoh, H.; Sekizawa, K. *International Journal of Radiation Oncology* Biology* Physics* **2003**, *56*, 7.

(100) Barendsen, G. *International Journal of Radiation Oncology* Biology* Physics* **1982**, *8*, 1981.

(101) Hunter, N.; Muirhead, C. R. *Journal of Radiological Protection* **2009**, *29*, 5.

(102) Kjellberg, R. N.; Hanamura, T.; Davis, K. R.; Lyons, S. L.; Adams, R. D. *New England Journal of Medicine* **1983**, *309*, 269.

(103) Steinberg, G. K.; Fabrikant, J. I.; Marks, M. P.; Levy, R. P.; Frankel, K. A.; Phillips, M. H.; Shuer, L. M.; Silverberg, G. D. *New England Journal of Medicine* **1990**, *323*, 96.

(104) Kjellberg, R. N.; Abe, M. In *Modern stereotactic neurosurgery*; Springer: 1988, p 463.

(105) Cherry, S. R.; Sorenson, J. A.; Phelps, M. E. *Physics in nuclear medicine*; Elsevier Health Sciences, 2012.

(106) Crook, J.; Esche, B.; Futter, N. *Urology* **1996**, *47*, 387.

(107) Arriagada, R.; Mouriesse, H.; Sarrazin, D.; Clark, R.; Deboer, G. *International Journal of Radiation Oncology* Biology* Physics* **1985**, *11*, 1751.

(108) Zoller, F.; Eisenhut, M.; Haberkorn, U.; Mier, W. *European journal of pharmacology* **2009**, *625*, 55.

(109) Watt, D.; Kadiri, L. *International journal of quantum chemistry* **1990**, *38*, 501.

(110) Neirinckx, R. D.; Canning, L. R.; Piper, I. M.; Nowotnik, D. P.; Pickett, R. D.; Holmes, R. A.; Volkert, W. A.; Forster, A. M.; Weisner, P. S.; Marriou, J. A. **1987**.

(111) Nath, R. *Brachytherapy Physics, eds. JF Williamson, BR Thomadsen, R. Nath* **1995**, 7.

(112) Williams, L. E.; DeNardo, G. L.; Meredith, R. F. *Medical physics* **2008**, *35*, 3062.

(113) Zalutsky, M. R. *Journal of Nuclear Medicine* **2006**, *47*, 1238.

(114) Enger, S. A.; Hartman, T.; Carlsson, J.; Lundqvist, H. *Physics in medicine and biology* **2008**, *53*, 1909.

(115) Harrison, L.; Blackwell, K. *The Oncologist* **2004**, *9*, 31.

(116) Bailey, D. L.; Townsend, D. W.; Valk, P. E.; Maisey, M. N. *Positron emission tomography*; Springer, 2005.

(117) Delbeke, D. *Journal of nuclear medicine: official publication, Society of Nuclear Medicine* **1999**, *40*, 591.
(118) Raju, M.; Eisen, Y.; Carpenter, S.; Inkret, W. *Radiation research* **1991**, *128*, 204.
(119) Morgenroth, A.; Vogg, A. T.; Mottaghy, F. M.; Schmaljohann, J. *Methods* **2011**, *55*, 203.
(120) McDevitt, M. R.; Sgouros, G.; Finn, R. D.; Humm, J. L.; Jurcic, J. G.; Larson, S. M.; Scheinberg, D. A. *European journal of nuclear medicine* **1998**, *25*, 1341.
(121) Haas, T.; Grant, J.; Dooley Iii, G. *Journal of Applied Physics* **1972**, *43*, 1853.
(122) Booze, J.; Paretzke, H.; Pomplun, E.; Olkol, P. *Radiation and environmental biophysics* **1987**, *26*, 151.
(123) Buchegger, F.; Perillo-Adamer, F.; Dupertuis, Y. M.; Delaloye, A. B. *European journal of nuclear medicine and molecular imaging* **2006**, *33*, 1352.
(124) Olsen, B. B.; Thisgaard, H.; Vogel, S.; Thomassen, M.; Kruse, T. A.; Needham, D.; Mollenhauer, J.; Flemming Høilund-Carlsen, P. *European Journal of Nanomedicine* **2013**, *5*, 181.
(125) Mamede, M.; Saga, T.; Kobayashi, H.; Ishimori, T.; Higashi, T.; Sato, N.; Brechbiel, M. W.; Konishi, J. *Clinical cancer research* **2003**, *9*, 3756.
(126) Porter, A. T.; Forman, J. D. *Cancer* **1993**, *71*, 953.
(127) Nag, S.; Kuske, R. R.; Vicini, F. A.; Arthur, D. W.; Zwicker, R. D. *Oncology (Williston Park, NY)* **2001**, *15*, 195.
(128) Patel, F. D.; Sharma, S. C.; Negi, P. S.; Ghoshal, S.; Gupta, B. D. *International Journal of Radiation Oncology* Biology* Physics* **1994**, *28*, 335.
(129) Stabin, M. G. *Cancer Biotherapy and Radiopharmaceuticals* **2003**, *18*, 611.
(130) Varma, V. M.; Beierwaltes, W. H.; Nofal, M. M.; Nishiyama, R. H.; Copp, J. E. *Jama* **1970**, *214*, 1437.
(131) Silverstein, M. N. *Archives of internal medicine* **1974**, *134*, 113.
(132) Falkmer, U.; Järhult, J.; Wersäll, P.; Cavallin-Ståhl, E. *Acta Oncologica* **2003**, *42*, 620.
(133) Kluetz, P. G.; Pierce, W.; Maher, V. E.; Zhang, H.; Tang, S.; Song, P.; Liu, Q.; Haber, M. T.; Leutzinger, E. E.; Al-Hakim, A. *Clinical cancer research* **2014**, *20*, 9.
(134) Volkert, W. A.; Hoffman, T. J. *Chemical reviews* **1999**, *99*, 2269.
(135) Seevers, R. H.; Counsell, R. E. *Chemical reviews* **1982**, *82*, 575.

