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Targeted delivery of anti-tuberculosis drugs to macrophages: targeting mannose receptors

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The development of systems for targeted delivery of anti-tuberculosis drugs is a challenge of modern biotechnology. Currently, these drugs are encapsulated in a variety of carriers such as liposomes, polymers, emulsions and so on. Despite successful *in vitro* testing of these systems, virtually no success was achieved *in vivo*, because of low accessibility of the foci of infection located in alveolar macrophage cells. A promising strategy for increasing the efficiency of therapeutic action of anti-tuberculosis drugs is to encapsulate the agents into mannosylated carriers targeting the mannose receptors of alveolar macrophages. The review addresses the methods for modification of drug substance carriers, such as liposomes and biodegradable polymers, with mannose residues. The use of mannosylated carriers to deliver anti-tuberculosis agents increases the drug circulation time in the blood stream and increases the drug concentration in alveolar macrophage cells. The bibliography includes 113 references.

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1. Introduction

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*). Every year, approximately 10 million people in the world become infected with tuberculosis and approximately 2 million people die; more over, 70% of the patients die within 10 years after mycobacteria get into the body.[1–4] Tuberculosis has been known since ancient times — the DNA of *M. tuberculosis* was identified in Egyptian mummies and a description of the symptoms can be found in the works of Hippocrates. In 1905, Robert Koch, a German microbiologist, was awarded the Nobel Prize for the discovery of mycobacterium, which was called 'Koch's bacillus'. Before the advent of antibiotics in the 1940s (streptomycin was discovered in 1944), hundreds of millions of people had died of tuberculosis. The appearance of active anti-tuberculosis drugs (ATDs) such as isoniazid, rifampicin, pyrazinamide and ethambutol in the 1960 – 1970s gave hope for quick and efficient eradication of this disease. Regretfully, this expectation did not come true, and tuberculosis, known as 'white plague', regained its position after 20 years of ATD application.[4] Currently, the World Health Organization recommends using a variety of combinations of ATDs of various groups for the treatment of tuberculosis.

According to the order No. 109 of the Russian Federation Ministry of Health dated March 21, 2003, anti-tuberculosis drugs are classified into two series: basic drugs (isoniazid, pyrazinamide, rifampicin, ethambutol and streptomycin) and reserve drugs (kanamycin, amikacin, capreomycin, cycloserine, rifabutin, *p*-aminosalicylic acid and fluoroquinolones). One more classification is based on the drug efficiency: the first line drugs (most efficient) are isoniazid and rifampicin, the second line ones (with medium efficiency) are ethambutol, streptomycin, ethionamide,

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pyrazinamide, kanamycin, cycloserine and some other, and the third line drugs (moderately efficient) include thiacetazone and *p*-aminosalicyclic acid. The second and third line agents are not only less active against mycobacteria, but are also more toxic and more expensive than the first line drugs.[5]

The treatment of tuberculosis requires a long period of chemotherapy (a minimum of 6 months) with a combination, as noted above, of several ATDs. The first phase of the therapy lasts for approximately 2 months and includes the intake of isoniazid, rifampicin and pyrazinamide. The second phase lasts for 4 months and includes therapy with isoniazid and rifampicin. The necessity of prolonged therapy is due to the life cycle of the bacilli that occur in the latent form (within granulomas). This promotes elimination of *M. tuberculosis* and prevents reactivation of the bacteria in glranulomas. If isoniazid and rifampicin resistance is found, the second and third line ATDs are prescribed, which requires even longer therapy (up to 2 years).[2,6]

The treatment of tuberculosis with ATDs is complicated by a number of factors. The first one is the resistance of *M. tuberculosis* to drug substances: there is multiple drug resistant tuberculosis (strains resistant to both isoniazid and rifampicin) and extensively drug resistant tuberculosis (strains resistant to isoniazid and rifampicin as well as any fluoroquinolone and any of the three second line injection agents).[5] The second complication is degradation of drug substances before they reach the focal point of infection. Isoniazid is prone to condensation with sugars *via* aldehyde and ketone groups and has low bioavailability in the acid medium of the stomach.[7] Rifampicin is hydrolyzed in the gastrointestinal tract to give 3-formylrifampicin or 1-amino-4-methylpiperazine, and its degradation is enhanced by isoniazid and yields the corresponding insoluble hydrazone.[6,8] Anti-tuberculosis drugs are unstable not only in the gastrointestinal tract, but also in the blood plasma: the half-life of rifampicin is 2 – 5 h, that of isoniazid is 1 – 7 h, and that of ethionamide is 2 h.[9] Third, it is virtually impossible to attain a high drug concentration in the foci of infection, which are usually macrophage cells or granulomas (containers for mycobacteria).[10] Fourth, long-term application of ATDs brings about a number of toxic effects. Because of feeling bad, patients often skip doses or completely abandon the therapy.

