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Nano-sized melphalan and sarcolysine drug delivery systems: synthesis and prospects of application

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Contents

I.	Introduction	783
II.	Demand for nano-sized melphalan and sarcolysine delivery systems	784
III.	Polymeric carriers melphalan and sarcolysine delivery systems	787
IV.	Dendrimers as melphalan carriers	791
V.	Melphalan inclusion complexes with cyclodextrins	800
VI.	Lipid nanocarriers for melphalan and sarcolysine	803
VII.	Melphalan conjugates with proteins	808
VIII.	Conclusion	810

Abstract. The results of experimental studies concerned with the development of nano-sized drug delivery systems for the antitumour drugs sarcolysine and melphalan are generalized. The structures and biological activities of nanocarriers in comparison with unmodified drugs are discussed. Particular attention is given to the liposomes containing lipid derivatives of sarcolysine and melphalan in the lipid bilayer. The bibliography includes 196 references.

I. Introduction

The efficiency of antitumour chemotherapy may be increased, among other methods, by using pharmaceutical forms based on nano-sized drug delivery systems. Nanocarriers transport the drug directly to the target tissue or cell and ensure the subsequent controlled drug release. Therefore, research dealing with 'nanodrugs', especially for cancer therapy, has been vigorously developed in the last decade.[1,2] By 2010, eleven antitumour pharmaceuticals based on nanoparticles have been registered for clinical use. These include liposomes with daunorubicin (DaunoXome), liposomal products with doxorubicin (Doxil/Caelyx and MCC465) and other. At least six agents are being tested in phase I or II clinical trials, and two agents have approached phase III trials.[3]

Inorganic nanoparticles (metals and metal oxides), polymeric nanoparticles, lipid nanocarriers, dendrimers, fullerenes, carbon nanotubes and other nano-sized carriers are potentially suitable for targeted drug delivery. The applicability of nanoparticles for medical purposes is largely determined by their biocompatibility and biodegradability.[4]

The behaviour of a nanoparticle bearing a drug in the body, including biodistribution, pharmacokinetics, target recognition, therapeutic effect, excretion of the major components and unfavourable action of the nanoparticle on the body are determined by a set of physicochemical and biological factors.[5] Among these, most important are nanoparticle size distribution, shape and rigidity (deformability) and the chemical nature, number and relative positions of functional groups on the nanoparticle surface.[5–7] No universal relationship was found between the nanoparticle size and circulation time in the blood. However, in some cases, the regularities of the *in vivo* behaviour of nanoparticles of the same chemical composition were elucidated as a function of their size.[5,8] For example, in the case of nondeformable polymeric nanoparticles coated by polyethylene glycol (PEG) fragments, the size that ensures prolonged circulation is < 150 nm. The increase in the size of the same particles to 200 – 250 nm leads to their fast excretion from the circulation owing to intense filtration through endothelium of the spleen venous sinuses.[5] It was believed that fine inert nanoparticles with size of < 20 nm tend to be retained for a long period in the circulating blood (and, hence, they are characterized by slow extravasation from vessels to the interstitial space).[8] Also, it was found that spherical quantum dots with a hydrodynamic diameter of $\leqslant 5.5$ nm are susceptible to fast renal excretion.[5]

The interaction of a nanoparticle with biological objects depends on the topology of the functional groups on the particle surface. When targeting fragments are present on the surface, nanoparticles can be used for targreted delivery

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of pharmacologically active agents.[6] The type and the number of functional groups in the outer layer of the nanoparticle determine the mechanism of nanoparticle insertion into the cell and intracellular location.

Despite the absence of universal relations between the nanoparticle size and the *in vivo* behaviour, it is known that local injections into the tumour require 30–100 nm particles. They do not tend to leak away through capillary walls in a healthy tissue and they are sufficiently small to avoid reticuloendothelial clearance.[9] Particles of 50–250 nm size are selectively accumulated in the tumour due to their passive targeted delivery to the tumour tissue.[9] This fact was described by Maeda[10] and called enhanced permeability and retention (EPR), *i.e.*, this is the effect of selective facilitated penetration of macromolecular compounds through leaky walls of tumour vessels and retention in the tumour. The EPR effect is induced by particular properties of solid tumours such as active angiogenesis and increased vessel density, enhanced production of vascular mediators, defective vessel structure, the presence of large pores in the vascular endothelium and poor lymphatic clearance of macromolecules and lipids from the interstitial tissue.[11]

Nanoparticles of >200 nm size are subjected to phagocytosis and are actively excreted with the participation of the reticuloendothelial system. Particles of this size activate the complement cascade and are excreted from the body faster than smaller nanoparticles.[11] The poloxamer 407 (copolymer consisting of a central block of polypropylene glycol flanked by two blocks of polyethylene glycol) coating of polystyrene 45 nm nanoparticles was found to increase the half-life of the particles in the circulating blood.[12]

Recently, animal experiments and clinical investigations demonstrated that medium-size nanoparticles (30 to 100 nm) can activate the complement cascade, which is manifested as immune response to administration of nanodrugs.[13] This induces fast nanoparticle decomposition and excretion from the body and excludes the possibility of effective therapeutic action of the drug they carry.

Thus, a topical problem in the development of nano-sized drug delivery systems is the design of stable biocompatible nanostructures with uniform size and morphology that decompose *in vivo* but are not destroyed before arrival to the target tissue and induce no immune response of the body. It is desirable that these systems contain targeting fragments and ensure the stability and prolonged release of the pharmacologically active agent.

The design of nanocarriers is especially important for those drugs that are unstable *in vivo*, poorly soluble in water and show high systemic toxicity. The role of nanoparticles in this case is to protect the drug from too early disintegration and undesirable interactions with biomolecules, to ensure boosted uptake by the target tissue and cell, to control the drug pharmacokinetics and distribution, and to overcome the drug resistance mechanisms of tumour cells.

Melphalan and sarcolysine have been successfully used in the therapy of tumours for a long time. However, some properties of these medicines restrict their efficiency; therefore, the development of nanocarriers for melphalan and sarcolysine would be highly desirable. A considerable body of information on the synthesis and properties of their derivatives has been accumulated. Sarcolysine derivatives are the subject of a review of Academician I L Knunyants and co-workers.[14] Another review[15] summarizes methods for the synthesis and properties of peptides and N-acyl derivatives of 4-[bis(2-chloroethyl)amino]phenylalanine accumulated in the period from publication of the review by Knunyants[14] up to 1995. One more review[16] considers the synthesis and properties of melphalan derivatives prepared up to 2003. However, none of the listed papers discusses the problems and specific features of melphalan and sarcolysine nanocarriers.

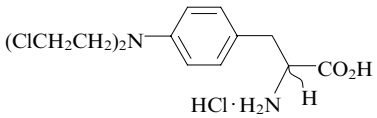
The present review considers the results of studies devoted to the synthesis and properties of nano-sized melphalan and sarcolysine carriers in the retrospective view and gives evaluation of the state of the art of the problem. Examples of testing of the antitumour activity of nano-sized melphalan and sarcolysine carriers in comparison with conventional dosage forms are analyzed.

II. Demand for nano-sized melphalan and sarcolysine delivery systems

1. Brief history of the development and application of sarcolysine and melphalan

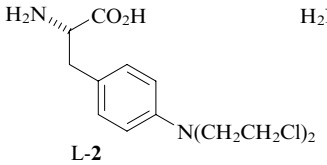
In the first half of the 1950s, the founder of tumour chemotherapy in Russia Academician L F Larionov[17–19] put forward the idea of possible applicability of natural amino acids for transportation of the bis(2-chloroethyl)-amino group (nitrogen mustard group) into tumour cells (theory of alkylating metabolites). Phenylalanine was chosen as the modification object. In 1953, *p*-[bis(2-chloroethyl)amino]-DL-phenylalanine hydrochloride (**1**) called sarcolysine was synthesized in the USSR under the supervision of Academician A S Khokhlov. Since the beginning of 1954, Academician L F Larionov and co-workers[20,21] studied its biological activity. In what follows, this amino acid residue is designated by SL.

Structures **1**, **2**



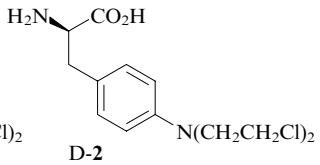
DL-**1**

(HCl·H-SL-OH)



L-**2**

(H-Mel-OH)



D-**2**

(H-Med-OH)

At the same time, in 1953, Bergel and Stock[22–27] in the UK synthesized *p*-[di(2-chloroethyl)amino]-DL-phenylalanine (merphalan) and its L-enantiomer (L-**2**, melphalan) and D-enantiomer (D-**2**, medpalan).

The activity of sarcolysine with respect to the spindle cell sarcoma in 45 rats was studied in detail;[28] sarcolysine was shown to quickly induce complete tumour regression. No such effect had been observed earlier for any compound; therefore, this agent was further studied in other tumours.[29,30]

A study of the antitumour activity of sarcolysine[30] and its enantiomers[31] in rats demonstrated that the biological activities of sarcolysine L-enantiomer (L-**1**) and D-isomer (D-**1**) were different. The antitumour activity was somewhat

lower for D-**1** than for L-**1**. The antitumour activity of melphalan (L-**2**) was also higher as compared with medphalan (D-**2**).[14]

However, a clinical investigation showed that in drug-resistant multiple myeloma patients, sarcolysine is more efficient than melphalan.[32]

Although according to clinical trials, sarcolysine was almost useless in the therapy of sarcomas, it was found to be highly efficient against malignant testicular tumours.[33] The clinical effect of sarcolysine chemotherapy as steady complete regression of seminoma metastases was obtained for the first time in the world history of medicine in the clinic of the Institute of Experimental Pathology and Cancer Therapy, USSR Academy of Medical Sciences (currently the N N Blokhin Russian Cancer Research Centre, Russian Academy of Medical Sciences).

Even in the initial phases of investigation, it was found that sarcolysine suppresses the hematogenesis and, when taken in large doses, causes changes in the blood-forming organs and in small intestine that are also typical of other chloroethylamines.[30] Since sarcolysine had high systemic toxicity,[34] the search for its derivatives that would have higher therapeutic index and broader range of antitumour activity was started almost immediately.

It happened that English and American scientists used melphalan in their works, while in the Soviet Union sarcolysine was used. Initially modification of sarcolysine was carried out by two research groups: a group headed by Academician I L Knunyants at the Institute of Organoelement Compounds, USSR Academy of Sciences, and a group headed by Academician A Ya Berlin in the chemical department of the Institute of Experimental and Clinical Oncology, USSR Academy of Medical Sciences. The antitumour activity of the synthesized products were studied under the direction of Academician L F Larionov.

The ethyl and isopropyl esters of sarcolysine and *N*-formyl- and *N*-acetylsarcolysine were among the first to be prepared.[35] Experiments in rodents showed that all four compounds differ in the antitumour activity, the esters being more toxic than sarcolysine, while acylation of the amino group resulted in a sharp decrease in the toxicity.

Bergel and Stock[36] and Larionov[37] prepared and studied melphalan-containing peptides. These works stimulated the development of effective antitumour agents by incorporation of sarcolysine and melphalan into peptides;[15] Ciphelin and Glutasar used in clinical practice were among these derivatives.

2. The significance of melphalan and sarcolysine in the today's chemotherapy of tumours

Since 1956, sarcolysine has been widely used in the clinical practice being a part of mono- and combination chemotherapy regimens for treatment of patients having testicular seminoma with single metastases, reticulosarcoma, malignant angioendothelioma, Ewing's sarcoma, multiple myeloma,[38] lymphoepithelial Schmincke's tumour and Hodgkin disease.[39]

Melphalan (as the commercial pharmaceutical product Alkeran) is used in the therapy of multiple myeloma, ovarian adenocarcinoma (late stages), lymphoepithelial Schmincke's tumour, localized limb malignant melanoma (especially in final stages), polycythaemia vera, neuroblastoma in children and refractory malignant blood diseases.[38] Melphalan shows antitumour activity with respect to breast, testicular and ovarian cancer but now it is not a first-line drug in the therapy of these diseases. Melphalan in combination with prednisolone (MP) still remains the basic combination in the therapy of multiple myeloma according to the chemotherapy protocol; no formulations that are unambiguously more effective than MP or new combinations based on MP have yet been found.[40–42]

It was shown in *in vitro* and *in vivo* experiments that melphalan tends to show a steeply ascending curve for the therapeutic effect *vs.* concentration;[43] therefore, it is suitable for high-dose chemotherapy where the amount of the administered drug is more than five times higher than the usual dose.

The studies aimed at increasing the efficiency of application of melphalan and sarcolysine by developing new administration protocols[44] and combining them with other antitumour agents[45] or endogenous bioregulators[46,47] are in progress.

The most comprehensive information on the melphalan pharmacology and pharmacokinetics was reported in the review by Jones,[48] who emphasized the importance of developing a dosage form for improving the therapeutic index of melphalan.

Melphalan is used as tablets under the trade name Alkeran and as a formulation for intravenous administration.

A sarcolysine dosage form — freeze-dried powder for the preparation of the solution for intravenous injections — was developed.[49] Sarcolysine-lyo is distinguished by lower toxicity and enhanced efficiency compared with the usual sarcolysine dosage form for parenteral administration.[50]

Thus, melphalan and sarcolysine remain demanded and actively used agents, especially for treating last-stage cancer and metastatic cancer.

3. Properties of melphalan and sarcolysine hampering their clinical use

a. Systemic toxicity

Melphalan causes serious side effects including haematotoxicity (leucopenia), gastrotoxicity, alopecia and allergic reactions.

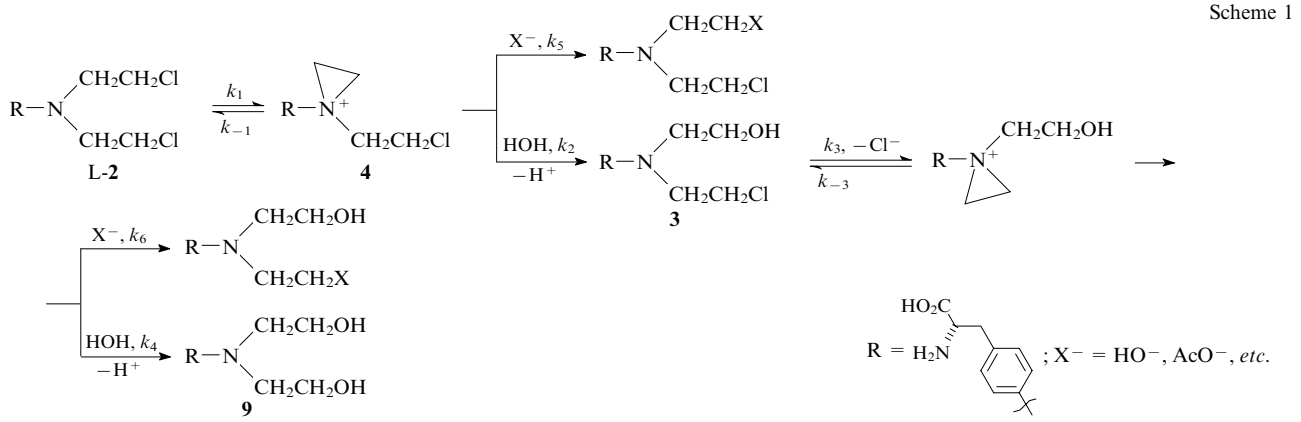
In ovarian cancer patients, melphalan therapy was found to cause in some cases secondary acute leukaemia. Subsequently, carcinogenic properties of melphalan were detected and it was demonstrated that the risk of acute leukaemia during four years after melphalan therapy is 200 times higher than the statistical mean.[51] According to recent investigations of the effect of melphalan on the mitosis, the carcinogenic activity of melphalan is due not only to its ability to promote gene mutations and chromosome breaks but also to the disruption of the formation process of new chromosomes during cell division.[52] Cardiotoxicity of melphalan in combination with fludarabine was revealed.[53]

The development of targeted delivery systems is of paramount importance for solving the melphalan and sarcolysine systemic toxicity problem. Particularly, the design of nanocarriers for these agents appears to be a rational and up-to-date approach to this issue.

b. Hydrolytic instability of sarcolysine and melphalan in water-containing media

Merphalan as a free base is almost insoluble in water, chloroform and diethyl ether, poorly soluble in ethanol,

Scheme 1



sparingly soluble in methanol and soluble in dilute mineral acids and in propylene glycol.[54] In water-containing media, it is hydrolyzed with the gradual replacement of chlorine atoms by hydroxyl groups and thus loses the alkylating properties. Due to instability in water, melphalan and sarcolysine have low bioavailability upon oral administration. In the case of intravenous administration, they rapidly decompose under the action of blood plasma proteins. The rate of alkaline hydrolysis of sarcolysine in aqueous ethanol in the presence of sodium chloride was studied.[55] Under these conditions, hydrolysis of sarcolysine was sharply retarded.

The kinetics of melphalan hydrolysis as a function of pH (1–13), ionic strength, temperature and chloride ion concentration (0–0.5 mol litre^{-1}) in buffer solutions was studied by reverse-phase HPLC.[56] The overall rate of hydrolysis was found to be limited by the transformation of melphalan into hydroxymelphalan **3**, which follows a monomolecular mechanism and involves the formation of iminium ion **4** (Scheme 1).

It was shown that the decomposition rate grows with increase in the pH of the medium or temperature. The best way for stabilizing melphalan in solutions for medical applications is to dissolve it in a 0.9% NaCl solution.[56]

In order to elucidate characteristic features of the major processes of intracellular detoxification of melphalan, HPLC studies of the kinetics of melphalan hydrolysis, phosphatolysis and conjugation with glutathione (non-catalyzed and catalyzed by glutathione S-transferase) were undertaken.[57] The pseudo-first-order rate constants for the reaction giving the iminium cation from melphalan and for the formation of all its monosubstituted derivatives (except for the monohydroxy-substituted melphalan) were found to be roughly equal. The enzyme interacts only with the iminium intermediate **4**, which is formed from melphalan.

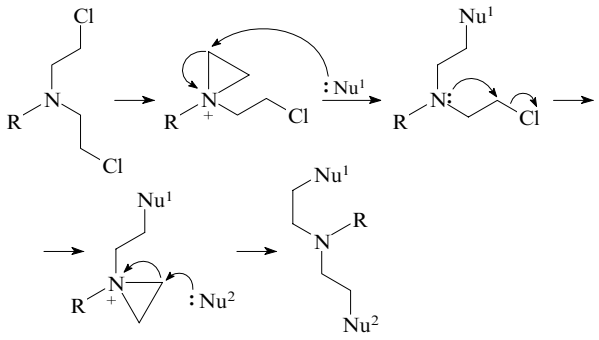
The instability of melphalan *in vivo* and fast hydrolysis in water-containing media stimulated target-oriented synthesis of hydrolytically more stable melphalan derivatives and water-soluble delivery systems including nanocarriers.

c. Formation of resistance of tumour cells to melphalan

The antitumour and carcinogenic activities of melphalan are determined by its ability to alkylate DNA molecules and DNA – protein complexes.[58] An advantage of melphalan and other alkylating agents over other antitumour drugs is that they are selective to tumour cells at any phase of the cell cycle, which is generally faster in the case of tumour cells.

The basicity of the nucleophilic atoms of nucleic bases determines the alkylation route. The guanine N(7) atom is most electronegative among all nitrogen atoms present in nucleic bases and, therefore, this atom is most prone to be attacked by the iminium intermediate.[59] The alkylation of nucleophiles Nu^1 and Nu^2 with bis(chloroethyl)amines $RN(CH_2CH_2Cl)_2$ is depicted in Scheme 2.

Scheme 2



Melphalan is a first-generation alkylating agent that is able to cross-link two complementary strands in the DNA major groove at the N(7) atom of guanine rather than to form a link on the same strand.[60] The alkylation of DNA molecules with melphalan has been studied in detail.[61–64]

There are several mechanisms for the formation of drug resistance of tumour cells to melphalan, in particular, repair of damaged DNA, detoxification with glutathione, and efflux of glutathione conjugates with the participation of multiple drug resistance proteins.

The repair of damaged DNA. The efficiency of the antitumour activity of melphalan and sarcolysine is affected by the ability of tumour cells to repair DNA. The nucleotide excision repair (NER) occurs by cutting out the site with damaged DNA nitrogenous bases and the subsequent restoration of the normal structure of the DNA molecule by synthetic recombination of the sequence and ligation of the complementary strand.[65] Repair is the key reason for the cross resistance of tumour cells to chemo- and radiation therapy. The types of tumours differ considerably in the ability to repair interstrand cross-links.