(136) Anderson, W. T.; Strand, M. *NCI monographs: a publication of the National Cancer Institute* **1986**, 149.

(137) Kwekkeboom, D. J.; Kooij, P. P.; Bakker, W. H.; Mäcke, H. R.; Krenning, E. P. *Journal of nuclear medicine: official publication, Society of Nuclear Medicine* **1999**, *40*, 762.

(138) Loh, K.-C.; Fitzgerald, P.; Matthay, K.; Yeo, P.; Price, D. *Journal of endocrinological investigation* **1997**, *20*, 648.

(139) Kato, I.; Ono, K.; Sakurai, Y.; Ohmae, M.; Maruhashi, A.; Imahori, Y.; Kirihata, M.; Nakazawa, M.; Yura, Y. *Applied radiation and isotopes* **2004**, *61*, 1069.

(140) Carlsson, J.; Sjoberg, S.; Larsson, B. S. *Acta Oncologica* **1992**, *31*, 803.

(141) Hopewell, J.; Morris, G.; Schwint, A.; Coderre, J. *Applied radiation and isotopes* **2011**, *69*, 1756.

(142) Ng, D. *Biomed Imaging Interv J* **2006**, *2*, e23.

(143) Wiseman, G. A.; White, C. A.; Witzig, T. E.; Gordon, L. I.; Emmanouilides, C.; Raubitschek, A.; Janakiraman, N.; Gutheil, J.; Schilder, R. J.; Spies, S. *Clinical cancer research* **1999**, *5*, 3281s.

(144) Vose, J. M. *The Oncologist* **2004**, *9*, 160.

(145) Macklis, R. M. In *Seminars in radiation oncology*; Elsevier: 2007; Vol. 17, p 176.

(146) Goldsmith, S. J. In *Seminars in nuclear medicine*; Elsevier: 2010; Vol. 40, p 122.

(147) Horning, S. J.; Younes, A.; Jain, V.; Kroll, S.; Lucas, J.; Podoloff, D.; Goris, M. *Journal of Clinical Oncology* **2005**, *23*, 712.

(148) Sharkey, R. M.; Brenner, A.; Burton, J.; Hajjar, G.; Toder, S. P.; Alavi, A.; Matthies, A.; Tsai, D. E.; Schuster, S. J.; Stadtmauer, E. A. *Journal of Nuclear Medicine* **2003**, *44*, 2000.

(149) Milenic, D. E.; Wong, K. J.; Baidoo, K. E.; Nayak, T. K.; Regino, C. A.; Garmestani, K.; Brechbiel, M. W. **2010**.

(150) Jelovac, D.; Emens, L. A. *Oncology* **2013**, *27*, 166.

(151) Abbas, N.; Bruland, Ø. S.; Brevik, E. M.; Dahle, J. *Nuclear medicine communications* **2012**, *33*, 838.

(152) Ciardiello, F.; Tortora, G. *New England Journal of Medicine* **2008**, *358*, 1160.

(153) Saki, M.; Toulany, M.; Sihver, W.; Zenker, M.; Heldt, J.-M.; Mosch, B.; Pietzsch, H.-J.; Baumann, M.; Steinbach, J.; Rodemann, H. *Strahlentherapie und Onkologie* **2012**, *188*, 823.