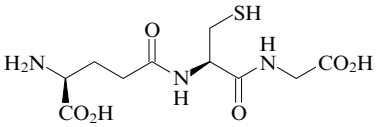
It was shown that melphalan adducts with DNA are repaired *via* NER.[66] Single-cell gel electrophoresis (SCGE) was used to quantitatively estimate the melphalan interstrand cross-links and the subsequent DNA repair in the blood plasma cells of treated (with tumour recurrence) and non-treated patients.[67] After melphalan treatment, DNA

cross-links were observed in the cells of both treated and non-treated patients. The content of DNA cross-links depended on the melphalan dose. The cells of non-treated patients did not show ability to repair.[67]

The enhanced repair of DNA interstrand cross-links along the Fanconi anemia (FA/BRCA) pathway was found to make a considerable contribution to the melphalan stability of the resistant multiple myeloma cells.[68] The violation of this mechanism results in restoration of the sensitivity of cells to alkylating agents.

Melphalan detoxification by glutathione. Glutathione (**5**, GSH) is a natural tripeptide, γ-glutamylcysteinylglycine synthesized in mammalian and human cells; its normal concentration is 1–10 mmol litre^{-1} (see Ref. 69).

Structure **5**

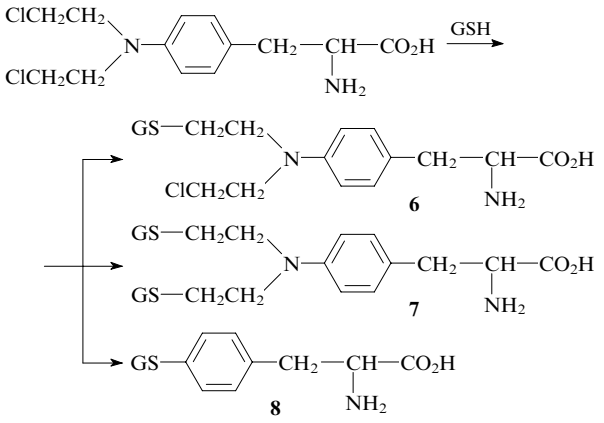


5 (GSH)

The resistance to tumour cells to antitumour agents is related to excessive concentration of GSH. The action of glutathione occurs *via* the reaction of melphalan with GSH and its detoxification upon hydrolysis of chloroethyl groups, which includes combined action of GSH, glutathione transferase and the glutathione S-conjugate efflux system. Melphalan-resistant tumour cells are characterized by increased amount of GSH and/or enhanced activity of γ-glutamylcysteine synthase (γ-GCS), which is involved in GSH biosynthesis.[69]

The formation of three melphalan–glutathione conjugates under catalysis by glutathione *S*-transferase during *in vitro* deactivation of melphalan was reported (Scheme 3).[70] Two of these products (**6** and **7**) are formed upon replacement of one or two chlorine atoms of the bis(chloroethyl)amino group. The third conjugate **8** is 4-(glutathionyl)phenylalanine arising upon replacement of the bis(chloroethyl)amino group.

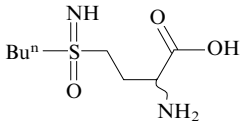
Scheme 3



The structure of melphalan conjugates with GSH was determined by liquid chromatography–electrospray ionization mass spectrometry.[71] The spontaneous reaction of melphalan with glutathione at 37 °C affords monoglutathionyl- and diglutathionylmelphalan with minor amounts of monohydroxymelphalan (**4**) and dihydroxymelphalan (**9**) (see Scheme 1) as impurities. It was shown that glutathione *S*-transferase is activated by melphalan by a mechanism dependent on the melphalan concentration and time.[71]

The combined use of melphalan and DL-buthionine-(*S*,*R*)-sulfoximine (**10**), an inhibitor of glutathione biosynthesis, brought about a much higher *in vitro* cytotoxicity of melphalan.[72] A number of methods for mitigation of the resistance to melphalan by using melphalan together with (*R*)-BSO as a γ-GCS inhibitor were developed in clinic.[73]

Structure **10**



10 [(*S*,*R*)-BSO]

Due to the joint action of all the above-mentioned factors, the melphalan bioavailability upon oral administration varies from 48% to 94%.[74] Melphalan and sarcolysine are inactivated by nucleophiles (water, thiols, proteins, *etc.*) *in vivo* and, as a result, they have low DNA affinity. Meanwhile, 10%–15% of melphalan is excreted unchanged in urine.[75]

Thus, melphalan and sarcolysine are distinguished by high systemic toxicity, variable bioavailability and instability in aqueous media; they poorly penetrate cell membranes and the blood–brain barrier; and tumour cells soon develop resistance to these agents in various ways. Therefore, the goals of design and production of nano-sized melphalan and sarcolysine delivery systems are to achieve direct delivery of these agents to the tumour tissue, tumour cells and tumour cell nuclei with a simultaneous decrease in the systemic toxicity of the drug; to protect the drugs from hydrolytic decomposition; to overcome the resistance of tumour cells to melphalan and sarcolysine by changing the mechanism of drug transport to the tumour cell and efflux from the cell; to increase the dosing efficiency by developing delivery systems with retarded programmed release of the active agent; and to improve oral bioavailability.

III. Polymeric carriers melphalan and sarcolysine delivery systems

The design of polymeric carriers is an early stage of the development of drug targeting systems. In relation to polymer particles, the mechanisms of uptake of micro- and nano-sized particles by cells, the particle distribution, and passive and active drug delivery to the target cells were investigated for the first time.

In order to increase the therapeutic index of sarcolysine and melphalan, experiments were performed on the synthesis and biological activity assays of their polymeric derivatives, which were either covalent conjugates of sarcolysine (melphalan) with a polymer or water-insoluble polymer particles containing physically adsorbed sarcolysine (melphalan).

The recognition of a drug covalently linked to a polymer (encapsulated in a polymer or sorbed by a polymer) is determined to a larger extent by the chemical structure of the polymer rather than of the free drug, because the drug fragment incorporated in a carrier is substantially screened by the polymer matrix. In the cell, the polymer can interact with various effector molecules and change the expression of multiple drug resistance (MDR) genes, protective mech-

anisms of the cell and apoptosis mechanism.[76] Due to the change in the endocytosis mechanism, polymer–drug conjugates and drugs physically sorbed by a polymer matrix are less sensitive to the functioning of MDR family proteins than the free drug outside the polymer.[77] Despite the listed advantages of polymers as drug carriers and the presence of an extensive bank of biocompatible polymeric compounds, only a few pharmaceutical products based on polymer nanoparticles are currently under clinical trials or are clinically used.[6, 78, 79] For example, in 2007, the US Food and Drug Administration (FDA) approved the drug Mircera for clinical use.[80] This is a nanoparticle composed of methoxypolyethylene glycol–epoetin-β and synthetic erythropoiesis-stimulating factor (ESA). In 2008, the drug Cimzia (certolizumab pegol) for the therapy of rheumatoid arthritis,[81] which is a polyethylene glycol-modified Fab antibody fragment specific to the human tumour necrosis factor TNF-α, was approved.

By now a number of polymeric derivatives of melphalan and sarcolysine have been prepared. They have not found clinical use as yet but they were used to study for the first time micro-sized delivery systems for these agents. Furthermore, covalent polymer conjugates of melphalan can serve as building blocks in the development of biodegradable polymer nanoparticles of a complex structure[82] with programmed drug release in a particular organelle of the target cell. This approach is especially important for melphalan, which is cytotoxic for normal cells. Melphalan must be targeted to the tumour cell nucleus that accommodates the main DNA target.

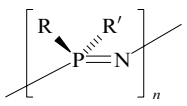
1. Solid polymeric preparations and microparticles containing melphalan

Polymeric preparations were initially designed as a means for prolonged contact action on the tumour with gradual release of the drug, which has been physically adsorbed by a water-insoluble polymer. The polymers (loaded with a free active cytotoxic substance) as films or fibres were injected directly into the tumour tissue. This was followed by microsphere type polymeric products, which were locally injected into the tumour as suspensions.

Polymeric microspheres and microcapsules containing the drug adsorbed by the polymer or encapsulated in the polymer shell are being developed as drug delivery systems (for example[83]). Polymeric microspheres and microcapsules are historic precursors of polymeric nanoparticles. The principles of their manufacture, data on the pharmacokinetics and pharmacodynamics of the drugs encapsulated therein serve as the basis for the development and study of polymeric nanocarriers.

Microspheres based on biocompatible polyphosphazenes containing melphalan were prepared.[84] Unsubstituted polyphosphazenes are inert inorganic polymers containing phosphazo groups non-degradable *in vivo*. For developing locally acting polymeric products, polyphosphazenes **11a–c** containing amino acid ester residues were synthesized.

Structures **11**



11a–c

R = R′ = $NHCH_2CO_2Et$ (**a**); R, R′ = $NHCH_2CO_2Et$, $NHCH(CO_2Et)(CH_2)_2CO_2Et$ (1:1) (**b**); $NHCH_2CO_2Et$, $NHCH(CO_2Et)(CH_2)_2Ph$ (1:1) (**c**)

Melphalan was incorporated into a polymer matrix by mixing polymer and melphalan solutions, the subsequent drying and grinding of the dry residue; the resulting powder was pressed into polymeric tablets to study the kinetics of melphalan release from the polymeric product upon implantation in mice.[85] The rate of melphalan release from the polymeric tablets was measured using a flow system that simulates the conditions of *in vivo* drug release from the polymer. The concentration of melphalan was determined by HPLC; the same method was used to register the formation of mono- and dihydroxymelphalan **9**.

The rate of melphalan release from polymeric tablets was shown to depend to a large extent on the chemical composition of the polymer. The slowest release of melphalan was observed for polymer **11b** (10% and 14% of the loaded amount of melphalan was released after a week at pH 7 and 5, respectively; from polymers **11a** and **11c**, 35% and 42%, and 12% and 14%, respectively, were released under the same conditions). It was shown that melphalan placed in a polymer matrix contacts the water-containing medium to a lesser extent than free melphalan and, hence, it is less hydrolyzed when embedded in the polymer.[85]

The tablet experiments carried out on a flow-through release system demonstrated that the form of the kinetic curve for the melphalan release from polymers **11a**,**b** is affected by both the polymer properties (diffusion coefficient, water penetration rate into the polymer, swelling kinetics and so on) and the presence of pores in the polymer.[86] The rates of melphalan and the melphalan methyl ester (H-Mel-OMe) release are affected by traces of the organic solvent and the initial amino acid esters used to prepare substituted phosphazenes **11a**,**b** remaining in the polymer.

Microparticles for parenteral administration containing melphalan methyl ester or melphalan physically absorbed by the polymer were manufactured based on polyphosphazene **11c**.[84] For the introduction of H-Mel-OMe into the polymer matrix, compound **11c** was dissolved in THF, and a solution of H-Mel-OMe in a methanol–THF mixture was added with stirring. The mixture was kept in an air flow to dryness, and the residue was dried in vacuum and powdered.

Microspheres were prepared by emulsion hardening. A suspension of the dry H-Mel-OMe/polymer powder in dichloromethane was sonicated until solid particles completely dissolved. In a reactor, water was mixed with the emulsifier Tween 80, the mixture was cooled down to 2–4 °C, and a solution of the H-Mel-OMe/polymer powder in CH_2Cl_2 was injected with vigorous stirring *via* an injection needle. The mixture was centrifuged, decanted and rapidly frozen in liquid nitrogen. Freeze drying yielded a microsphere powder. The same procedure was used to prepare melphalan-containing microspheres.

It was shown that by changing the stirring velocity and time, duration of evaporation, the polymer : drug ratio and the volume ratio of the solvents used, it is possible to obtain microspheres of 20 to 100 μm size and to vary their quality (density of the polymer matrix, microparticle surface area, the uniformity of geometric size).

Flow type setup experiments demonstrated that H-Mel-OMe is released from the microspheres during 24 h in higher concentrations than those observed for tablets made of the same polymer and containing the same amount of H-Mel-OMe in proportion to the polymer weight.[86] Thus, polymeric microspheres are more efficient means for

prolonged release of the pharmacologically active component than polymeric implants.

The antitumour activities of the microspheres, polymeric preparations and therapeutic solutions of melphalan and its methyl ester with the same concentration were studied on the model L1210 lymphoid leukaemia of DBA_2 mice. For intraperitoneal administration, a suspension of microspheres in a phosphate-buffered physiological saline solution containing Tween 80 was made. It was shown that the application of polymeric microspheres encapsulating melphalan or its methyl ester induces more pronounced therapeutic effect than the use of polymeric preparations and injections of melphalan or melphalan methyl ester, respectively.[84]

2. Covalent polymer–sarcolysine and polymer–melphalan conjugates

Synthesis of polymer conjugates of cytotoxic antitumour drugs is an important line in the development of targeted drug delivery systems.[87–89] By 2006, sixteen polymer–drug covalent conjugates were accepted for clinical use or were under clinical or preclinical trials of various phases.[88] The first polymer conjugate of the antitumour agent Paclitaxel with polyglutamic acid under the trade name Xyotax appeared in the pharmaceutical market in 2007.[90] Polymer fragments are contained in many nanocarriers,[79,91] in particular, in liposomes, antibodies and their conjugates, viral and non-viral vectors, polymer–drug and polymer–peptide conjugates and so on.[92]

A multifunctional system for drug delivery into a damaged tissue or organ can be obtained based on a biologically compatible polymer molecule (Fig. 1).

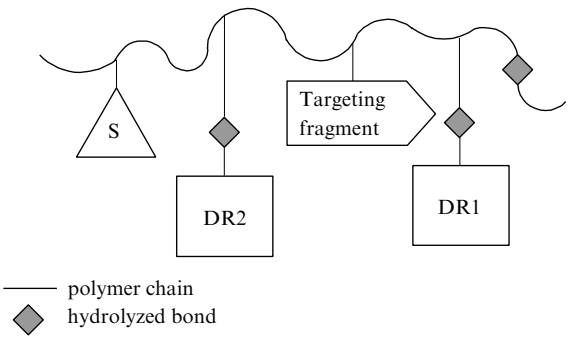


Figure 1. Polymeric prodrug model.
DR1 and DR2 are pharmacologically active agents (drugs), S is the solubilizing group, and targeting fragment is the fragment for targeted drug delivery to the damaged organ or tissue, for example, antibody or specific polypeptide.

Molecules of one or several drugs are attached to the macromolecule of a biodegradable biocompatible polymer *via* hydrolytically labile covalent bond in order to stabilize the drugs and improve the solubility and bioavailability, change the pharmacodynamics and vary the therapeutic effect. A solubilizing group is introduced to impart the desired physicochemical properties, for example, good solubility. One or several targeting groups,[93] for example, an antibody or antibody fragment,[94] are attached for the targeted delivery. After elimination of the pharmacologically active agents, the polymer is either excreted from the body or decomposes to give non-toxic products.

The key views on the mechanisms of delivery and intracellular transformations of polymeric prodrugs have formed under the influence of studies of Ruth Duncan and coworkers.[87,95]

The properties of a polymer–drug conjugate are largely determined by properties of the linker, *i.e.*, the unit that links the polymer and the pharmacologically active agent. The linker must be stable in the blood stream but be hydrolyzed before the conjugate is excreted from the body. The rate constant for the hydrolysis of the linker depends on its chemical nature, the polymer structure and conditions of the medium,[96] *e.g.*, acidity.[97]

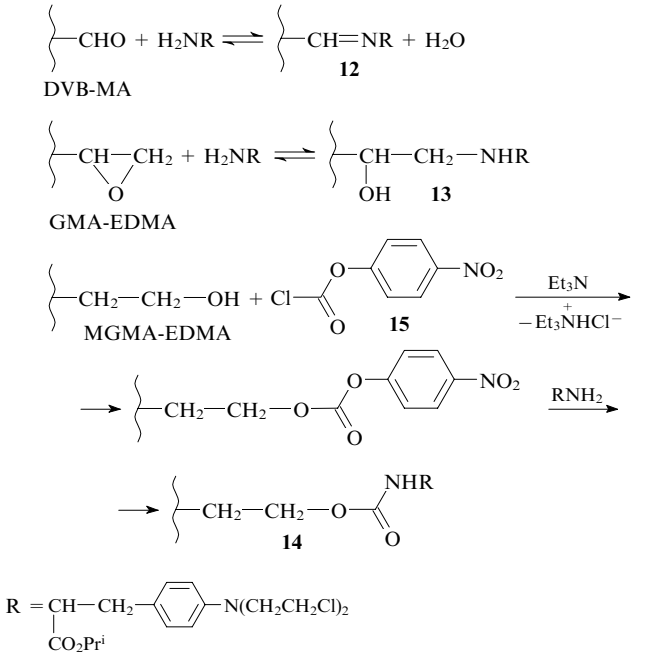
The degree of penetration of the conjugate from the blood vessel into the tumour depends on the polymer molecular mass.[98]

Water-soluble polymers are successfully used to devise polymeric drug delivery systems. Examples are polyethylene glycol (PEG) and its ethers,[99] polymer and copolymers of *N*-(2-hydroxypropyl) methacrylamide (HPMA),[100–103] polyglutamic acid (PGA), lactic and glycolic acid copolymer (PLGA), polylactide (PLA)[82] and other.

Historically, water-insoluble polymeric derivatives of sarcolysine were the first to be synthesized. Covalent attachment of sarcolysine isopropyl ester to functional groups on the polymer surface yielded water-insoluble polymeric derivatives of sarcolysine **12**–**14**.[104]

The study was performed using the cross-linked copolymer of 1,4-divinylbenzene with methacrylic aldehyde (DVB-MA, particle size from 125 to 160 μm), commercially available glycidyl methacrylate copolymer with ethylene dimethacrylate (GMA-EDMA, particle size from 125 to 160 μm) and (2-hydroxyethyl) methacrylate copolymer with ethylene dimethacrylate (MGMA-EDMA, particle size from 80 to 125 μm).[104] The reaction of sarcolysine isopropyl ester with free aldehyde (in DVB-MA) or epoxide (in GMA-EDMA) groups in copolymer molecules gave sarcolysine derivatives **12** and **13** containing an aldimino

Scheme 4



and hydroxyalkyl group, respectively (Scheme 4). Derivative **14** containing an urethane group was synthesized *via* intermediate reaction of the polymer with *p*-nitrophenyl chloroformate (**15**).

The rate of entry of the active component (sarcolysine isopropyl ester) into the blood and in the tumour from the conjugate is determined by the chemical stability of the bond between the polymer fragment and the drug molecule. If the bond between the polymer and the drug molecule is stable against hydrolysis, the pharmacotherapeutic agent is not released in the active form and cannot exhibit its properties *in vivo*. The highest rate of hydrolysis of compounds **12**–**14** was observed in acid media. In the phosphate-buffered (pH 2) dioxane–water (1 : 1) mixture, 65% of the aldimino groups of polymer **12** are hydrolyzed within 1 h to release the drug.[104]

The antitumour activity of compounds **12**–**14** was studied against to transplantable tumours (L1210 leukaemia, P388 leukaemia, Ehrlich ascites carcinoma, NK/Ly lymphoma, intramuscular Lewis lung carcinoma, sarcoma 180) in four lines of mice.[105] Prior to administration of the drugs, their stable suspensions in physiological saline solution were prepared by treating compounds **12**–**14** by emulsifier Tween 80.

A single-shot intraperitoneal administration of compound **12** into mice with L1210 leukaemia in a dose equivalent to 150 mg of sarcolysine per kg of mouse weight resulted in average increase in life span (ILS) by 75.9%. Since sarcolysine is released from the polymer conjugate over a long period of time, the dose used was rather high. The average ILS for mice treated with free sarcolysine (orally, 5.5 mg kg^{-1}) for 5 days was 73.1%.[105] However, the polymeric preparation **12** was introduced as a single dose, while sarcolysine was given for 5 days.

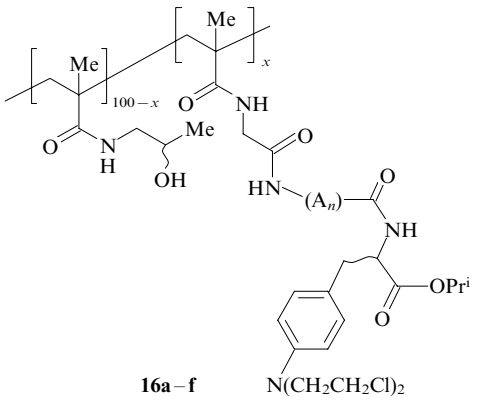
Compound **12** was more active than sarcolysine against the NK/Ly tumour and Ehrlich ascites carcinoma and was equivalent to sarcolysine against the P388 tumour.[105] However, against Lewis lung carcinoma, compound **12** had a lower antitumour activity than sarcolysine. Generally it was found that the attachment of the sarcolysine ester to the polymeric carrier substantially reduces the drug toxicity and, hence, increases the maximum tolerated dose 12–15-fold and increases the half-lethal dose (LD_{50}) 9-fold compared to sarcolysine.[105]

Water-soluble polymeric derivatives of sarcolysine were prepared; this not only changed the quality of *in vivo* application of sarcolysine-bearing polymers but also provided grounds to consider polymer conjugates as systems of targeted sarcolysine delivery to tumour cells. HPMA conjugates containing doxorubicin and melphalan on the same polymer backbone were synthesized in the 1980s.[102] Flanagan working in Duncan's laboratory established the immune-stimulating activity of the melphalan conjugate with the HPMA copolymer.[102] The tumour samples taken from rats with transplanted Walker (W256) carcinosarcoma four days after the introduction of the melphalan conjugate with the HPMA copolymer were found to contain more marcrophages, B-cells and T-cells than the tumour tissues of non-treated animals or animals treated with melphalan.

In some works, sarcolysine was not attached directly to free functional groups of the polymer but was connected to the polymer *via* a linker, for example, a short peptide. The attachment of drugs or their derivatives to the polymeric carrier *via* a peptide linker ensures the controlled drug release in cell lysosomes where peptide hydrolysis takes place.

Conjugates of sarcolysine with HPMA copolymer — methacryloyl glycine dipeptides (**16a**–**f** and **17a**,**b**) in which sarcolysine was attached to the polymer *via* a peptide linker was first synthesized by Duncan and co-workers.[106]

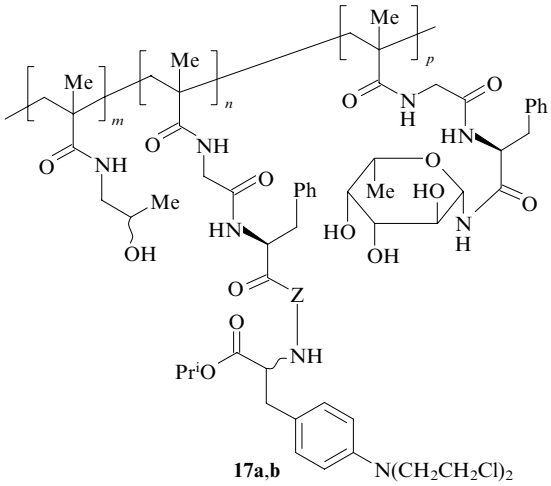
Structures **16**



16a–**f**

$NH(A_n)C(O)$ = Leu-Gly (**a**), Leu-Leu-Gly (**b**), Phe-Gly (**c**), Phe-Leu-Gly (**d**), Leu-Ala(SO_2NH_2) (**e**), Phe-Ala(SO_2NH_2) (**f**)

Structures **17**



17a,**b**

Z = CH_2 (**a**), $CHBu^iC(O)NHCH_2$ (**b**)

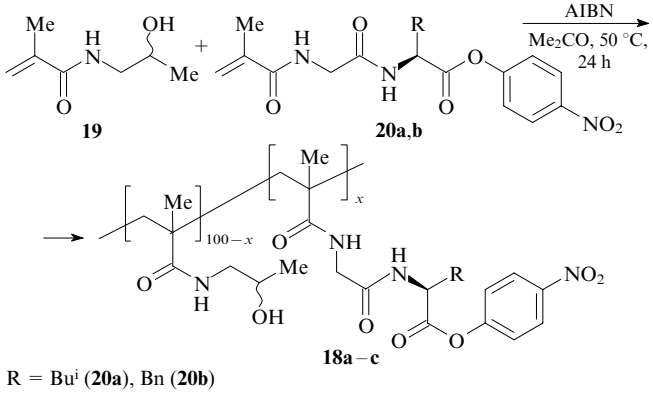
The initial copolymers **18a**–**c** were prepared by azobis(isobutyronitrile) (AIBN)-initiated radical polymerization of *N*-(2-hydroxypropyl) methacrylamide (**19**) and *p*-nitrophenyl esters of *N*-methacryloyl dipeptides **20a**,**b**, which were synthesized by a method reported earlier [107] (Scheme 5).

The initial sarcolysine di- and tripeptides **21a**–**f** were synthesized from sarcolysine isopropyl ester and appropriate amino acids or dipeptides by the dicyclohexylcarbodiimide (DCC) method (Scheme 6).

Compounds **16a**–**f** were prepared by aminolysis of polymeric precursors **18a**,**b** containing *p*-nitrophenyl groups with sarcolysine di- and tripeptide **21b**,**d**,**f**.

Polymers **17a**,**b** were synthesized by aminolysis of polymeric precursor **18c** with fucosylamine followed by addition of short peptides **21b** or **21d**, respectively, in DMSO. Fucosylamine was introduced into the molecule as the

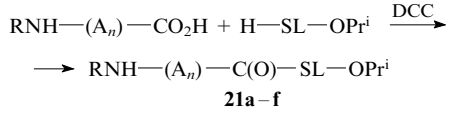
Scheme 5



R = Bu^i (**20a**), Bn (**20b**)

Compound **18**	R	x (%)	M_w
a	Bu^i	6.99	32 000
b	Bn	7.70	36 000
c	Bn	9.78	25 000

Scheme 6



Compound **21**	R	NH(A$_n$)C(O)	Compound **21**	R	NH(A$_n$)C(O)
a	Boc	Gly	**d**	HCl·H	Leu-Gly
b	HCl·H	Gly	**e**	Cbz	Ala(SO$_2$NH$_2$)
c	Boc	Leu-Gly	**f**	H	Ala(SO$_2$NH$_2$)

Boc is *tert*-butoxycarbonyl, Cbz is benzyloxycarbonyl

targeting fragment, because the L1210 cells are known to have a membrane receptor that recognizes fucose residues.

Studies of the *in vitro* hydrolysis of polymeric prodrugs **16a–f** under the action of tritosomes (a mixture of lysosomal enzymes from the rat liver) or cathepsin B, a lysosomal enzyme, demonstrated that with both enzymes, the highest rate of sarcolysine release was observed from polymeric derivatives with tetrapeptide linkers **16b** and **16d**. Under the action of the above enzymes, the major portion of the sarcolysine ester was isolated in the active form, *i.e.*, with the retained di(2- chloroethyl)amino group.[106]

As expected, human L1210 leukaemia cells were less sensitive *in vitro* to polymer conjugates **16** and **17** than to sarcolysine.[106]

The reported method[106] was used to synthesize HPMA copolymers containing tyrosine amide and melphalan or sarcolysine isopropyl ester residues (1 mass % – 10 mass %) attached to the polymer chain *via* the peptide side chain (compounds **22a–h**, **23a–e**).[108]

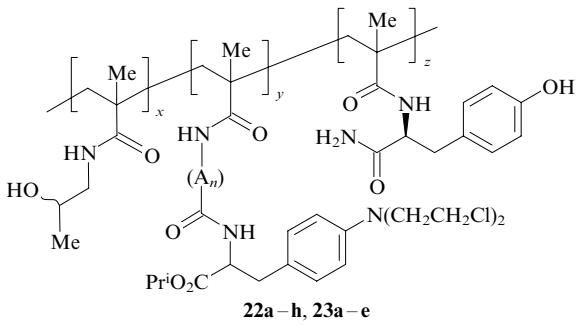
The amino acid composition and the length of the peptide linker determine the rate of enzymatic elimination of melphalan from the polymer. Compounds **22d** and **22c** with pentapeptide and tetrapeptide linkers, respectively, underwent faster enzymatic hydrolysis *in vitro* to give melphalan than polymer **22b** with the tripeptide linker. Although the rate of melphalan release from the polymer varied depending on the enzyme used for hydrolysis, the rate of cleavage of the linker between melphalan and the polymeric carrier decreased in all cases in the series **22c** ⩾ **22d** > **23a** > **22b** > **22a** (not hydrolyzed).[108] Evidently, as the length of the linker increases, the peptide bond being hydrolyzed becomes spatially more accessible for the enzyme.

It was shown in animal experiments,[108] that the ^{125}I-labelled polymer conjugate P-Gly-Phe-Gly-Mel-OPr^i (**22f**, P is polymer) has much slower clearance from plasma, slow excretion and much higher concentration in the tumour than melphalan.

The polymeric derivatives of melphalan may be aggregated in solution, depending on the number of melphalan moieties attached to the polymeric molecule and on the concentration of the polymer conjugate in solution. The aggregation affects the pharmacokinetics of polymeric prodrugs and the action mechanism *in vivo*. The results of *in vitro* experiments indicate that polymers containing about 10 mass % of melphalan are less active against the Walker sarcoma than polymers with lower melphalan contents.[108]

It was found that polymers that are faster disintegrated *in vitro* are also distinguished by the highest antitumour activity *in vivo*.[108] The HPMA conjugates containing non-destroyable stable linkers do not show antitumour activity *in vivo*.[92]

Structures **22**, **23**



z = 1 mol.%

Compound **22**	NH(A$_n$)C(O)	Drug
a	Gly	Mel
b	Gly-Gly	Mel
c	Gly-Leu-Gly	Mel
d	Gly-Phe-Leu-Gly	Mel
e	Gly	SL
f	Gly-Phe-Gly	SL
g	Gly-Leu-Gly	SL
h	Gly-Phe-Leu-Gly	SL

Compounds **23a–e**: NH(A$_n$)C(O) = Gly-Phe-Gly, the drug is melphalan; Mel-OPr^i : TyrNhH$_2$ (mol.): 6,1 (**a**), 0.6 (**b**), 1.5 (**c**), 2.4 (**d**), 5.1 (**e**)

IV. Dendrimers as melphalan carriers

Dendrimers are branched polymeric macromolecules with a size of up to 10 nm characterized by high density of terminal functional groups on the surface and compact globular structure. They have a number of advantages over other nano-sized delivery systems: low polydispersity index, a multitude of attachment sites for the drug molecule

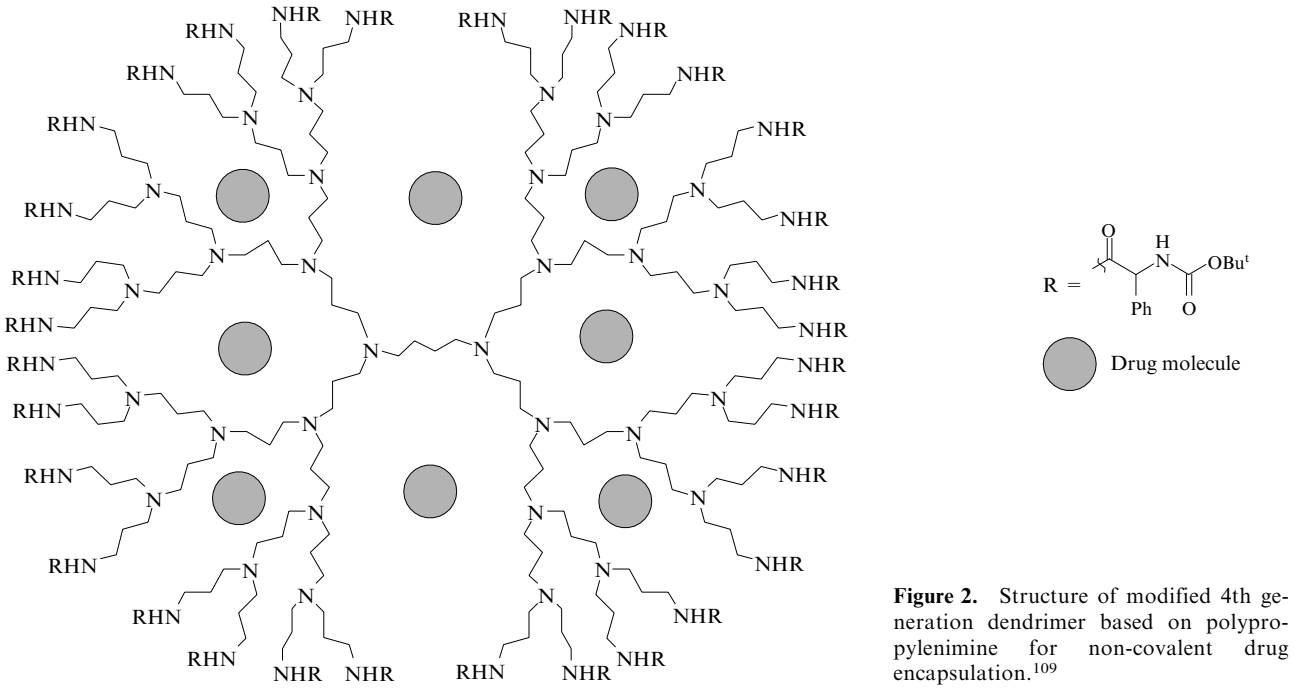


Figure 2. Structure of modified 4th generation dendrimer based on polypropylenimine for non-covalent drug encapsulation.[109]

and for marker, solubilizing and other units, and strictly controlled chemical structure and globule size.[109] Several products based on dendrimers are under clinical trials and some have been already registered as pharmaceuticals.[110] Dendrimers are characterized by the number of branching points from the central core (or the number of layers) called dendrimer generation (G*n* is the *n*th generation dendrimer). Dendrimers based on biocompatible monomers decompose *in vivo* as a result of a single initiating reaction to give numerous molecules of the pharmacologically active agent.[111] In the design of dendrimers for the diagnosis and therapy of cancer, considerable advances have been made during the last 5 years.[112] Dendrimer carriers were prepared for doxorubicin, paclitaxel, methotrexate, campotecan and other antitumour agents; in *in vivo* and *in vitro* experiments, they exhibited much higher antitumour activity than the corresponding free drugs.[112] The dendrimer based on L-lysine with disodium 2-(3,6-disulfo-1-naphthyloxy)acetate residues as the drug — microbicide SPL7013 — is under clinical trials.[113] A broad range of activities against human immunodeficiency virus was revealed for the SPL7013 agent (the dosage form is VivaGel) active against HIV-1 and HSV-2 viruses.[114]

A drug can be either covalently attached to the functional groups on the dendrimer surface or encapsulated in the dendrimer inner cavities (Fig. 2).[109]

By covalent attachment of a drug molecule to a dendrimer with an appropriate selection of the linker, it is possible to obtain a system in which the drug is released in a type of chain reaction: the enzymatic hydrolysis of the bond with the trigger group initiates a sequence of transformations involving self-immolative linkers (SIL), destruction of the dendrimer and release of a multitude of the drug molecules. The trigger group can be located in any part of the dendrimer (either inside or on the surface) and should easily split-off under physiological conditions.[115]

The spontaneous self-immolation is observed, in particular, for polysubstituted aromatic linkers, for example, benzyl alcohol derivatives containing electron-donating substituent (NH_2 or OH group) in the *ortho*- or *para*-position relative to the benzyl CH_2 group. The self-immolation of this linker occurs as a dearomatization reaction with an electron pair transfer. The electron-donating substituent is necessary for decreasing the energy barrier for this process. For example, an *in vitro* experiment was performed to study the release of taxol upon enzymatic hydrolysis of polymer conjugate **24** containing a branched AB_3 type linker based on 2,4,6-trihydroxymethylaniline attached to the polymer *via* the short Gly-Phe-Leu-Gly-Phe-Lys peptide chain and *p*-aminobenzyl alcohol as the SIL (Scheme 7).[115]

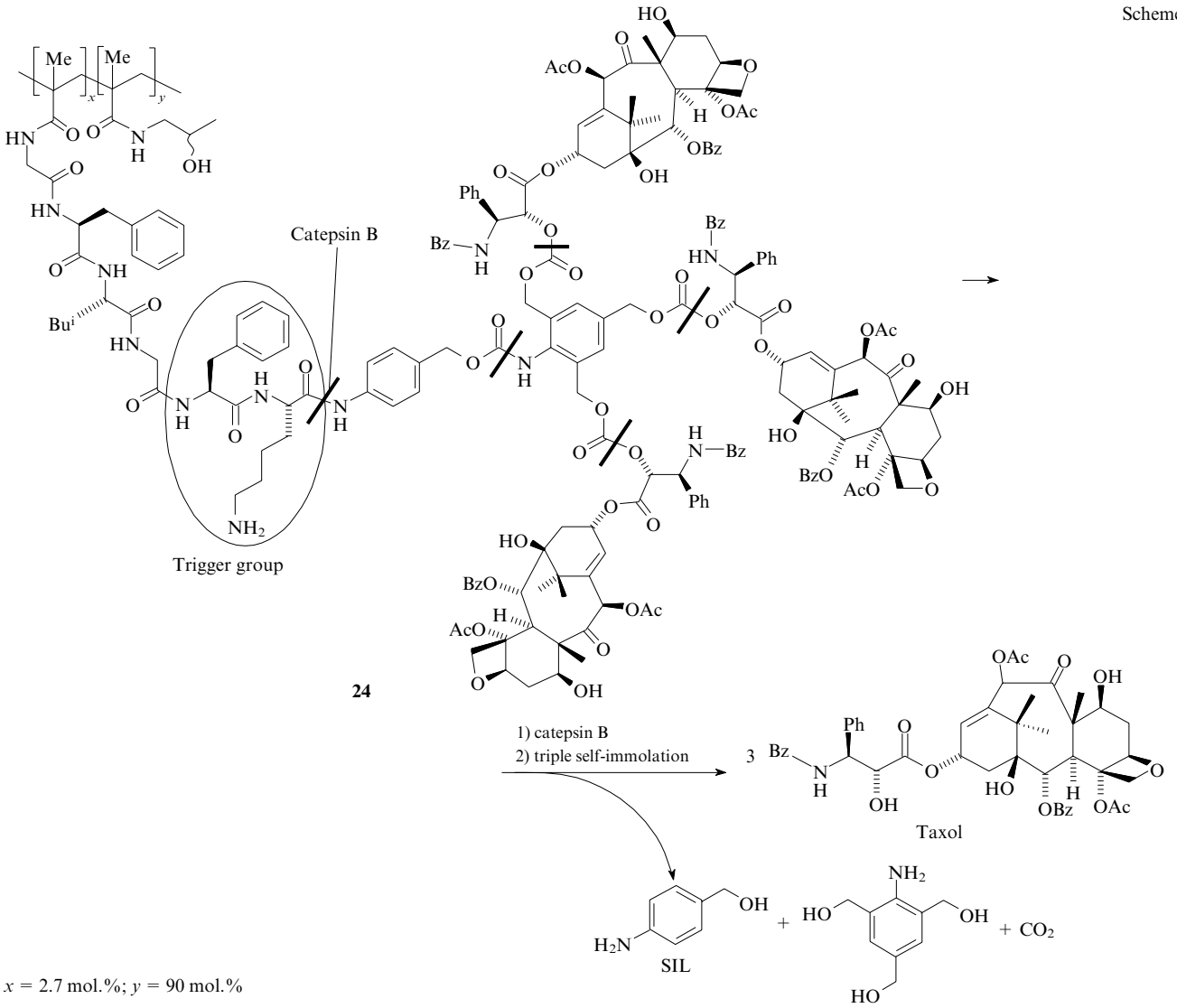
It was shown that the introduction of a linear SIL (for example, *p*-aminobenzyl alcohol) into the structure of the polymer–drug conjugate for increasing the distance between the polymer and parts of the drug molecule (in order to improve the accessibility of the bond to be hydrolyzed for the enzyme) leads to a substantial increase in the rate of catepsin B-induced hydrolysis of polymer conjugates. Dendrimer **24** showed a much higher antitumour activity than the corresponding polymer–drug linear conjugates or compounds containing no SIL.[115]

The destruction of the dendrimer brings about exponential increase in the concentration of the active agent with time. Dendrimers have considerable advantages over derivatives that contain one pharmacologically active fragment provided that the enzyme that initiates the sequence of transformations is present in the tumour in a relatively small quantity.