(154) Johnston, J. B.; Navaratnam, S.; Pitz, M. W.; Maniate, J. M.; Wiechec, E.; Baust, H.; Gingerich, J.; Skliris, G. P.; Murphy, L. C.; Los, M. *Current medicinal chemistry* **2006**, *13*, 3483.

(155) Cai, W.; Chen, K.; He, L.; Cao, Q.; Koong, A.; Chen, X. *European journal of nuclear medicine and molecular imaging* **2007**, *34*, 850.

(156) Eilers, R.; Gandhi, M.; Patel, J.; Mulcahy, M.; Agulnik, M.; Hensing, T.; Lacouture, M. E. *Journal of the National Cancer Institute* **2010**, *102*, 47.

(157) Reubi, J. C.; Mäcke, H. R.; Krenning, E. P. *Journal of Nuclear Medicine* **2005**, *46*, 67S.

(158) Kwekkeboom, D. J.; de Herder, W. W.; van Eijck, C. H.; Kam, B. L.; van Essen, M.; Teunissen, J. J.; Krenning, E. P. In *Seminars in nuclear medicine*; Elsevier: 2010; Vol. 40, p 78.

(159) Arnold, R.; Trautmann, M.; Creutzfeldt, W.; Benning, R.; Benning, M.; Neuhaus, C.; Jürgensen, R.; Stein, K.; Schäfer, H.; Bruns, C. *Gut* **1996**, *38*, 430.

(160) Bergsma, H.; van Vliet, E. I.; Teunissen, J. J.; Kam, B. L.; de Herder, W. W.; Peeters, R. P.; Krenning, E. P.; Kwekkeboom, D. J. *Best Practice & Research Clinical Gastroenterology* **2012**, *26*, 867.

(161) Faintuch, B. L.; Teodoro, R.; Duatti, A.; Muramoto, E.; Faintuch, S.; Smith, C. J. *Nuclear medicine and biology* **2008**, *35*, 401.

(162) Kato, Y.; Yashiro, M.; Fuyuhiro, Y.; Kashiwagi, S.; Matsuoka, J.; Hirakawa, T.; Noda, S.; Aomatsu, N.; Hasegawa, T.; Matsuzaki, T. *Anticancer research* **2011**, *31*, 3369.

(163) Speer, T. W. *Targeted radionuclide therapy*; Lippincott Williams & Wilkins, 2012.

(164) Vaidyanathan, G.; Zalutsky, M. R. *Physics in medicine and biology* **1996**, *41*, 1915.

(165) O'donoghue, J.; Wheldon, T. *Physics in medicine and biology* **1996**, *41*, 1973.

(166) Panyutin, I. G.; Winters, T. A.; Feinendegen, L. E.; Neumann, R. D. In *Molecular Nuclear Medicine*; Springer: 2003, p 697.

(167) Sedelnikova, O. A.; Luu, A. N.; Karamychev, V. N.; Panyutin, I. G.; Neumann, R. D. *International Journal of Radiation Oncology* Biology* Physics* **2001**, *49*, 391.

(168) Kassis, A. I. *Journal of Nuclear Medicine* **2003**, *44*, 1479.

(169) Griffiths, G. L.; Govindan, S. V.; Sgouros, G.; Ong, G. L.; Goldenberg, D. M.; Mattes, M. J. *International journal of cancer* **1999**, *81*, 985.

(170) Howell, R. W. *Medical physics* **1992**, *19*, 1371.

(171) Stein, O.; Gross, J. *Endocrinology* **1964**, *75*, 787.

(172) Borgman, M. P.; Coleman, T.; Kolhatkar, R. B.; Geyser-Stoops, S.; Line, B. R.; Ghandehari, H. *Journal of Controlled Release* **2008**, *132*, 193.

(173) Hofer, K.; Keough, G.; Smith, J. M. *Current topics in radiation research quarterly* **1978**, *12*, 335.

(174) Sedelnikova, O. A.; Panyutin, I. G.; Thierry, A. R.; Neumann, R. D. *Journal of nuclear medicine: official publication, Society of Nuclear Medicine* **1998**, *39*, 1412.

(175) Yang, W.; Zhao, J.-G.; Li, X.-Y.; Gong, S.-L.; Cao, J.-P. *Radiation and environmental biophysics* **2010**, *49*, 705.

(176) Calabresi, P.; Cardoso, S. S.; Finch, S. C.; Kligerman, M. M.; Von Essen, C. F.; Chu, M. Y.; Welch, A. D. *Cancer research* **1961**, *21*, 550.