The outer functional groups of the dendrimer can bear, apart from pharmacologically active agents, targeting fragments, diagnostic (for example, visualizing) elements or biomarkers for evaluating the efficiency of therapy.[116]

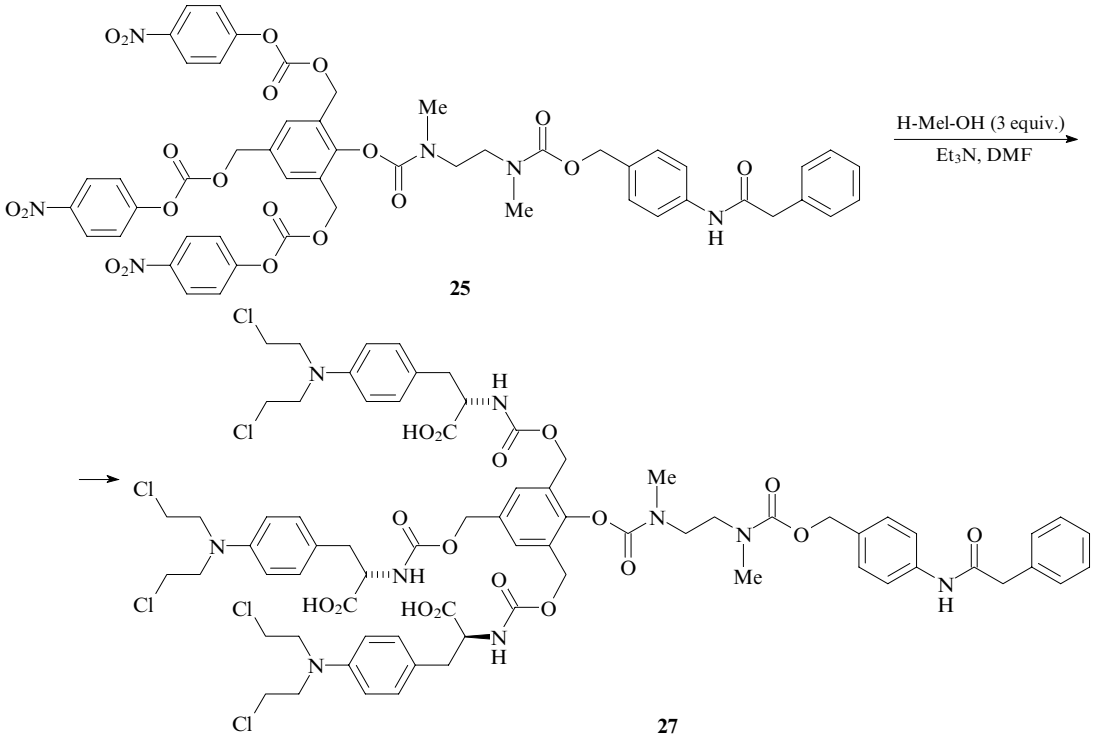
Precursors **25** and **26** were used to synthesize branched AB_3 type molecules **27** and **28** with covalently attached melphalan (Schemes 8 and 9).[117,118] Tricarbonate **25** was

Scheme 7

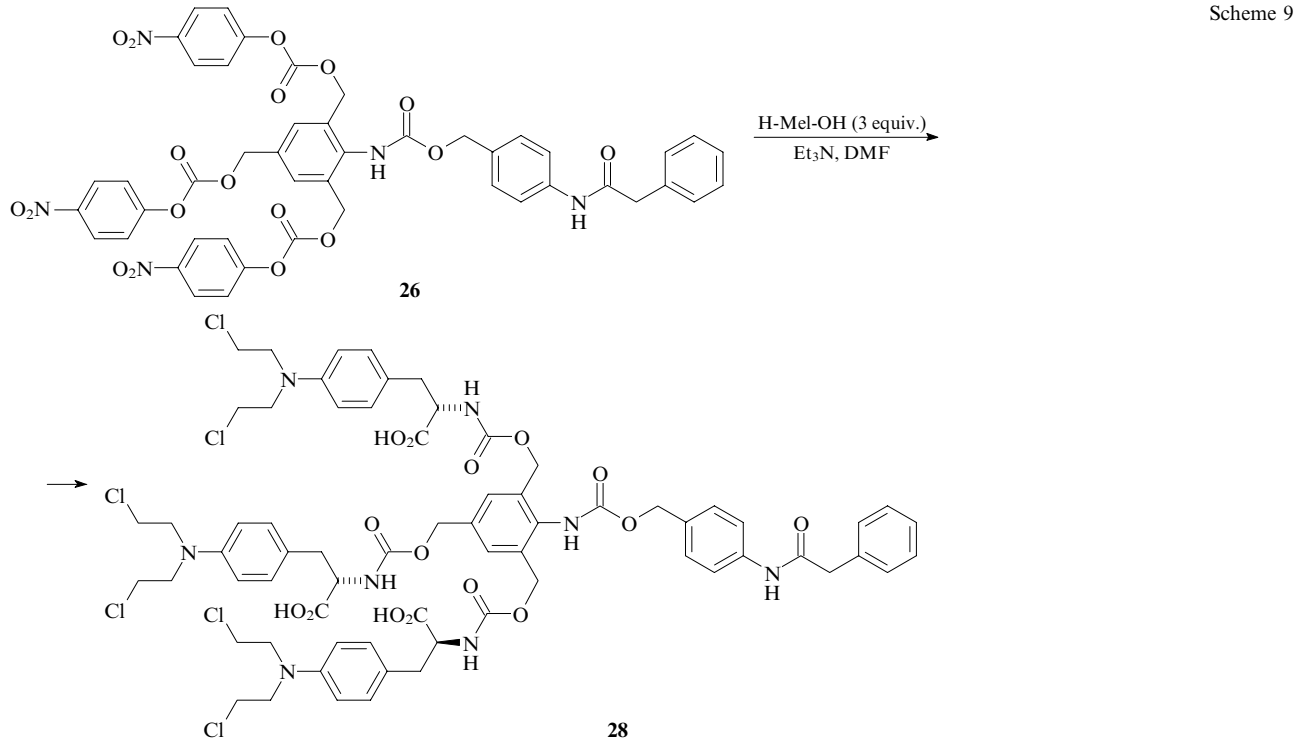


x = 2.7 mol.%; y = 90 mol.%

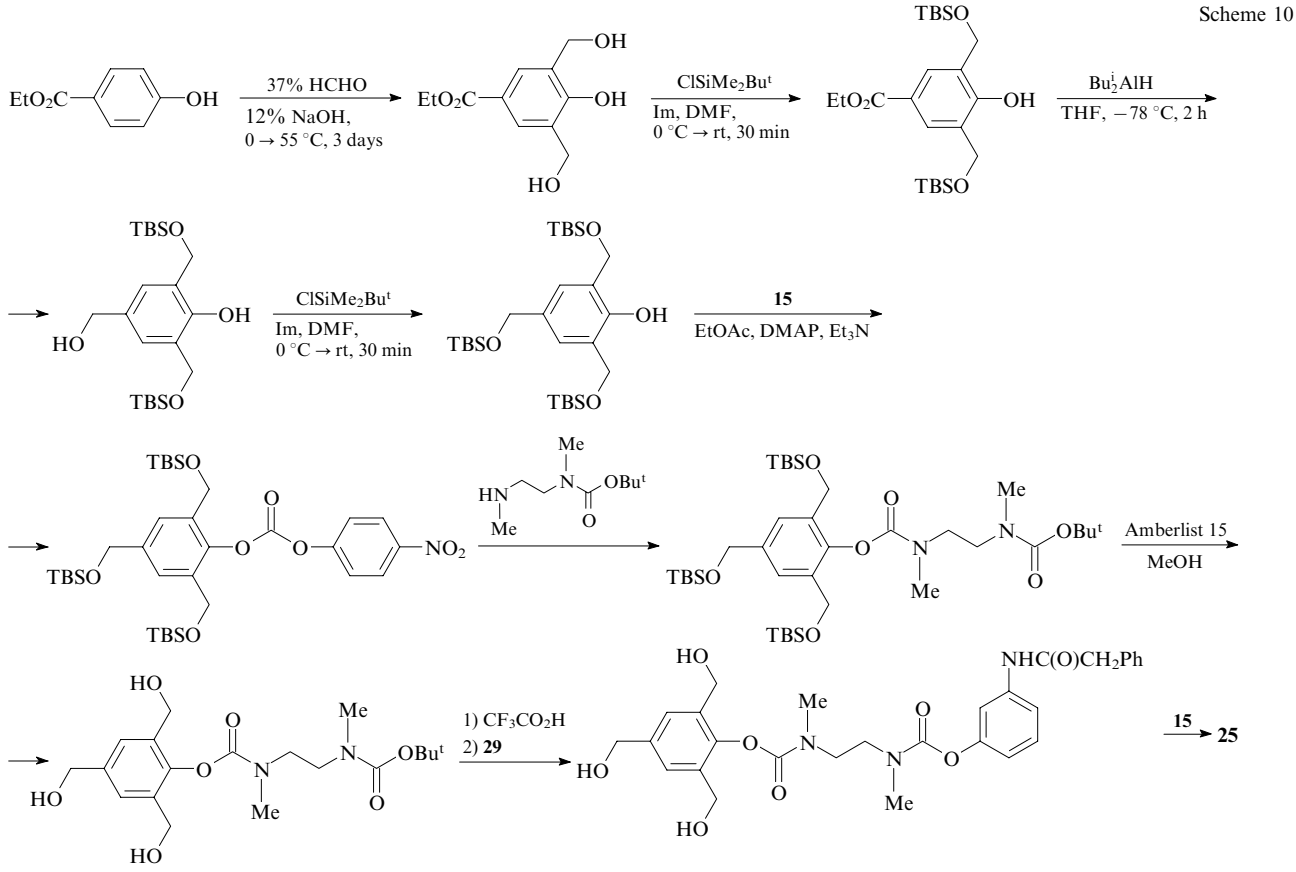
Scheme 8



Scheme 9



Scheme 10

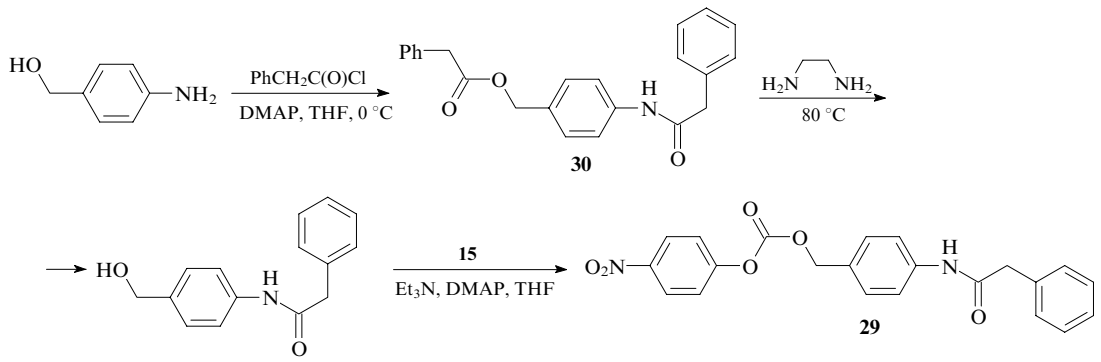


Im is imidazole, TBS = Bu^tMe_2Si, DMAP is 4-(dimethylamino)pyridine

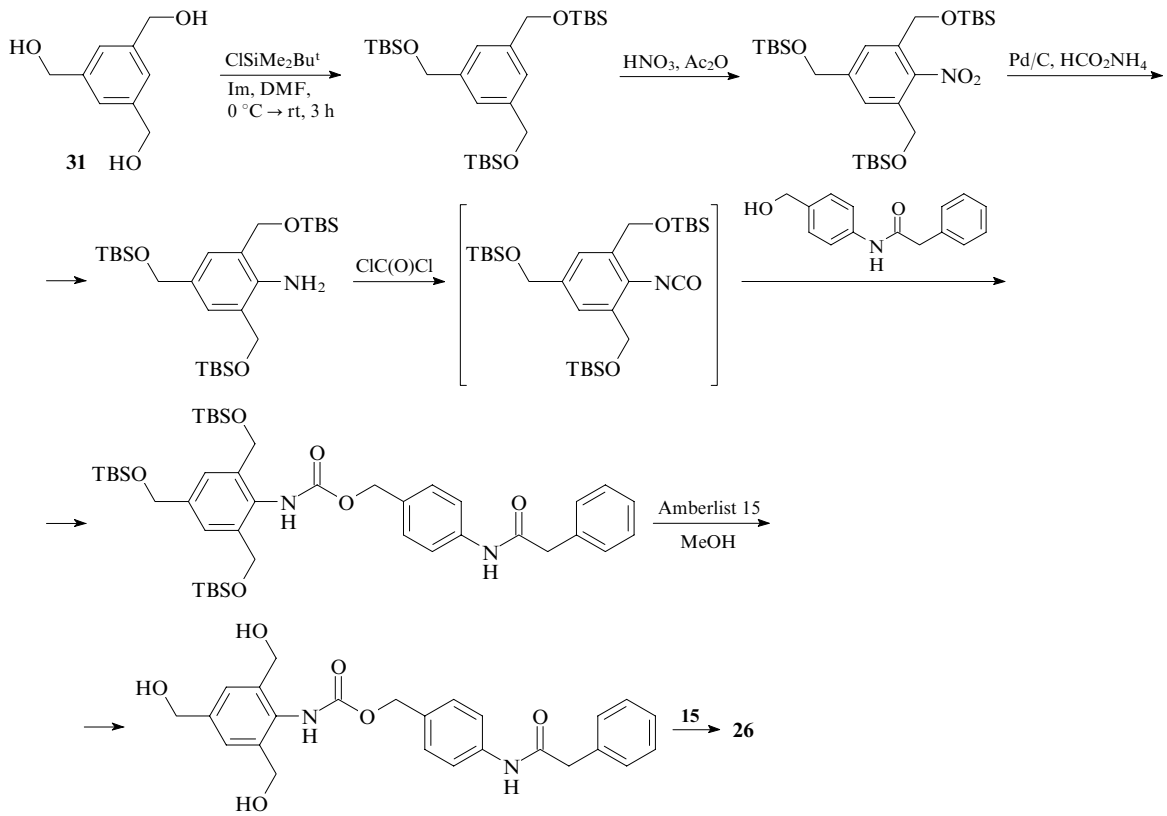
obtained from ethyl 4-hydroxybenzoate in 10 steps (Scheme 10).

Carbonate **29** serving as a linker was prepared by, first, conducting the acylation of 4-aminobenzyl alcohol with phenylacetyl chloride in the presence of DMAP to give 4-aminobenzyl alcohol **30**.[119] Compound **30** was heated with ethylenediamine to give 4-(*N*-phenacetylamino)benzyl

Scheme 11



Scheme 12



alcohol, which reacted with *p*-nitrophenyl chloroformate (**15**) to give compound **29** (Scheme 11).

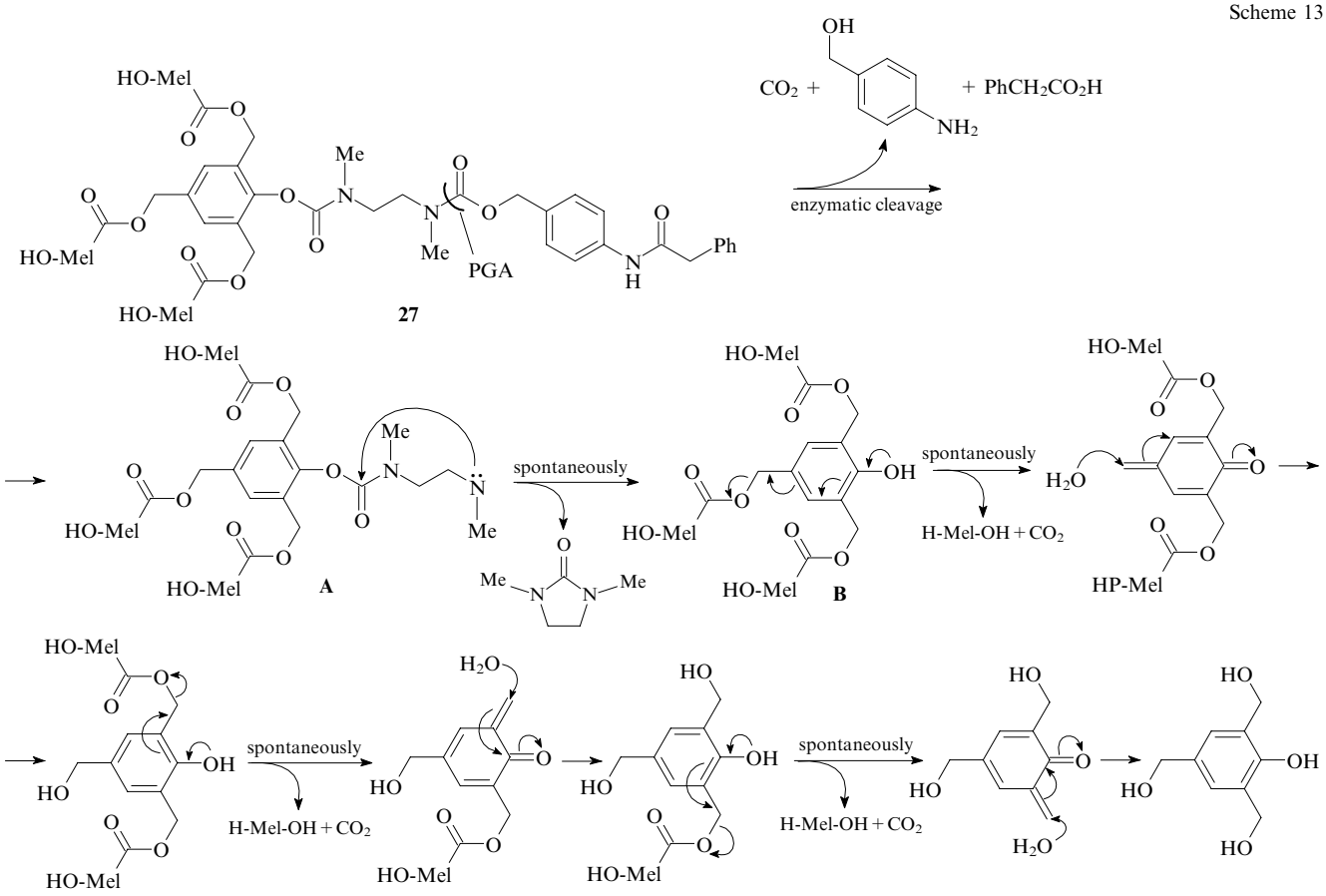
Compound **26** was prepared from 1,3,5-tri(hydroxymethyl)benzene (**31**) in seven steps (Scheme 12).[117]

The antitumour activities of compounds **26** and **27** were determined in the acute lymphocytic leukaemia (MOLT-3) cell line in the presence and in the absence of penicillin G-amidase (PGA) in a concentration of 1 μmol litre^{-1}. In the absence of PGA, which promotes decomposition of compounds **26** and **27**, both prodrugs are considerably less active against MOLT-3 cells than melphalan.[118] In the presence of PGA, the half-maximal inhibitory concentration (IC_{50}) decreases 17-fold for compound **26** and 200-fold for compound **27** as compared with the value observed without the enzyme. In the presence of PGA, compound **27** is almost as active against MOLT-3 as melphalan and has the properties of a melphalan prodrug, *i.e.*, a compound that is not active in the blood flow and decomposes to give melphalan in enhanced PGA expression zones.

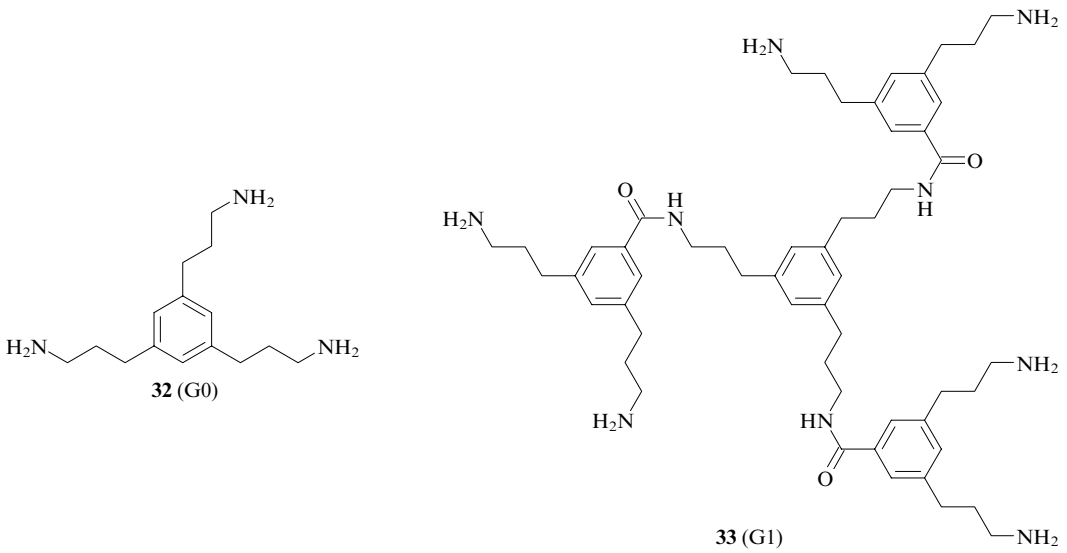
The mechanism of decomposition of compounds **26** and **27** was described.[118] The process starts with elimination of phenylacetic acid catalyzed by enzymes, elimination of azaquinomethide and decarboxylation. This gives intermediate **A**, which undergoes intramolrecular cyclization to split off a dimethylurea derivative. Phenol **B** thus formed eliminates three melphalan residues one by one (Scheme 13). The rate-limiting step is cyclization of amine **A**. For accelerating the drug release, compound **27**, which decomposes without the cyclization step, was prepared.

Compounds **26** and **27** are poorly soluble in water; therefore, no encouraging results for the application of these compounds *in vivo* are available to date. However, the mere fact that they have been synthesized is significant. They can serve as starting compounds to prepare dendrimer globules as melphalan nanocarriers with strictly determined surface architecture.

Scheme 13



Structures **32**, **33**

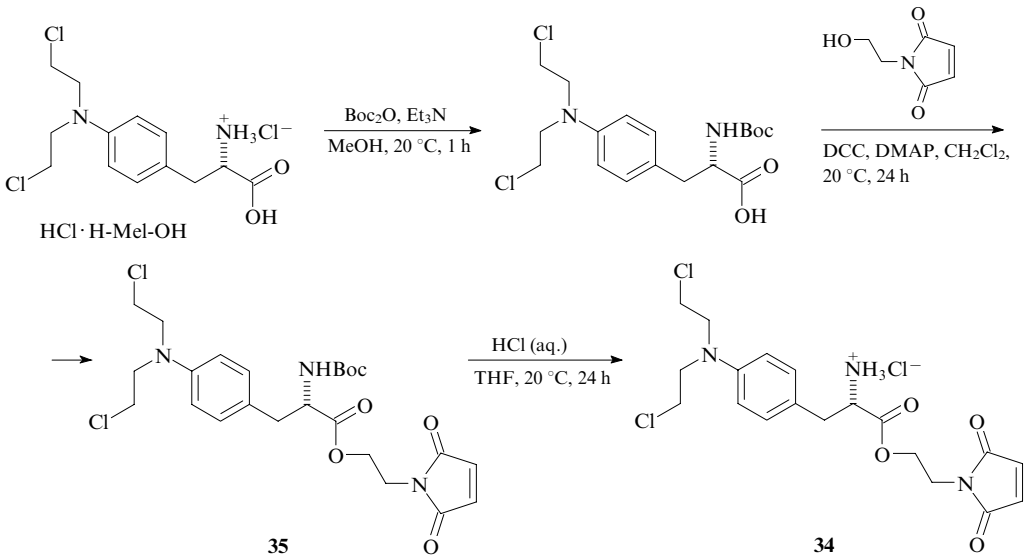


Melphalan was attached, either directly or *via* a cleavable maleimide spacer, to dendrimers, 1,3,5-tris(3-aminopropyl)benzene (**32**) and 3,5-bis(3-aminopropyl)-*N*-(3-{3,5-bis[3-{3,5-bis(3-aminopropyl)benzoylamino}propyl]phenyl}propyl)benzamide (**33**).[120] These two dendrimers showed no cytotoxic activity in the *in vitro* measurement of the antitumour activity in human MCF-7 and MDA-MB-231 cell lines. Schemes 14–16 show the synthesis of melphalan ester **34** (*via* Boc derivative **35**) to be attached to dendrimer molecules through a spacer and the synthesis of dendrimer–melphalan conjugates **36** and **37** *via* Boc-protected precursors **38** and **39**, respectively.

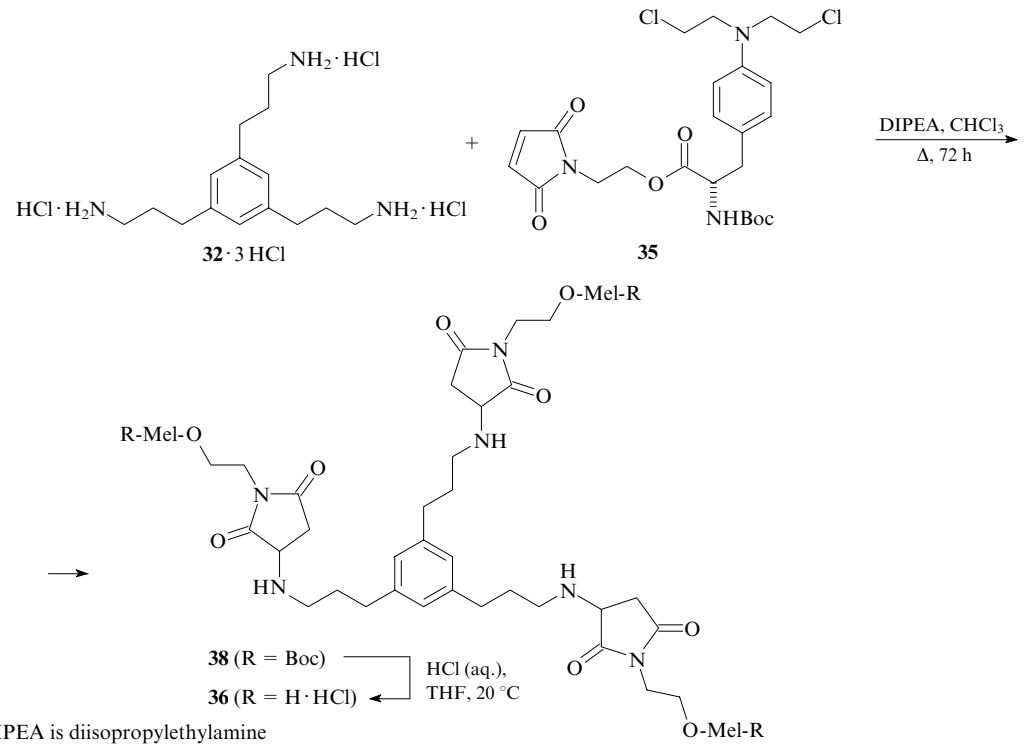
The protective groups of compounds **38** and **39** were removed by treating a THF solution of these compounds with aqueous HCl.

None of compounds **36**–**39** exhibited antiproliferative activity that would exceed this activity of melphalan in any cell line. This might be related to insufficient rate of *in vivo* hydrolysis of the linker in **36**–**39** and/or to the presence of Boc groups that block the melphalan amino group in

Scheme 14



Scheme 15

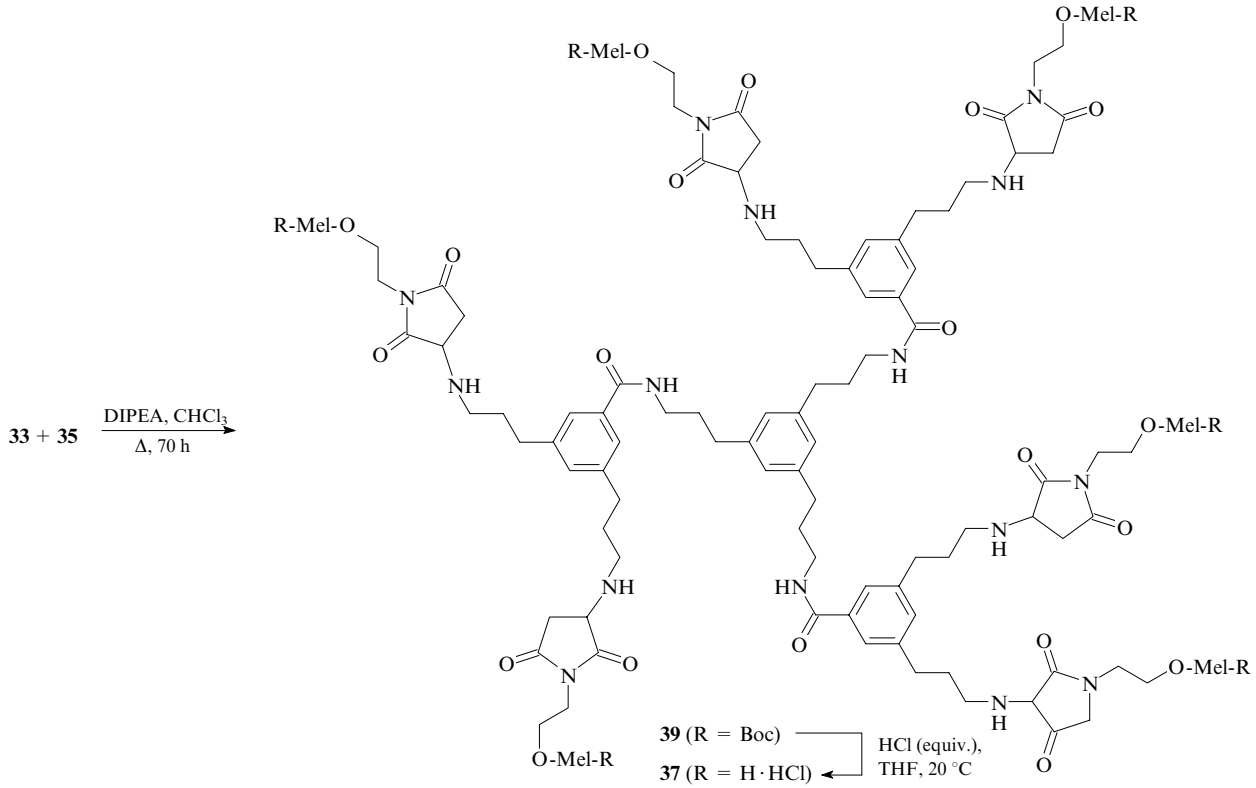


DIPEA is diisopropylethylamine

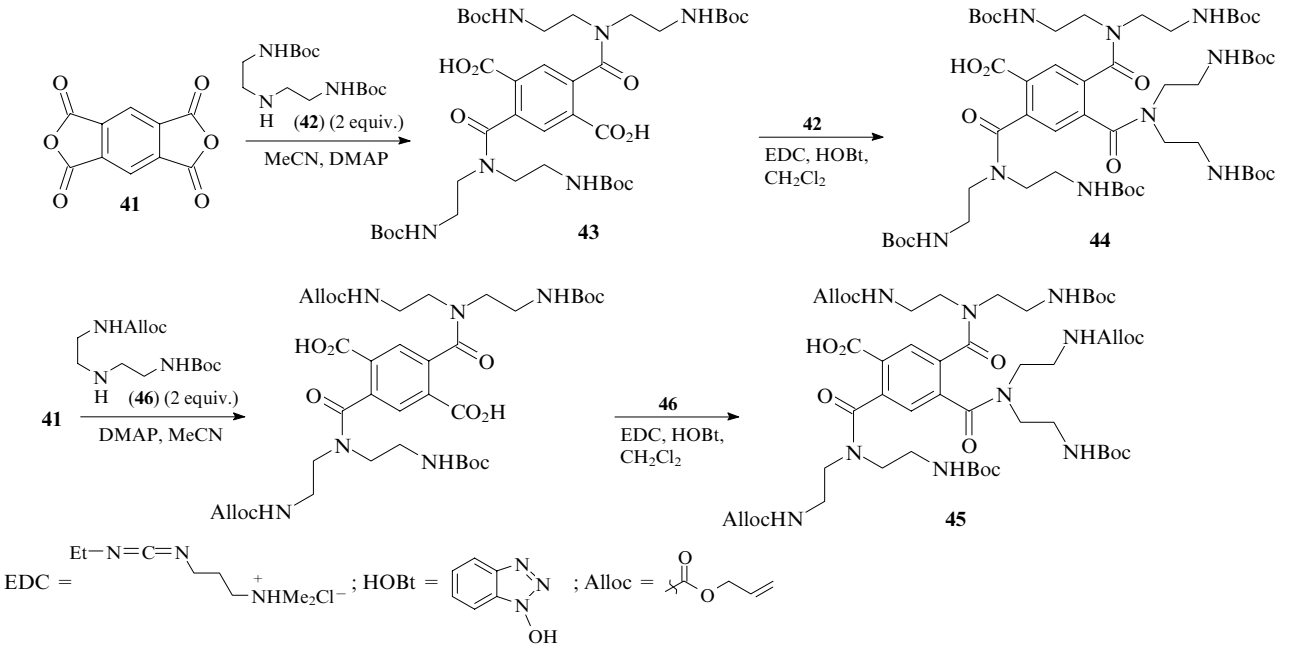
compounds **38** and **39**. However, it is quite probable that these melphalan delivery systems may provide advantages *in vivo* by improving biodistribution and accumulation in the tumour.

A method for the preparation of first generation dendrimers is the reaction of a low-molecular-mass core containing at least two active functional groups (two sites for attachment) with a monomer unit bearing three sites of attachment. Every monomer unit added provides at least two attachment sites for subsequent chemical modification. This principle was used in the development of multifunctional platforms for transport of several pharmacologically active agents to synthesize dendrimers containing, apart from the melphalan residue, doxorubicin and etoposide moieties.[116] The 36-valent dendrimer **40** for the transport of two pharmacologically active agents (18 fragments of each) was prepared by solid-state organic synthesis techniques (Schemes 17 and 18). The reaction of pyromellitic dianhydride (**41**) with a twofold excess of di(*tert*-butoxycarbonyl)-*N*-(2-aminoethyl)ethane-1,2-diamine (**42**) followed by the reaction of the resulting 1,4-bis[di(*N*-*tert*-butoxycarbonyl)aminoethyl]amide of pyromellitic acid (**43**) with an equimolar amount of the same reagent gave rise to the hexa(*tert*-butoxycarbonyl) derivative, 1,2,4-tris[di(*N*-*tert*-butoxycarbonyl)aminoethyl]amide of pyromellitic acid (**44**). Compound **45** for the assembly of a dendrimer was obtained in a similar way using *N*-allyloxycarbonyl-*N*′-*tert*-butoxycarbonyl-*N*″-(2-aminoethyl)ethane-1,2-diamine (**46**).

Scheme 16

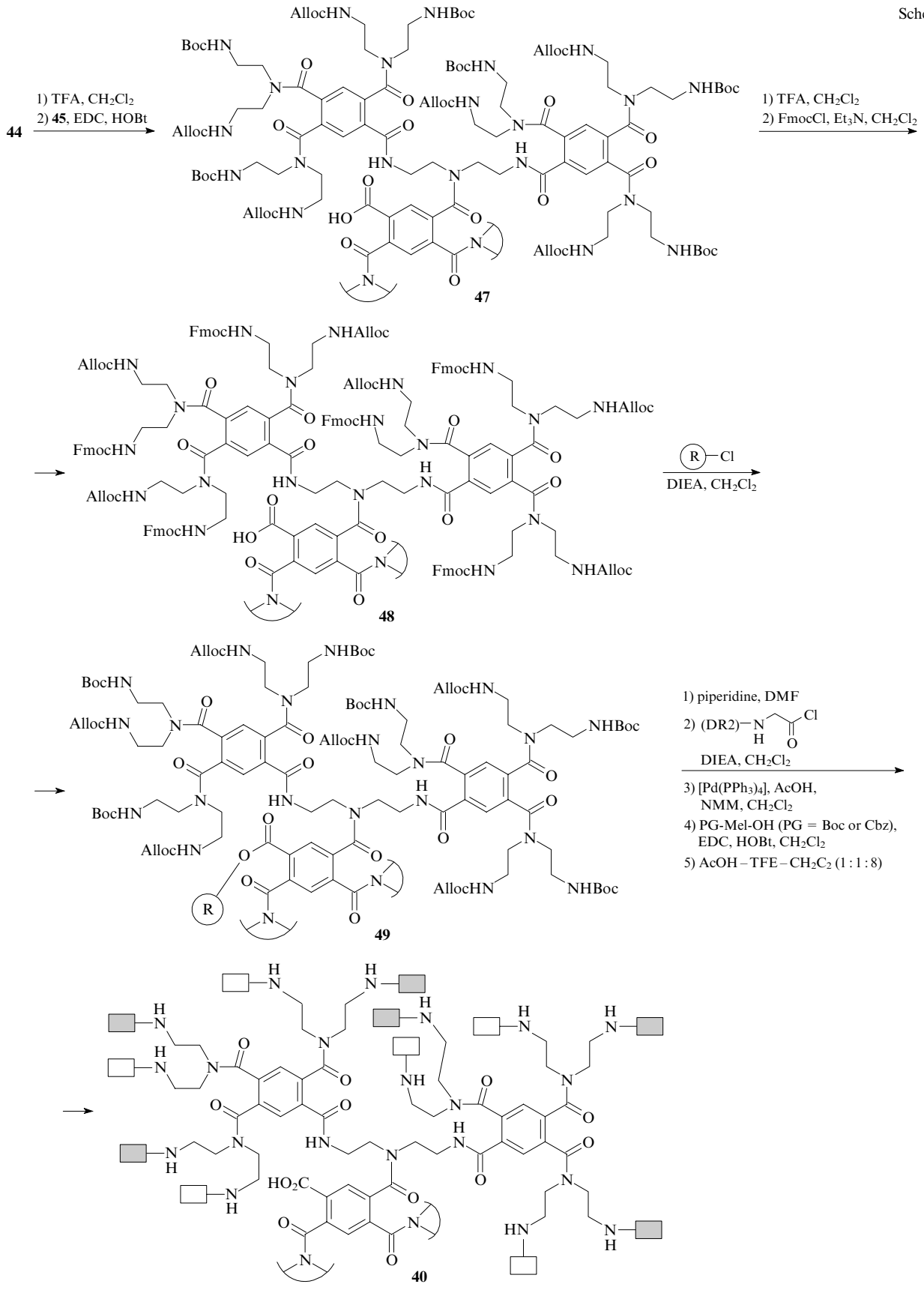


Scheme 17



The removal of all Boc groups of compound **44** and the subsequent reaction with compound **45** furnished dendrimer **47** (Scheme 18 shows the structure of one of the three dendrons), which was converted to 36-valent dendrimer **48** by removing Boc groups and introducing Fmoc protecting groups. Compound **48** was supported on a solid material (trityl chloride polymer, R–Cl). This gave compound **49**, which was then loaded successively by pre-activated drugs through the formation of amide bonds or urea fragments. After being detached from the polymer, dendrimer **40** was grafted to another support. The patent cited[116] does not specify the type of support and does not give the biological activity data for the synthesized compounds.

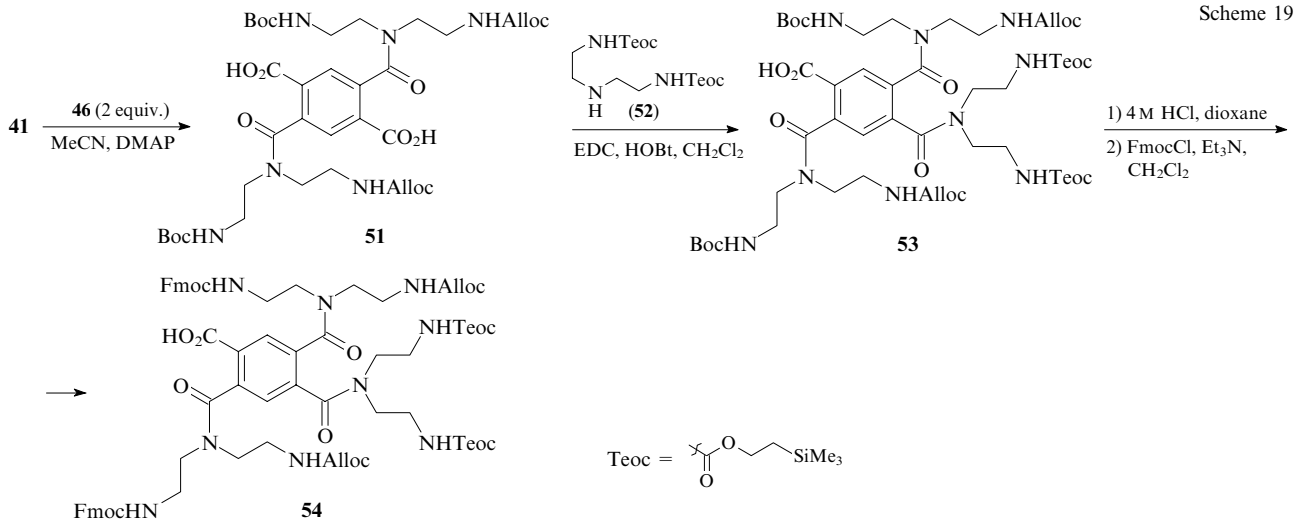
Scheme 18



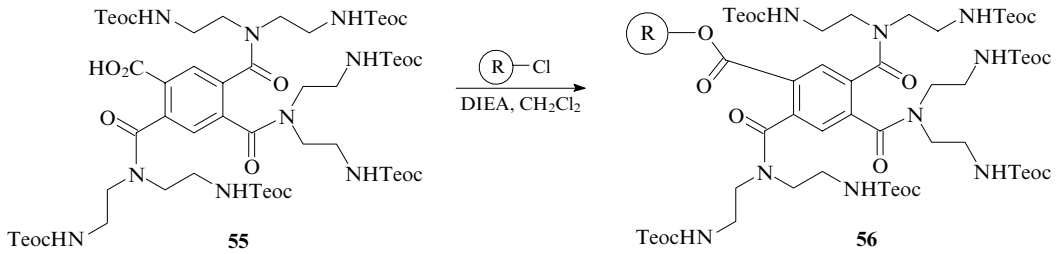
R—Cl = PS—(Ph, Ph, Cl) (PS is polystyrene); here and in Scheme 21 = O—C(=O)—N(H)—(DR2) (DR2 is doxorubicin), = Mel-Boc(Cbz);

NMM is *N*-methylmorpholine, TFA = CF_3CO_2H, TFE = CF_3CH_2OH

Scheme 19



Scheme 20



Also, 36-valent dendrimer **50** for loading with three pharmacologically active agents was synthesized (Schemes 19–21).[116] This dendrimer with three types of N-protecting groups was obtained starting from pyromellitic anhydride, which regioselectively reacted with substituted ethane-1,2-diamine **46** to give a mixture of isomers; the mixture was separated by flash chromatography. The reaction of isomer **51** with di-*N*-Teoc-*N*-(2-aminoethyl)-ethane-1,2-diamine **52** afforded compound **53** with three different *N*-protecting groups. After replacement of the Boc group by the Fmoc group with retention of the other protecting groups, structural unit **54** was synthesized.