(177) Welch, A. D.; Prusoff, W. H. *Cancer chemotherapy reports. Part 1* **1960**, *6*, 29.

(178) Mester, J.; DeGoeij, K.; Sluyser, M. *European Journal of Cancer* **1996**, *32*, 1603.

(179) Kassis, A. I.; Wen, P. Y.; Van den Abbeele, A. D.; Baranowska-Kortylewicz, J.; Makrigiorgos, G. M.; Metz, K. R.; Matalka, K. Z.; Cook, C. U.; Sahu, S. K.; Black, P. *Journal of Nuclear Medicine* **1998**, *39*, 1148.

(180) Kassis, A. I.; Dahman, B. A.; Adelstein, J. S. *Acta Oncologica* **2000**, *39*, 731.

(181) Kassis, A. I.; Fayad, F.; Kinsey, B. M.; Sastry, K. S.; Adelstein, S. J. *Radiation research* **1989**, *118*, 283.

(182) Lobachevsky, P. N.; Martin, R. F. *International journal of radiation biology* **2004**, *80*, 915.

(183) Balagurumoorthy, P.; Xu, X.; Wang, K.; Adelstein, S. J.; Kassis, A. I. *International journal of radiation biology* **2012**, *88*, 998.

(184) Kassis, A. I.; Harapanhalli, R. S.; Adelstein, S. J. *Radiation research* **1999**, *151*, 167.

(185) Ickenstein, L. M.; Edwards, K.; Sjöberg, S.; Carlsson, J.; Gedda, L. *Nuclear medicine and biology* **2006**, *33*, 773.

(186) Gardette, M.; Papon, J.; Bonnet, M.; Desbois, N.; Labarre, P.; Wu, T.-D.; Miot-Noirault, E.; Madelmont, J.-C.; Guerquin-Kern, J.-L.; Chezal, J.-M. *Investigational new drugs* **2011**, *29*, 1253.

(187) Gardette, M.; Viallard, C.; Paillas, S.; Guerquin-Kern, J.-L.; Papon, J.; Moins, N.; Labarre, P.; Desbois, N.; Wong-Wah-Chung, P.; Palle, S. *Investigational new drugs* **2014**, 1.

(188) Hillyar, C. R.; Cornelissen, B.; Vallis, K. A. *Therapeutic delivery* **2014**, *5*, 319.

(189) Abikhzer, G.; Keidar, Z. *European journal of nuclear medicine and molecular imaging* **2014**, *41*, 67.

(190) Anthony, L. B.; Sinha, P. In *Management of Pancreatic Neuroendocrine Tumors*; Springer: 2015, p 127.

(191) Forssell-Aronsson, E.; Bernhardt, P.; Nilsson, O.; Tisell, L.-E.; Wängberg, B.; Ahlman, H. *Acta Oncologica* **2004**, *43*, 436.

(192) De Jong, M.; Valkema, R.; Jamar, F.; Kvols, L. K.; Kwekkeboom, D. J.; Breeman, W. A.; Bakker, W. H.; Smith, C.; Pauwels, S.; Krenning, E. P. In *Seminars in nuclear medicine*; Elsevier: 2002; Vol. 32, p 133.

(193) Wang, M.; Caruano, A. L.; Lewis, M. R.; Meyer, L. A.; VanderWaal, R. P.; Anderson, C. J. *Cancer research* **2003**, *63*, 6864.

(194) Ginj, M.; Hinni, K.; Tschumi, S.; Schulz, S.; Maecke, H. R. *Journal of Nuclear Medicine* **2005**, *46*, 2097.

(195) Fasih, A.; Fonge, H.; Cai, Z.; Leyton, J. V.; Tikhomirov, I.; Done, S. J.; Reilly, R. M. *Breast cancer research and treatment* **2012**, *135*, 189.

(196) Costantini, D. L.; McLarty, K.; Lee, H.; Done, S. J.; Vallis, K. A.; Reilly, R. M. *Journal of Nuclear Medicine* **2010**, *51*, 1084.

(197) Järver, P.; Mäger, I.; Langel, Ü. *Trends in pharmacological sciences* **2010**, *31*, 528.

(198) Bailey, K. E.; Costantini, D. L.; Cai, Z.; Scollard, D. A.; Chen, Z.; Reilly, R. M.; Vallis, K. A. *Journal of Nuclear Medicine* **2007**, *48*, 1562.

(199) Cornelissen, B.; Darbar, S.; Kersemans, V.; Allen, D.; Falzone, N.; Barbeau, J.; Smart, S.; Vallis, K. A. *Nuclear medicine and biology* **2012**, *39*, 1142.