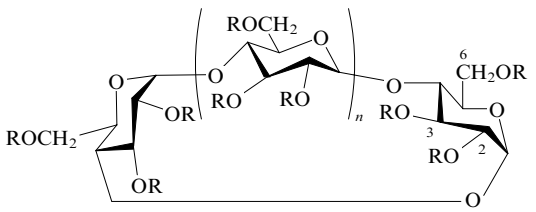
The structural block **55** synthesized similarly to compound **44** was linked to the Cl-trityl polymer *via* a COOH group to give compound **56** (Scheme 20). Then all Teoc groups of compound **56** were removed by treatment with TBAF in THF or with KF in DMF. The product was linked by a standard method to the base, compound **54** (Scheme 21). This yielded compound **57**, a branched platform on the polymeric support containing 36 amino groups protected by different groups (Boc, Alloc and Teoc). The removal of Boc groups and subsequent reaction with *N*-Boc(*N*-Cbz)-protected melphalan gave compound **58**. The removal of the Alloc group, the reaction of the free NH_2 groups of the resulting amine with *p*-nitrophenyl chloroformate and the subsequent condensation with doxorubicin (DR2) resulted in compound **59**.

The subsequent removal of the Teoc groups and condensation with etoposide *p*-nitrophenyl carbonate (DR3) to give the carbamate bond produced compound **50** (see Scheme 21). The detachment from the polymer afforded a dendrimer with 36 residues of three pharmacologically active agents (12 residues of each); the dendrimer can be linked to targeted carriers through the CO_2H group. The *in vitro* kinetics of release of these drugs during hydrolysis of compound **50** was studied. Unfortunately, the results of this study are not presented in the patent, nor the data on the biological activities of the synthesized compounds.

V. Melphalan inclusion complexes with cyclodextrins

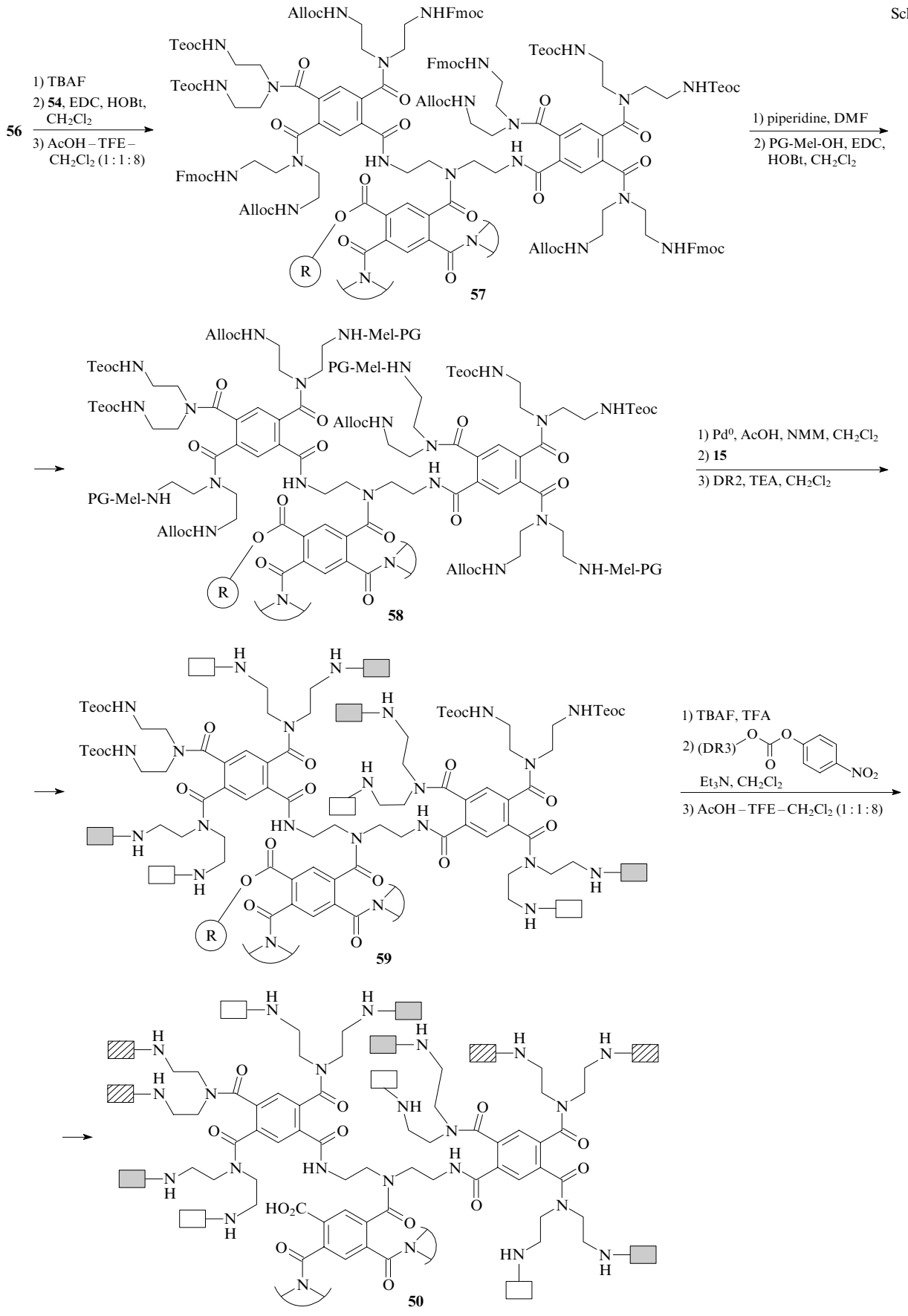
Cyclodextrins (CD) are nanoparticles with hydrophilic surface and hydrophobic inner cavity. They are used to increase water solubility and stability of drugs. Some dosage

Structure of cyclodextrins



Compound	R	*n*	Designation
α-Cyclodextrin	H	4	α-CD
β-Cyclodextrin	H	5	β-CD
γ-Cyclodextrin	H	6	γ-CD
2-Hydroxypropyl-β-cyclodextrin	$CH_2CH(OH)Me$ or H	5	2-HP-β-CD
Sulfobutyl ether of β-cyclodextrin	$(CH_2)_4SO_3Na$ or H	5	SBE-β-CD

Scheme 21



PG = Boc, Cbz; DR3 is activated etoposide; TBAF = Bu^n_4NF; = O O (DR3)

forms are inclusion complexes formed by a drug with dextrin.[121] Cyclodextrins are known to improve the pharmacodynamics and the therapeutic effect of many antitumour agents.[122–124] Non-covalent inclusion complexes of melphalan with cyclodextrins were obtained.[125–130]

Pharmaceutical compositions are prepared using α-, β- and γ-CD and β-CD derivatives.[131]

Since rotation about the 1–4 bonds between the glucose fragments is absent, the shape of the cyclodextrin molecule resembles a truncated cone (Fig. 3).[132]

Cyclodextrins form inclusion complexes with drugs,[132] in which the low-molecular-mass hydrophobic drug molecule is located in the inner cavity of the cyclodextrin molecule. The physical and chemical properties and the biological activity of these non-covalent complexes considerably differ from those of the free drug or cyclodextrin. They can serve to improve the water solubility of the drug, increase the dissolution rate and increase the drug stability in solution.

The formation of the inclusion complex of melphalan with 2-hydroxypropyl-β-cyclodextrin in aqueous solutions is documented.[125] In order to elucidate the influence of cyclodextrin on the solubility of melphalan in water, supersaturated solutions of melphalan in aqueous solutions of 2-HP-β-CD of various concentrations were prepared using short-term sonication. The suspensions were kept at 30 °C until melphalan precipitated, and the amount of melphalan in the solution was determined by HPLC. The melphalan solubility in water was found to substantially increase upon the addition of an aqueous solution of 2-HP-β-CD.

A kinetic study of melphalan decomposition as a function of pH of the medium, 2-HP-β-CD concentration and temperature established that the melphalan stability in water increases upon the formation of the inclusion complex with 2-HP-β-CD. This is expressed as a substantial decrease in the rate constant for melphalan decomposition in the presence of cyclodextrin. With temperature rise, the stabilizing and solubilizing effects of cyclodextrin decrease.[125]

The influence of 2-HP-β-CD and β-cyclodextrin sulfobutyl ether on the melphalan stability was studied by HPLC, UV spectroscopy and NMR spectroscopy.[126] Both cyclodextrins were shown to substantially decrease the rate of melphalan hydrolysis. The rate constant for decomposition of melphalan at 37 °C in the complex with 2-HP-β-CD decreases 17.3-fold, while in the complex with SBE-β-CD, it decreases 96.4-fold.

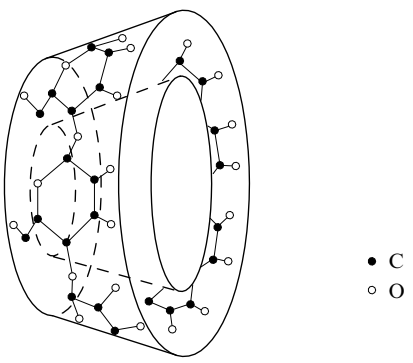


Figure 3. Conical hollow shape of the β-cyclodextrin molecule containing 7 glucose residues.[132]

In the hydrolysis of the nitrogen mustard group of melphalan, the formation of the aziridinium cation involves the charge separation and a transition state that is much more polar than the initial molecule. It is evident that in the case of the melphalan inclusion complex with cyclodextrin, the non-polar inner cavity of cyclodextrin does not provide the microenvironment required for the formation of the polar transition state and, hence, melphalan decomposition slows down.[126]

By comparison of the band intensities in the UV spectra and fluorescence intensities of inclusion complexes and free melphalan, it was shown that melphalan complexed with SBE-β-CD is likely to have a more hydrophobic environment than melphalan complexed with 2-HP-β-CD. According to the NMR data, including 2D nuclear Overhauser effect NMR (NOESY), the location of melphalan inside SBE-β-CD differs from the location inside 2-HP-β-CD.[126] In the former of mentioned cyclodextrins, melphalan is located more deeply and has cyclodextrin binding sites that differ from the binding sites found in the inclusion complex with 2-HP-β-CD.

It was also ascertained that SBE-β-CD is more prone to bind to neutral substrates than HP-β-CD;[133] therefore, it stabilizes melphalan more efficiently.

The melphalan decomposition rate was found to substantially decrease when an aqueous solution of SBE-β-CD or HP-β-CD was used as the solvent for Alkeran without preliminary use of the organic solvent mixture supplied with the pharmaceutical product for the initial dissolution of the powder.[127] The half-life period of melphalan in a normal saline solution increased up to 5 h upon the addition of SBE-β-CD or HP-β-CD as compared with 1.7 h observed upon preliminary dissolution in the auxiliary organic solvents alone. In Alkeran freeze-dried together with SBE-β-CD, melphalan remains stable for more than a year at 5 °C (after 60 weeks, the degree of melphalan decomposition was only 4%).[127]

An *in vitro* experiment in a HUVEC endothelial cell culture demonstrated that HP-β-CD- and SBE-β-CD-based melphalan pharmaceutical compositions injure the vein endothelium to a lesser extent than melphalan solutions obtained with organic co-solvents.[128]

The pharmacokinetic parameters of a melphalan specimen prepared from the commercial Alkeran product and solvents supplied with Alkeran and a melphalan specimen obtained by dissolving Alkeran in a solution of cyclodextrin SBE-β-CD were studied in Sprague Dawley rats.[129] The melphalan half-life period in the body, distribution volume, clearance and degree of renal excretion do not differ significantly for the two pharmaceutical compositions in question. This result is consistent with earlier data indicating the lack of influence of cyclodextrins on the drug pharmacokinetics upon intravenous administration. Perhaps, the SBE-β-CD–melphalan complex is unstable in the circulating blood and soon dissociates to release melphalan without having time to affect its pharmacokinetics but substantially increasing the hydrolytic stability of melphalan during the preparation of the solution and administration of this agent.

A pharmaceutical composition containing melphalan hydrochloride, a buffer and a cyclodextrin (mainly SBE-β-CD) was patented; the dissolution of the composition in water for injection gives a solution for parenteral administration in which melphalan decomposes by 2% during 5 h at 25 °C.[130] It was shown that the solubility of

melphalan hydrochloride in a solution of $SBE_{6.5}$-β-CD (concentration 125 mmol litre^{-1}) at pH 7.5 is ~35 mg ml^{-1}, whereas melphalan hydrochloride is poorly soluble in water (< 2 mg ml^{-1}).

The patent [130] describes the intermediate results of phase IIa clinical trials of a new cyclodextrin-based pharmaceutical composition of melphalan in the therapy of multiple myeloma patients. This composition was suitable for parenteral administration and at least as efficient for the therapy of multiple myeloma as Alkeran. However, it is much more convenient than Alkeran for the high-dose melphalan therapy of patients being prepared for autologous stem cell transplantation.[130] The clinical trials of a new melphalan pharmaceutical form based on cyclodextrin $SBE_{6.5}$-β-CD are in progress.[134]

VI. Lipid nanocarriers for melphalan and sarcolysine

1. Melphalan nanoemulsion

The increase in the bioavailability of poorly water soluble drugs upon application of lipid dosage forms is due to several reasons, including the lymphatic transport of the drug in the lipid carrier, which makes it possible to avoid first-pass metabolism of the drug.[135] Lipid dosage forms enhance the absorbability of the drug in the gastrointestinal tract. In recent years, emulsion-based medicines have found wide use.[136] Nanoemulsions are dispersions of lipids in water or dispersions of water in a non-polar compound in which the drops of the drug-bearing disperse phase are stabilized by a film composed of a surfactant and a co-surfactant (additional surfactant), which differs from the first surfactant in chemical structure. The addition of co-surfactant reduces the surface tension of the interfacial film, promotes the formation of spherical emulsion drops and provides a barrier to coalescence of the emulsion, resulting in a decrease in the free energy needed for the emulsion to form. The nanoemulsion drop size is 20–500 nm. The physical stability of nanoemulsions can be markedly increased by selecting appropriate surfactants.[137]

A method for the preparation of a stable nanoemulsion containing melphalan in the lipophilic phase was developed.[138] The components were chosen considering their pharmaceutical acceptability and toxicity. The best physical characteristics and the highest rate of melphalan release *in vitro* were found for the following composition: Capmul MCM (10 vol.%), Tween 80/Trancutol (3 : 1 ratio, 35 vol.%), water (55 vol.%).

Mouse experiments showed that the bioavailability of melphalan in a nanoemulsion is 4.83-fold higher that the bioavailability of melphalan in a suspension. According to the results of comparative analysis of absorption of melphalan in a nanoemulsion by various organs within 2 h after administration, the use of nanoemulsions leads to selective accumulation of melphalan in ovaries, unlike melphalan used as a suspension.[138]

2. Liposomes loaded with melphalan and lipid derivatives of sarcolysine and melphalan

Liposomes are nano-sized particles (about 30 to 1000 nm) consisting of amphiphilic monomers (usually phospholipids), which are self-assembled in solution to form a closed bilayer membrane where the hydrophilic parts of the molecules point towards hydrated membrane surfaces. Liposomes are hollow vesicles with a lipid membrane, which are used as drug carriers. The pharmacotherapeutic properties of drugs in the liposomal form are, most often, substantially superior to those of free drugs.[139] Depending on the lipophilicity, a drug can be dissolved in the aqueous contents of the liposome inner cavity, reside inside the liposome in the crystalline state or be included in the membrane bilayer.

By varying the composition of lipids containing fatty acid residues with different chain lengths, it is possible to adjust either temperature- or pH-controlled release of the drug from the liposome.[140, 141]

Liposmes enter a cell by means of endocytosis (Fig. 4).[141] In addition, they are adsorbed by the cell membrane either specifically (through ligand–receptor interaction if the liposome contains a targeting fragment) or non-specifically or are fused with the membrane and eject their contents into the cytoplasm (see Fig. 4).[139]

The endocytosis of empty liposomes of distearylphosphatidylcholine (with deuterated stearic acid residues) in MCF-7 breast carcinoma cells was visualized using Raman spectroscopy.[142]

Liposomes are the first nano-sized drug delivery systems. The first nanodrug, Doxil (liposomal doxorubicin), was developed by Barenholz and Gabizon in the mid-1980s [143] and was registered by the FDA in 1995. Doxil was prepared by active loading (remote loading) of nano-sized liposomes using the transmembrane gradient of ammonium sulfate (Fig. 5).

The active loading still remains the method of choice for producing high-capacity nano-sized liposomes as water-soluble drug carriers where the drugs are weak amphiphilic bases or acids (for weak acids, a concentration gradient of calcium or sodium acetate is used).[144] The salt gradient can be created by passive encapsulation of the gradient-forming salt into liposomes during their manufacture and replacement of the salt in the external medium by an isotonic NaCl or saccharose solution. The operating force of the gradient, of, *e.g.*, ammonium sulfate is caused by the pronounced difference between neutral compounds and ions in the

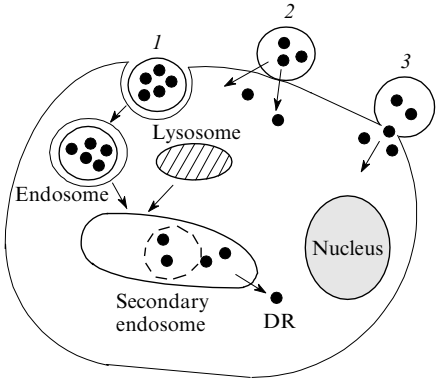


Figure 4. Methods of transport of the liposome content into the cell.[139]
(*1*) Endocytosis: formation of the primary endosome, the subsequent formation of the secondary endosome by fusion of the primary endosome with a lysosome, liposome breakdown by lysosomal enzymes, and drug efflux to the cell; (*2*) liposome adsorption on the membrane and easy diffusion of the drug to the cytoplasm; (*3*) fusion of the lipid double layer of the liposome with the cell membrane. DR is drug.

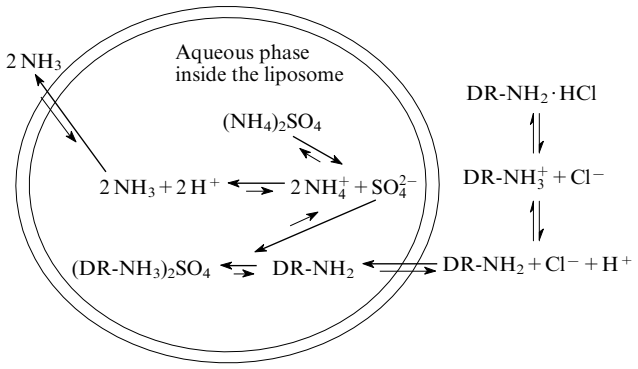


Figure 5. Remote loading of the drug (amphiphilic weak base) into liposomes by means of ammonium sulfate gradient.[144]
The concentration of $(NH_4)_2SO_4$ inside the liposome is approximately 1000 times higher than in the external environment. Neutral drug ($DR\text{-}NH_2$) penetrates the membrane and is retained owing to ionization and formation of insoluble salt with the counter-ion inside the liposome. DR is drug.

ability to penetrate the membrane lipid bilayer (see Fig. 5): SO_4^{2-}, $NH_4^+ \ll H^+ \ll NH_3$.

The very high permeability of the bilayer for an ammonia molecule leads to its fast diffusion to the environment after formation inside the liposome, resulting in lowering of the pH in the inner space of the liposome. The amphiphilic drug molecules, weak bases, are able to penetrate the bilayer in the neutral form and enter the liposome. However, having acquired a positive charge in the acid medium inside the liposome, the drug molecules are no longer able to penetrate the bilayer and are entrapped in the inner cavity of the liposome, in particular, as sulfates. The counter-ion can be selected to ensure the formation of an insoluble drug–counter-ion salt in the liposome and thus to provide controlled drug release from the liposome, *e.g.*, as a function of temperature.[144]

Using analytical computer programmes and nine drugs — weak amphiphilic acids and bases — together with an extensive experimental database on the dependence of the efficiency of liposome remote loading on the conditions and physicochemical properties of the drug, a decision tree was developed for selecting the optimal parameters to attain the maximum degree of remote loading of the drug into liposomes.[144]

Kirby and Gregoriadis [145] developed a dehydration–rehydration method for preparation of liposomes according to which the lipid is first dissolved in an organic solvent ($CHCl_3$, CH_2Cl_2, MeOH or EtOH), the lipid solution is dried on a rotary evaporator, and a lipid film is formed on the wall of the evaporator flask. The addition of water or an aqueous buffer solution to the dry lipid film and subsequent shaking gives rise to multilamellar liposomes. The liposomes thus obtained are mixed with an aqueous solution of the drug either containing additives or not, then the mixture is dehydrated by freeze drying. The subsequent repeated hydration of the dried material affords dehydration/rehydration vesicles containing the drug in the inner cavity. In order to reduce the average size of the drug-loaded liposomes, the liposome suspension is subjected to microfluidization using dedicated equipment and then purified from the non-encapsulated drug by centrifugation or chromatography. The method of dehydration/rehydration vesicles was applied to prepare liposomes from cationic lipids such as [(2,3-dioleoyloxy)prop-1-yl]trimethylammonium, {[2,3-di(hexadecyloxy)]prop-1-yl}trimethylammonium and [(2,3-dioleoyloxy)prop-1-yl]triethylammonium chlorides in order to provide intracellular delivery of polynucleotides for gene therapy.[146]

The introduction of PEG residues into lipids that form liposomes may retard the clearance of liposomal medicines and thus prolong the action of the entrapped drug.[147] Liposomes coated by PEG fragments have been called STEALTH liposomes for their inertness with respect to immune systems that protect the organism (*i.e.*, for the stealth to avoid this systems).[148]

Currently, clinical chemotherapy uses liposomal forms of doxorubicin [Doxil or Caelyx (PEG-coated liposomes), Myocet], daunorubicin (DaunoXome),[149,150] vincristine (Onco TCS), lurtotecan (NX211)[151] and some other. Liposomal forms for some drugs have been developed in Russia.[152]

Liposomes as drug delivery systems are attractive, first of all due to their biocompatibility.[153,154]

The drawbacks of the liposomal dosage forms include fast decomposition in the blood flow and capture of liposomes by the cells of reticuloendothelial system.[139] These problems are solved by introducing functional groups on the liposome surface, which give rise to new properties: (i) long-term circulation in the blood and accumulation in the solid tumour tissue; (ii) specific recognition and bonding to the target tissue or cells with the participation of the surface-grafted ligand; (iii) response to local stimulating characteristics (efflux of the drug or specific action on the cell membrane at particular pH and/or temperature at the lesion site provided that pH- and/or temperature-sensitive elements have been grafted on the liposome); (iv) ability to penetrate inside the cells bypassing the lysosomal degradation path, which is used for effective intranuclear drug delivery [for this purpose, the liposome surface is additionally modified by peptides that penetrate into the cell, *e.g.*, TAT (HIV-1 trans-acting translational activator) and VP22 (cell-penetrating herpes simplex virus protein)].[151]

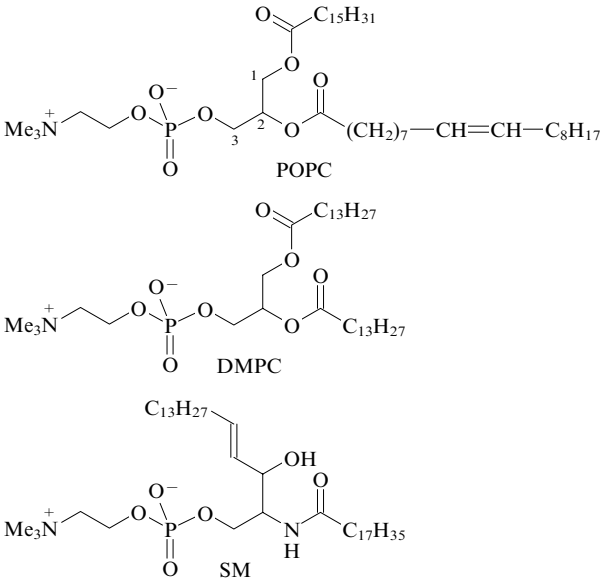
However, in recent 2–3 years, researchers' attention has been focused not on the development of methods for introducing targeting fragments in liposomes but rather on overcoming the immune response of the human body to liposomes (and also to other nanoparticles) both containing and not containing targeting fragments. It was ascertained that PEGylated liposomes activate the complement system due to the presence of a negative charge on the oxygen atom of the phosphate group in PEGylated phospholipids.[13] Anionic liposomes can activate the complement by either classical or alternative path (or both) depending on the structure of the polar head group of the liposome-forming phospholipid.[13] The packing density of functional groups and hydrophobic/hydrophilic liposome domains determines the predominant path of complement activation after recognition by the factor C1q. The immune response is also triggered by regularly repeated epitopes on the liposome surface (targeting residues).[13]

The key difficulties faced by the development of liposomal drug delivery systems are insufficient drug entrapment capacity, fast efflux of water-soluble drugs from liposomes in the presence of blood components, low physical and chemical stability of liposomes. Liposomes incompletely protect unstable drugs from decomposition. Liposomal dosage forms preclude strict control over the

administered dose. Liposomes are unsuitable for oral administration, as phospholipids decompose in the gastrointestinal tract.[155] A number of interesting solutions have been proposed in recent years to increase the liposome stability and protect the phospholipids from enzymatic decomposition, in particular, coating of liposomes by finer silica nanoparticles. For the ratios SiO_2 : lipid > 3.4 (a solid particle layer has formed on the liposome surface), good stability of liposomes against enzymatic decomposition *in vitro* was observed (< 20% in 45 min).[155]

In early works, attempts were made to fabricate liposomes containing melphalan in the inner cavity. Monolamellar liposomes of diameter 30–60 nm were prepared, which consisted of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) or sphingomyelin (*N*-stearoyl-4-sphingenyl-1-phosphocholine) (SM) with or without cholesterol (Ch) and contained melphalan in the inner space.[156]

Structures of phosphatidylcholines and sphingomyelin



Solutions of phospholipids in organic solvents were concentrated on a rotary evaporator to give a thin lipid layer. Then the lipids were dispersed by shaking the flask with a lipid film together with the buffer solution and salts and melphalan or [^{14}C]-melphalan.[156] The resulting compositions were subjected to sonication, then conditioned, and finally centrifuged to remove multilayer structures and membrane fragments. It was shown that the degree of melphalan entrapment into POPC-liposomes increases following the increase in the content of cholesterol in the lipid layer. Entrapment of melphalan into liposomes is most pronounced when Ch and phospholipid are present in equimolar amounts.

A study of the pharmacodynamics of [^{14}C]-labelled melphalan demonstrated that its clearance from blood slows down when the liposomal dosage form is administered intravenously or intraperitoneally compared to the clearance of melphalan after intravenous administration. The melphalan clearance observed upon the intravenous administration of liposomal forms depends on the presence of Ch in the liposome lipid layer. The higher the Ch content in POPC-liposomes, the more pronounced the retardation of clearance.[156] When POPC-liposomes with cholesterol were used, the melphalan release rate was the lowest in the range of liposomes studied, and thus they can be suitable carriers for prolonged melphalan release.

The qualitative and quantitative composition of phospholipids determines also the rate of melphalan excretion from the body: melphalan loaded into SM-liposomes without Ch remains in the circulating blood upon intravenous administration in mice for a considerably longer period than that entrapped in POPC- or DMPC-liposomes without Ch.[156]

POPC and Ch liposomes with a 8.1% average entrapment efficiency of melphalan were obtained.[157] Kinetic measurements of melphalan release from liposomes *in vitro* in phosphate-buffered saline (PBS) at 37 °C showed that 70% of melphalan is released from the liposome during the first 4 h, while only 20% is liberated during the next 24 h. In rat experiments, immediately after the introduction of melphalan-loaded liposomes, the melphalan concentration in the ipsilateral lymph nodes was 200 and 100 times higher than in the blood plasma and contralateral lymph nodes, respectively. Twenty four hours after the injection of liposomes, the difference between the melphalan contents in the mentioned tissues decreased by about an order of magnitude. In experiments in rat 13762 mammary adenocarcinoma, it was found that the same antitumour effect was induced by liposomal melphalan in a dose of 0.125 mg kg^{-1} or free melphalan in a dose of 1 mg kg^{-1} .

The tissue distribution of POPC-liposomes with melphalan was studied using ^{14}C-melphalan and ^{3}H-phosphatidylcholine.[158] When large liposomes (up to 250 nm) or small liposomes with up to 34 nm size have been injected subcutaneously into rat thighs, the melphalan concentration in the ipsilateral lymph nodes after 24 h was 10 times higher for fine than for large liposomes. This result must be due to the fact that large liposomes remained near the site of injection and, therefore, the concentration of melphalan in lymph nodes increased insignificantly compared with its blood concentration. It is noteworthy that the blood concentration of melphalan was relatively low upon the injection of both types of liposomes.

Generally, the experiments on liposomes carried out in the 1980s[156–158] demonstrated for the first time how difficult it is to achieve an optimal relationship between the liposome size and appropriate melphalan loading.

One of approaches to provide drug targeting to the tumour is to use temperature-sensitive liposomes together with local heating of the tissue — hyperthermia.[159] Attempts were made at targeted melphalan delivery to the tumour tissue using liposomes as melphalan delivery systems and local heating of the tumour, which promotes the breakdown of thermally sensitive liposomes in the tumour with release of the active agent.[160] In bilayers made of distearoylphosphatidylcholine (DSPC) and dipalmitoylphosphatidylcholine (DPPC) mixtures, the phase transition of the gel to liquid crystalline phase occurs at temperatures close to the clinical hyperthermia temperature; therefore, these layers are used to encapsulate drugs to be used for chemotherapy together with hyperthermia. The melting point of the DPPC bilayer is 42 °C, that of the DSPC bilayer is 60 °C.

The DSPS and DPPC with melphalan were prepared by the method described by Kirby and Gregoriadis.[156]

The antitumour efficiency of liposomes dosage forms in comparison with melphalan was studied in B16F10 melanoma in C57B1/7 mice; the animals were divided into the

following groups: control; hyperthermia at 42 °C for 1 h; melphalan (3 mg kg^{-1}); melphalan (3 mg kg^{-1}) plus hyperthermia; thermally sensitive liposomes with melphalan; thermally sensitive liposomes with melphalan plus hyperthermia. All medications were administered on the 15th day after tumour transplantation.

In mice treated with free melphalan together with hyperthermia, the tumour size considerably decreased compared with the mice treated with melphalan. The combination of hyperthermia and chemotherapy with thermally sensitive melphalan-loaded liposomes resulted in considerable regression of the tumour (the tumour volume decreased 1.5-fold on the 30th day compared with the initial volume observed on the 15th day of the experiment) and an increase in the survival rate.[160]

Mouse experiments showed also considerable enhancement of the antitumour activity of melphalan against the B16F10 melanoma when melphalan has been encapsulated into egg phosphatidylcholine and cholesterol liposomes, which are used together with local hyperthermia.[161]

Of considerable interest are liposomes containing a lipid drug derivative in the lipid bilayer.[162, 163] Sarcolysine and melphalan are relatively hydrophilic and cannot by themselves be retained in the cell membrane. It is of interest to consider the synthesis of amphiphilic derivatives of drug molecules containing a lipid anchor. It is assumed that as the molecule has entered the cell, its active cytotoxic part splits off by phospholipases, esterases and other intracellular enzymes.

The inclusion of a lipophilic prodrug into the liposome membrane simplifies the manufacture of the liposomal dosage form. The delivery of cytotoxic drugs as lipid derivatives incorporated in the lipid bilayer of a liposome provides the following advantages: reduces the loss of the drug in the circulating blood and upon the liposome interaction with the cell; lipid derivatives are capable of direct transmembrane transport, which can fundamentally change the mechanism of transport into the cell and the intracellular traffic of the pharmaceutical and facilitate the liposome unloading.

A lipid derivative of sarcolysine, racemic 1,2-dioleoyl-3-sacrolysylglycerol (**60**), was synthesized as a mixture of 4 diastereomers.[163] The reaction of *N*-*tert*-butoxycarbonylsarcolysine with dioleoylglycerol in the presence of DCC and 4-pyrrolidinopyridine (4-PPy) gave dioleoylsacrolysylglycerol (**60**) after isolation of the condensation product **61** and removal of the Boc group by treatment with trifluoroacetic acid in dichloromethane (Scheme 22). Later on, the same researchers used a similar procedure to convert *N*-*tert*-butoxycarbonylmelphalan to 1,2-dioleoyl-3-melphalanylglycerol as a mixture of two diastereomers (*S* ,*R* + *S*,*S*)-**60** and used this product to study the physicochemical properties of the liposomal dosage form.[164]

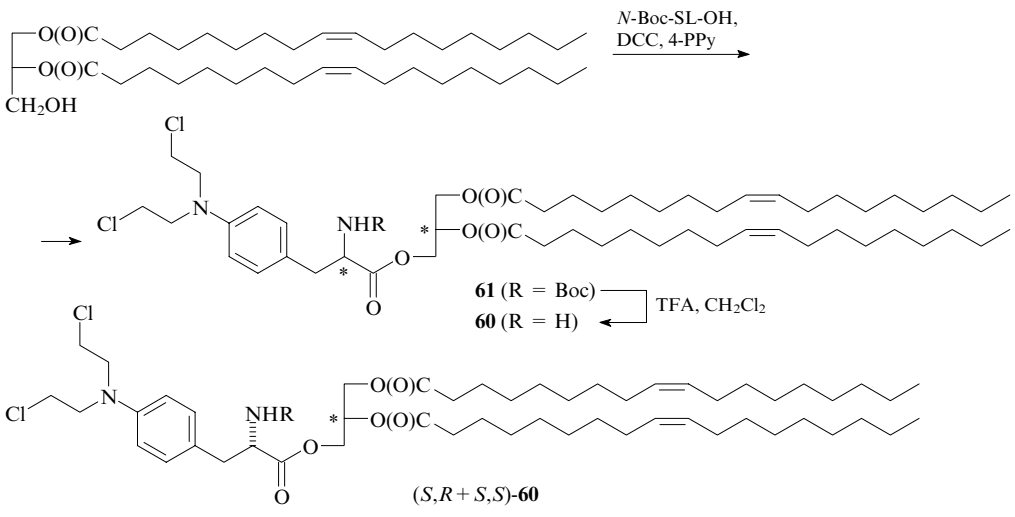
Liposomes containing natural phospholipids and compound (*S*,*R* + *S*,*S*)-**60** in the bilayer were prepared by extrusion of a PBS suspension of hydrated lipid films composed of egg phosphatidylcholine (ePC) : phosphatidylinositol (PI) : lipophilic prodrug = 8 : 1 : 1 through polycarbonate membrane filters with a pore diameter of 100 nm.[164] It was shown that melphalan diglyceride ester was fully incorporated into monolamellar liposomes ~100 nm in diameter. The dispersions were retained for several days without substantial aggregation. The dispersions of medicinal liposomes can be deeply frozen and stored at −70 °C; prior to use, they can be unfrozen and treated on an ultrasonic bath for a short period to fully restore the composition and particle size.

Comparison of HPLC analysis data for the reaction mixture resulting from keeping liposome formulation of compound (*S*,*R* + *S*,*S*)-**60** in a phosphate-buffered physiological saline solution or in human blood plasma did not reveal any distinctions.[165] Thus, blood plasma esterases do not cleave compound (*S*,*R* + *S*,*S*)-**60** to give melphalan and/or products of its hydrolysis. Hence, the lipid melphalan derivative would be hydrolyzed inside the cell rather than in the circulating blood. The intracellular hydrolysis of prodrug (*S*,*R* + *S*,*S*)-**60** is one of the factors decreasing the systemic toxicity of melphalan.

Of much interest is the design of liposomes containing a targeting fragment.[166] Enhanced expression of lectins, specific proteins that bind carbohydrates, was established for malignant cells. This fact is related to tumour metastatic spread.[167] The excessive expression of lectins by cancer cells compared with normal cells can be used for liposome targeting to tumours by means of special structure elements — targeting carbohydrates. Some glycoconjugates were synthesized and evaluated as vector structure elements of liposomes bearing a lipophilic sarcolysine prodrug.[168–170]

Liposomes from natural phospholipids loaded with lipid compound **60** and containing lipophilic carbohydrate vectors were prepared: α-L-fucose conjugates (vector 1) and *N*-acetyl-β-D-galactosamine (vector 2) with a polyacryl-

Scheme 22



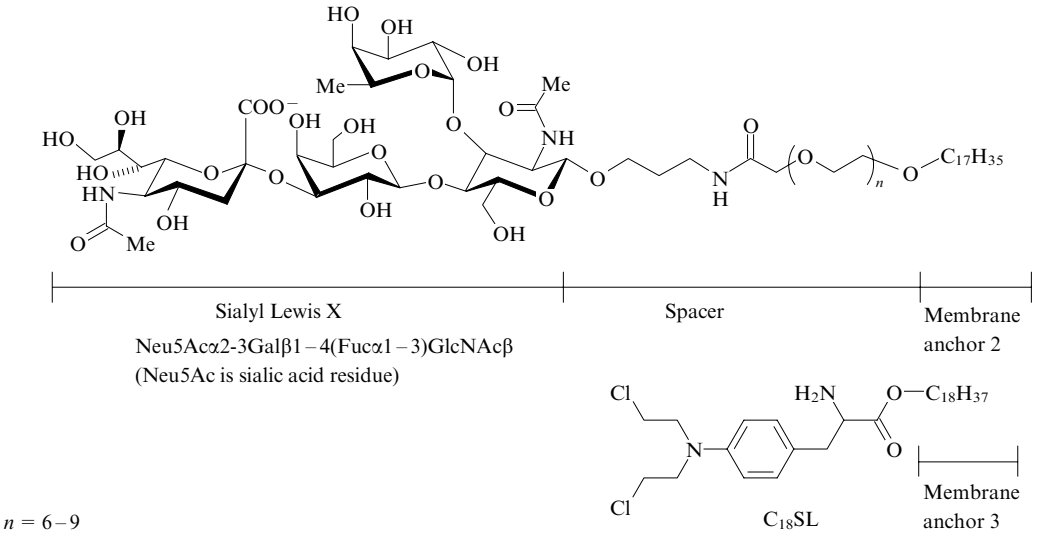


Figure 6. Structure of the vector SiaLe^X and lipophilic sarcolysine prodrug C_{18}SL.

amide matrix bearing dioleoylphosphatidylethanolamine residues for anchoring to the membrane.[168] The *in vivo* antitumour activity was estimated by the increase in the average life span of BDF1 mice with model P388 leucosis (intraperitoneal introduction of test drugs 48 h after transplantation of cells and once again after 72 h). Sarcolysine in a dose of 7.0 mg kg^{-1} induced a 113% ILS. Free liposomes were inactive. The introduction of liposomes with compound **60** in an equivalent dose according to the same schedule resulted in a 163% ILS; treatment with liposomes with carbohydrate vector 1 resulted in a 213% ILS, while in the case of vector 2, this value was more than 243% ILS.[168]

In order to reveal carbohydrate ligands for tumour cell lectins (HL-60 and human lung adenocarcinoma ACL), screening was performed in the series of fluorescently-labelled carbohydrate–polyacrylamide–fluorescent tag conjugates.[169] The specificity of binding of carbohydrate probes to the cells was estimated at a qualitative level by observing fluorescence under a microscope. The active ligands found for the studied two cell lines are Galβ and trisaccharides HSO_3-Le^X [Le^X = 3Galβ1–4(Fucα1–3)GlcNAcβ, where Gal is D-galactose, Fuc is D-fucose, Glc is D-glucose] and HSO_3-Le^A [Le^A = 3Galβ1–3(Fucα1–4)GlcNAcβ]. Then liposomes were prepared based on a mixture of matrix phospholipids, phosphatidylcholine–phosphatidylinositol–cholesterol–tocopherol, with compound **60** (10% of basic lipids) and carbohydrate–polyacrylamide–membrane anchor (0.4 mol.%) conjugates. Liposomes provided with active vectors were found to bind to cells 50%–80% more efficiently than liposomes without carbohydrate conjugates. This results in a considerable increase in the *in vitro* cytotoxicity of targeted liposomes compared with liposomes without a vector (2–4-fold).[169]

The *in vivo* testing was carried out for liposomes containing, in the lipid bilayer, a lipophilic conjugate of the tetrasaccharide SiaLe^X and the lipophilic sarcolysine derivative C_{18}SL (Fig. 6)[169] synthesized from *N-tert*-butoxycarbonylsarcolysine and octadecan-1-ol in the presence of DCC and 4-pyrrolidinopyridine, as reported.[163] The tetrasaccharide SiaLe^X (Sialyl Lewis X) is a ligand of selectins, cell adhesion molecules (glycoproteins), which are involved in a multitude of (patho)physiological processes and serve as key participants of inflammation development, tumour growth and metastasis.[171] The SiaLe^X vector (see Fig. 6) was synthesized as a conjugate with a surfactant containing a PEG fragment. This gave liposomes of various composition based on natural lipids, phosphatidylcholine and phosphatidylinositol, which contained the lipophilic merphalan prodrug C_{18}SL.[170]

The schematic view of liposomes bearing sarcolysine lipid conjugates (similarly, for melphalan lipid derivatives) and carbohydrates are shown in Fig. 7.