(200) Janib, S. M.; Moses, A. S.; MacKay, J. A. *Advanced drug delivery reviews* **2010**, *62*, 1052.

(201) Welch, M. J.; Hawker, C. J.; Wooley, K. L. *Journal of Nuclear Medicine* **2009**, *50*, 1743.

(202) Zhang, R.; Xiong, C.; Huang, M.; Zhou, M.; Huang, Q.; Wen, X.; Liang, D.; Li, C. *Biomaterials* **2011**, *32*, 5872.

(203) Cammilleri, S.; Sangrajrang, S.; Perdereau, B.; Brixy, F.; Calvo, F.; Bazin, H.; Magdelenat, H. *European journal of nuclear medicine* **1996**, *23*, 448.

(204) Hara, E.; Makino, A.; Kurihara, K.; Ueda, M.; Hara, I.; Kawabe, T.; Yamamoto, F.; Ozeki, E.; Togashi, K.; Kimura, S. *Journal of nanoparticle research* **2013**, *15*, 1.

(205) Chang, M.-Y.; Seideman, J.; Sofou, S. *Bioconjugate chemistry* **2008**, *19*, 1274.

(206) Sofou, S.; Kappel, B. J.; Jaggi, J. S.; McDevitt, M. R.; Scheinberg, D. A.; Sgouros, G. *Bioconjugate chemistry* **2007**, *18*, 2061.

(207) Bandekar, A.; Zhu, C.; Jindal, R.; Bruchertseifer, F.; Morgenstern, A.; Sofou, S. *Journal of Nuclear Medicine* **2014**, *55*, 107.

(208) Fonge, H.; Lee, H.; Reilly, R. M.; Allen, C. *Molecular pharmaceutics* **2009**, *7*, 177.

(209) Fondell, A.; Edwards, K.; Ickenstein, L. M.; Sjöberg, S.; Carlsson, J.; Gedda, L. *European journal of nuclear medicine and molecular imaging* **2010**, *37*, 114.

(210) Gedda, L.; Fondell, A.; Lundqvist, H.; Park, J. W.; Edwards, K. *Journal of Nuclear Medicine* **2012**, *53*, 480.

(211) Bolton, A. *International Journal of Radiation Applications and Instrumentation. Part A. Applied Radiation and Isotopes* **1988**, *39*, 533.

(212) Bunnett, J. *Quarterly Reviews, Chemical Society* **1958**, *12*, 1.

(213) Weichert, J. P.; Van Dort, M. E.; Groziak, M. P.; Counsell, R. E. *International Journal of Radiation Applications and Instrumentation. Part A. Applied Radiation and Isotopes* **1986**, *37*, 907.

(214) Klapars, A.; Buchwald, S. L. *Journal of the American Chemical Society* **2002**, *124*, 14844.

(215) Foster, N.; Dannals, R.; Burns, H.; Heindel, N. *Journal of Radioanalytical and Nuclear Chemistry* **1981**, *65*, 95.

(216) Seymour, L.; Duncan, R.; Strohalm, J.; Kopeček, J. *Journal of biomedical materials research* **1987**, *21*, 1341.

(217) Simone, E. A.; Zern, B. J.; Chacko, A.-M.; Mikitsh, J. L.; Blankemeyer, E. R.; Muro, S.; Stan, R. V.; Muzykantov, V. R. *Biomaterials* **2012**, *33*, 5406.

(218) Wilbur, D. S. *Bioconjugate chemistry* **1992**, *3*, 433.
(219) Kučka, J.; Hrubý, M.; Lebeda, O. *Applied radiation and isotopes* **2010**, *68*, 1073.
(220) Behr, T. M.; Gotthardt, M.; Becker, W.; Behe, M. *Nuklearmedizin. Nuclear medicine* **2002**, *41*, 71.
(221) Yeung, T. K.; Hopewell, J. W.; Simmonds, R. H.; Seymour, L. W.; Duncan, R.; Bellini, O.; Grandi, M.; Spreafico, F.; Strohalm, J.; Ulbrich, K. *Cancer chemotherapy and pharmacology* **1991**, *29*, 105.
(222) Facchinetti, T.; Geroni, C.; Fumagalli, A.; Giuliani, F. *Drugs under experimental and clinical research* **1985**, *12*, 657.
(223) Chytil, P.; Etrych, T.; Kříž, J.; Šubr, V.; Ulbrich, K. *European Journal of Pharmaceutical Sciences* **2010**, *41*, 473.

APPENDIX - PUBLICATIONS D1 - D5