BLRB mice with high frequency of spontaneous mammary cancer were used to test the antitumour activities of the described liposomes.[170] Models of this mouse mammary

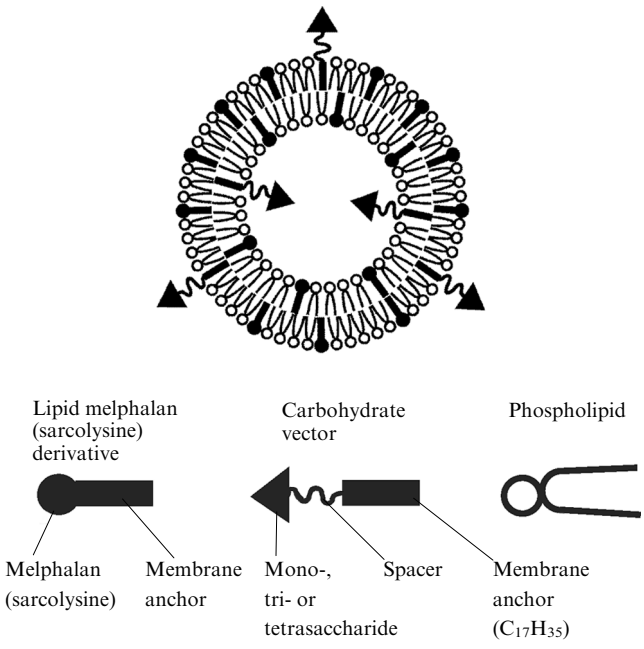


Figure 7. Schematic view of liposome with lipid sarcolysine (melphalan) derivative and carbohydrate vector.

tumour most adequately reflect the situation of hereditary forms of breast cancer in women, provide complete information on the efficiency of the antitumour drugs being tested. In the study of oncogenesis, 4.5-month old male BLRB mice were subcutaneously injected, into the right flank, with a suspension of tumour cells obtained from mammary adenocarcinoma of syngeneic female mice.[170] The effects on the tumour of sarcolysine, liposomes containing only matrix lipids, C_{18}SL liposomes with equivalent sarcolysine dose, liposomes with C_{18}SL and SiaLe^X and liposomes with only the SiaLe^X vector were compared by the oncometric method. In the case of mice treated with C_{18}SL-SiaLe^X liposomes, the most pronounced decrease in the tumour size and the rate of tumour growth were obtained as compared with not only the control but also with the group treated with C_{18}SL liposomes having no carbohydrate vector. The survival rate of mice of the group treated with C_{18}SL-SiaLe^X liposomes was much higher than of mice of other groups. The vector liposomes without sarcolysine and empty liposomes have little effect on the tumour growth. The obvious advantage of the method of targeting cytotoxic liposomes by a carbohydrate vector over the liposomes devoid of the targeting fragment was demonstrated.

The modification of the liposome surface by functional groups can crucially affect the biological compatibility of these nanoparticles. The most illustrative example is the use of PEG coating to shield the surface of liposomes or other nanoparticles from proteins of the circulating blood. Despite the commonly accepted view that PEG is nontoxic and not immunogenic, it was found that PEGylated liposomes (including Doxil) cause the hypersensitivity syndrome or pseudo-allergy related to activation of the complement system.[172]

The complex supramolecular structure of a liposome implying a specific relative orientation of functional groups on the liposome surface can considerably affect the biological cascades in the body.

Hemocompatibility tests were carried out for ePC-PI-liposomes loaded with (*S*,*R*+*S*,*S*)-**60** and the SiaLe$^{X/A}$-vector [liposomes Mel-L-SiaLe^A and Mel-L-SiaLe^X; the tetrasaccharide SiaLe^A, like SiaLe^X, is a selectin ligand] or without the vector (Mel-L) and for liposomes devoid of PI (Mel-L-SiaLe^A/PI).[173] The effects of liposome components on the principal blood components and parameters were estimated by keeping liposomes with human whole blood. Phosphatidylinositol was eliminated from the liposome to determine how much the hydrated groups of the PI polar head shield the liposome from interaction with biological objects.

Liposomes were prepared by the method reported earlier.[164, 165] The average diametres of the liposomes Mel-L, Mel-L-SiaLe^A, Mel-L-SiaLe^X and Mel-L-SiaLe^A/PI were 85 ± 2, 85 ± 1, 90 ± 3, 92 ± 1 nm, respectively, the liposomes being stable for at least 2 weeks at 4 °C.[173]

Thus it was ascertained that neither the red blood cell or platelet count nor the morphology of these elements change upon treatment of blood with liposomes of any of the types. Liposomes did not cause hemolysis and did not affect the coagulation pathway (blood coagulation was studied and the prothrombin time and activated partial thromboplastin time were calculated).[173]

The degree of activation of the complement system by nanoparticles may be estimated by analyzing blood samples for the C3a peptide component of the complement, which is formed upon execution of any complement activation pathway.[174] It was shown by enzyme immunoassay that liposomes loaded with the lipid derivative of melphalan (*S*,*R*+*S*,*S*)-**60** do not affect the contents of the key components of the complement system C3a or the complex SC5b-9. Targeting carbohydrates, as well as PI, do not affect the liposome inertness with respect to the studied biological cascades.[173]

To conclude this Section, we would like to note that liposomes with carbohydrate vectors reported in several publications[168–170, 173] are the only targeting merphalan carriers obtained to date that not only have a stable nanostructure but also exhibit higher *in vitro* and *in vivo* antitumour activity than the non-targeted liposome specimens. Furthermore, these liposomes do not affect the principal parameters of human blood and do not activate the complement system; therefore, they are promising for detailed *in vivo* studies and further preclinical trials.

VII. Melphalan conjugates with proteins

1. Conjugates with antibodies

Antibodies have found use as selective nano-sized means for the delivery of cytotoxic drugs into tumour cells containing the corresponding antigens on the surface. The targeted delivery of antitumour agents was attained by attachment of drugs, radioisotopes and cytotoxins to antibodies as transport elements, either directly or *via* a linker.[175] In particular, this was done using monoclonal antibodies (MAbs) that specifically bind to the tumour antigens. The monoclonal antibodies are highly specific and are directed against one antigenic determinant (epitope).

In some early works, chemical addition of melphalan molecules to immunoglobulins was performed. Melphalan was attached to immunoglobulin G (IgG) of ICR mice.[176] Immunoglobulin IgG is a Y-shaped glycoprotein consisting of four amino acid chains, two heavy (H-chains) and two light (L-chains), and a carbohydrate chain.[177] Each peptide chain of the antibody comprises mainly two molecular regions, the variable (V) and constant (C) regions (Fig. 8).[178] The antibody interacts with a particular site of the antigen molecule by the variable region. After the formation of the antibody–antigen complex with the participation of V-regions, the IgG constant regions initiate a sequence of biochemical transformations, in particular, activation of the complement system.

The melphalan conjugate with murine IgG exhibited a substantial antitumour activity against sarcoma 180 and Erlich carcinoma in ICR mice.[176] By a similar condensation of melphalan with human IgG, the K-18 conjugate was obtained. Using ^{14}C-labelled melphalan and K-18, it was found that after oral administration in mice, the conjugate is absorbed in gastrointestinal tract and is selectively accumulated in the Erlich carcinoma tumour.[179] The K-18 conjugate demonstrated a considerable *in vivo* antitumour efficiency against MX-1 breast cancer, MK-2 gastric cancer and LC-10 human lung cancer in murine xenografts and was considered as a potential antitumour medicine.[180] Experiments in mice showed a considerable antitumour effect of K-18 used in combination with hyperthermia against Lewis lung carcinoma.[181]

In order to overcome the non-specific toxicity of melphalan, *N*-acetylmelphalan (AM), which is 75 times less toxic *in vitro* than melphalan, was synthesized, acetylmelphalan conjugates with various monoclonal antibodies

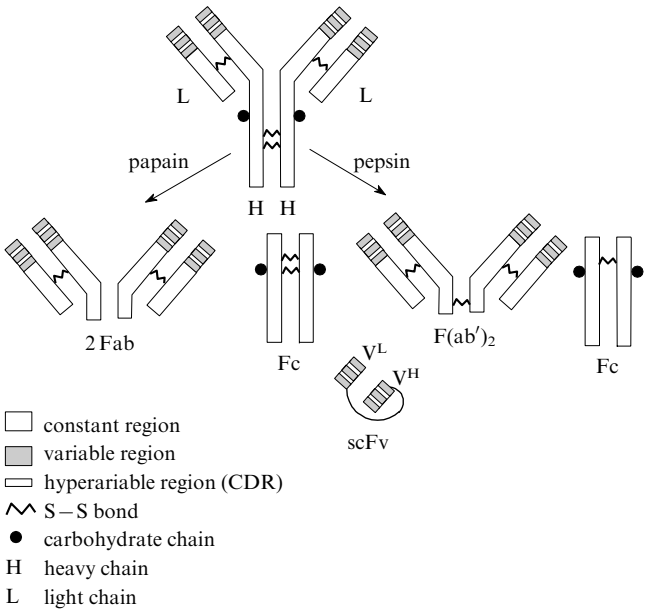


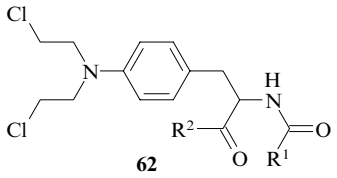
Figure 8. Schematic view of immunoglobulins IgG and their fragments obtained by enzymatic hydrolysis.[178]
Fab is the antigen-binding fragment, Fc is crystallizable fragment, scFv is the single-stranded variable fragment.

[anti-Ly2.1 specific to murine Ly2.1 antigen and A3C6 (anti-TFR) specific to human transferrin receptor TFR] were obtained, the AM content in the conjugates was quantitatively estimated.[182] The antibody conjugates were prepared by adding a solution of activated AM ester in DMF to a solution containing antibodies purified by affinity chromatography. The mixture was kept at room temperature. The precipitated protein was separated by centrifugation, and AM that has not reacted with free amino groups of the antibody was separated by gel filtration. It was shown that up to 30 AM molecules can be added to the antibody without substantial loss of its properties and solubility. The *in vitro* cytotoxicity of the anti-Ly2.1 and anti-TFR conjugates with AM was determined for Ly-2-positive E3 cells [cloned murine thymoma ITT(1)75NS CEM] and TFR-positive CE human cells. In both cases, the conjugate cytotoxicity was much higher than the cytotoxicity of AM but lower than the melphalan cytotoxicity. It was proven for the conjugates that AM penetrates into the cell either being delivered by the antibody or upon conjugate endocytosis rather than by specific LAT-mediated phenylalanine transport. The cytotoxicity of the conjugates is determined by the ability of the antibody to bind to antigens on the cell surface.[182] It was found in experiments in mice that the conjugates have a stronger *in vivo* antitumour activity than melphalan and AM.[182]

The synthesis of the conjugates formed by melphalan derivatives with antibodies was patented.[183]

Monoclonal antibodies specific to breast, brain, lung, pancreatic and intestine tumours or melanoma were used. Compounds of the general formula **62** were attached to the free amino groups of the antibody. The conjugates were synthesized by the method reported in Ref. 182. The covalent AM–anti-Ly-2.1 conjugate proved to inhibit the growth of the murine E3 tumour *in vivo* more efficiently than free AM and the anti-Ly-2.1 antibody. The AM–

Structure **62**



62

$R^1 = (CHR^4)_nCH_2R^3$ (R^3, R^4 = H, Alk, Ar, CO_2H, OH, NH_2; $n = 1–10$); R^2 are the residues activating the melphalan CO_2H-group (oxysuccinimide, acyloxy, oxysulfosuccinimidyl, azide, *p*-nitrophenyloxy)

anti-Ly-2.1 conjugate was non-toxic for CBA mice at the highest dose tested (16 mg kg^{-1}), and it was approximately 3 times more effective upon intravenous administration than upon intraperitoneal administration.[183]

It is known that the use of a part of the antibody molecule may provide the targeting effect similar to the effect achieved with the intact antibody. Antibody fragments are obtained by treating them with various enzymes and named depending on what part of the antibody molecule has been separated (see Fig. 8).[178]

In order to reduce the non-specific binding through Fc receptors, AM was attached to the $F(ab')_2$ antibody fragments.[184] The $F(ab')_2$ fragments were obtained by pepsin hydrolysis of IgG in acid medium. The AM–$F(ab')_2$ conjugates containing up to 20 AM molecules exhibited specific *in vitro* cytotoxicity; the AM–$F(ab')_2$ and AM–IgG conjugates had similar *in vivo* antitumour activities. The immunogenic activity of AM–$F(ab')_2$ fragments was lower than that of the whole antibody,[184] which is due to several reasons.[185]

According to results of mouse experiments, the antitumour effect of melphalan immunoconjugates can be enhanced by introducing hydralazine 24 h after the introduction of the conjugate.[186]

Subsequently, the same authors used two antibodies, 49-11.1 (IgG2a) specific to the murine Ly-2.1 antigen and 5034-29.5 (IgG1) specific to the murine Ly-3.1 antigen, for the delivery of two drugs.[187] Both antibodies were isolated from murine ascites by affinity chromatography. One MoAb was conjugated with *N*-acetylmelphalan by the method described earlier.[182] Idarubicin was attached to the second antibody by the same method. The use of a combination of conjugates (different antibodies and different drugs) provides more pronounced antitumour effect than the combined administration of free drugs without antibodies.[187]

Phase I clinical trials were performed for the conjugates of *N*-acetylmelphalan with monoclonal antibodies MoAb 30.6, I-1 or JGT for therapy of intestine cancer.[188] These conjugates (the appropriate antibody conjugate for particular patient was selected based on immunochemical assays of the primary tissue of patient's tumour) were injected into arteria hepatica of 10 patients with extensive intestine cancer disseminated into the liver in a dose of 1000 mg m^{-2}, which corresponded to 20 mg m^{-2} of *N*-acetylmelphalan. This does not bring about significant side effects that are usually induced by administration of melphalan. However, all patients showed immune response to the murine antibodies present as parts of the conjugates. A considerable improvement of the general condition as a result of treatment and clinical effect were noted for all patients; in 33% of the cases, liver MRI showed some inhibition of tumour growth.

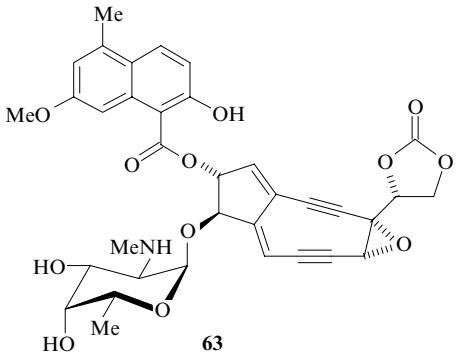
Despite the good results obtained for melphalan conjugates with antibodies in mice, these nano-sized melphalan carriers still have not found clinical use, which may be due to too small number of drug molecules that can be attached to an antibody without loss of their properties, high immunogenic activity of these conjugates and their high cost.

Generally, the method of direct conjugation of the cytostatic with the antibody is experimentally rather topical,[189–191] although it has a number of considerable drawbacks [178] and is not used in clinical practice.

2. Conjugates with other proteins

Neocarzinostatin (NCS) is a new antitumour antibiotic (isolated from the culture *Streptomyces carzinostaticus*), which represents 1 : 1 complex of the protein apo-NCS and chromophore **63** the naphthalenecarboxylate moiety of which participates in binding to the protein and simultaneously determines the intercalation into DNA, which accounts for the cytotoxicity of neocarzinostatin.[192, 193] Currently, NCS derivatives are used in Japan as antitumour drugs.[192] A lot of effort is made to identify the non-natural ligands of the apoprotein (apo-NCS) and to use it for the design of nano-sized drug delivery systems in order to stabilize unstable drug molecules. Neocarzinostatin is selectively transported to the tissues of some tumours.[192]

Structure **63**



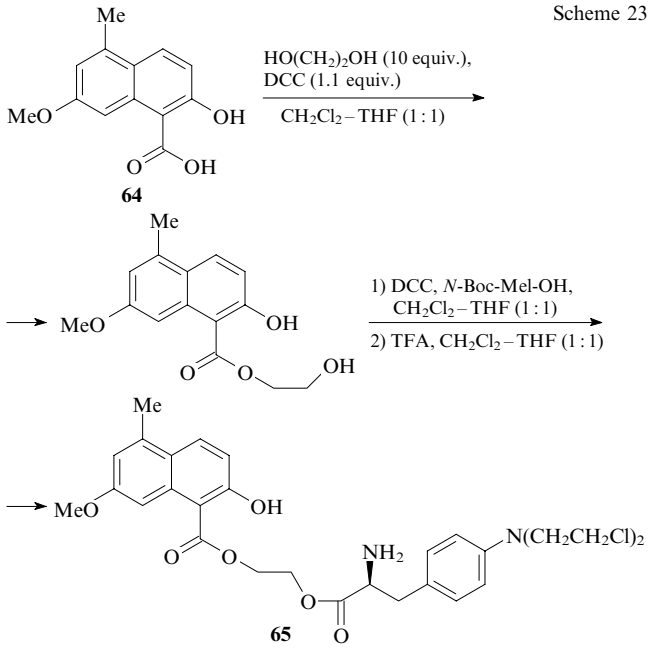
63

In order to stabilize melphalan and enhance its interaction with DNA, the possibility of melphalan attachment to 2-hydroxy-5-methyl-7-methoxy-1-naphthoic acid (**64**) followed by formation of the complex with the protein apo-NCS was considered.

The condensation of *N-tert*-butoxycarbonylmelphalan with acid **64** was carried out by the carbodiimide method.[192] First, compound **64** was condensed, in the presence of DCC, with ethylene glycol serving as a short flexible linker, and then the reaction with *N*-Boc-protected melphalan was carried out also in the presence of DCC. The subsequent removal of the Boc-group in the acid medium gave ester **65** (Scheme 23).

A study of the cytotoxicity on K562 leukaemia cells showed that ester **65** is approximately 4 times more cytotoxic than melphalan and forms adducts with DNA with an alkylation degree higher than that observed upon the action of melphalan. The addition to naphthoate does not change the melphalan selectivity with respect to alkylation of guanine-enriched nucleic acid sequences. According to ^{1}H, ^{15}N and HSQC NMR studies of binding of compound **65** to the protein part of neocarzinostatin, ester **65** occupies the same binding site of apo-NCS as non-modified naphthoate

Scheme 23



in natural neocarzinostatin. Hydrolysis of ester **65** with and without apo-NCS was investigated by reverse phase HPLC.[194] It was found that non-covalent conjugation with the protein stabilizes the melphalan derivative **65**. However, treatment of K562 cells with both apo-NCS and ester **65** did not reveal substantial changes in the IC_{50} value of compound **65**.

VIII. Conclusion

At the current stage of development of biotechnology and medicine, it is beyond doubt that the success of application of pharmaceutical products is largely determined not only by the chemical structure of the pharmacologically active agent and the dosage form but also by the way of delivery to the target tissues, cells or subcellular structure. Despite the massive efforts directed to the design of nanocarriers for drug delivery and considerable progress in this field made in the last 15 years, only few nanodrugs have found clinical use.[195] Experimental data are still insufficient to make general conclusions concerning the increase in the efficiency of nanodrugs as compared with conventional antitumour drugs. In some cases, the use of nanocarriers enhances the therapeutic action, while in other cases, only palliative effect was revealed.[196]

Melphalan, a drug widely used in practical chemotherapy, has not lost its value. Its clinical efficiency considerably depends on the duration of exposure of the target tissue, the dosage form and the way of administration. The design of nanodrugs based on melphalan (sarcolysine) may overcome its drawbacks such as poor solubility and fast hydrolysis in aqueous media, optimize the duration of its release *in vivo* in the active form and thus the duration of exposure of the target tissue, decrease the systemic toxicity and overcome the mechanisms of tumour cell resistance to melphalan.

The preparation of new biocompatible and biodegradable nano-scale melphalan carriers is an actively developing area in the design of new antitumour agents in which considerable advances have been made. Nano-sized structures were developed that solve, to different extents, the problems of dose optimization and increase in the antitu-

mour efficiency of melphalan and sarcolysine. In relation to polymeric sarcolysine and melphalan derivatives, the regularities of selective transport of microparticles into tumour-damaged tissue were estimated for the first time; the importance of the rate of drug release from the carrier for high therapeutic index to be achieved is demonstrated. The inclusion complexes of cyclodextrins with melphalan provide solution to the problem of poor drug solubility and instability in aqueous media; they have almost reached the clinical use stage. Dendrimers containing a melphalan residue on the globule surface are examples of nanoparticles with a known surface architecture; in view of the important effect of a nanoparticle 'functional relief' (and its low predictability and uncertainty for nanocarriers as a whole) on the therapeutic action, this issue is of large interest. Targeted liposomes containing a lipophilic melphalan (or sarcolysine) derivative in the lipid bilayer were developed. Liposomes provided with carbohydrate vectors are the first non-immunogenic targeted melphalan nanocarriers.

Among the melphalan nanocarriers obtained to date, inclusion complexes of melphalan with cyclodextrins, which considerably increase the drug solubility and make it possible to avoid the use of organic co-solvents, and liposomes containing a lipophilic sarcolysine (melphalan) derivative in the lipid bilayer approached most closely the clinical application.

The considered data attest to good prospects of the design of nano-scale pharmaceutical drugs based on melphalan and sarcolysine.

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