Thus, it is necessary to design ATD delivery systems that would ensure high concentrations of drug substances in the focus of infection, mitigate the toxic effects and decrease the time of therapy[10] and also would allow overcoming the antibiotic resistance and increase the ATD stability, solubility and absorbability in the gastro intestinal tract.[9] The function of these systems is targeted drug delivery to granulomas and macrophages followed by fast release of ATDs within these particles, which is expected to induce cell immune response and kill the bacteria. Quite a number of carriers for ATD delivery have been developed to solve this problem; unfortunately, none of the currently developed carriers has a 100% efficiency.

The following abbreviations are used in the review:
DPPC — dipalmitoylphosphatidylcholine,
IL — interleukin,
IFNγ — gamma-interferon,
PLG — poly(lactide-co-glycolide),
ATD — anti-tuberculosis drug,
PEG — polyethylene glycol,
SOD — superoxide dismutase,
TNFα — tumour necrosis factor-alpha,
PC — phosphatidylcholine,
CL — cholesterol,
CD — cyclodextrin.

2. Anti-tuberculosis drug delivery systems: key achievements and problems

The most scientifically and practically significant results have been obtained with the use of liposomes (or lipid particles) and biodegradable polymers as carriers for ATD delivery. Liposomes are self-assembling structures consisting of amphiphilic lipid molecules, which form one or several concentric bilayers. In the bilayers, the polar groups of lipids point to the aqueous phase and the hydrophobic groups point inside the bilayers. Owing to this structure, liposomes can encapsulate both hydrophilic and hydrophobic molecules. The liposome particle diameter varies from 20 nm (unilamellar vesicles) to 50 μm (multilamellar vesicles). Liposomes were used for the first time as carriers of therapeutic agents in 1970,[10] five years after they were discovered. Liposomes are employed most widely for encapsulation of anticancer and antimicrobial drugs. The benefits of liposomes include biocompatibility, biodegradability, the lack of toxicity, easy preparation, the possibility of encapsulating a broad range of compounds, control of particle size and the possibility of surface modification.[10 – 12]

Encapsulation of ATDs into liposomes composed of the same lipids as the lung surfactant may provide the targeted delivery of ATDs to alveolar macrophages, which are able to absorb lung surfactant lipids and prevent their excessive accumulation in alveolas. More than a half of lipid content of the lung surfactant is phosphatidylcholine (PC), in particular, dipalmitoylphosphatidylcholine (DPPC); the other components are phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine, cholesterol (CL), triglycerides, unsaturated fatty acids and sphingomyelin.[13,14] As a rule, one or several of these lipids are present in the liposomes used for targeted ATD delivery. It has been found that rifampicin is embedded in DPPC membranes and accumulated in the mass of fatty acid residues.[15] Zaru *et al.*[16] succeeded in selecting the molar ratio PC/CL = 7 : 1,† at which a high degree (70%) of rifampicin entrapment in multilamellar vesicles is attained.

The *in vitro* and *in vivo* effects of liposomes with encapsulated ATDs are determined by not only their composition, but also by the particle size and surface charge. Chono *et al.*[17] have shown that the uptake of

† The liposome components are separated by slash (/) in this review.

liposomes (7 : 2 : 1 mol. PC/CL/dicetyl phosphate) by macrophage cells (NR8383) *in vitro* increases with increasing particle diameter in the 100 to 1000 nm range and then remains invariable until the liposome size is 2000 nm (incubation of macrophage cells with liposome particles for 2 h at 37 °C). The causes for this phenomenon are unknown. When liposomes are administered into lungs of laboratory rats (through nasal cavities), the uptake of particles by alveolar macrophages is enhanced with increasing particle diameter from 100 to 2000 nm, with the 1000 – 2000 nm particles being taken up most intensively (the particle content of macrophages was determined 2 h after the administration). The macrophage uptake of liposomes is induced by their opsonization by lung surfactant proteins. The degree of curvature of liposome surface decreases with increasing particle size; in the authors' opinion, this promotes more efficient opsonization and phagocytosis.

The effect of the liposome charge on the properties of encapsulated ATDs cannot be interpreted unambiguously. The negative charge of dicetyl phosphate present on the surface of PC/CL (7 : 3 mol.) liposomes is favourable for better delivery of rifampicin to alveolar macrophages in which the rifampicin concentration is 1.5 – 3 times higher than that of rifampicin that is free or incorporated in non-modified liposomes. Meanwhile, it has been shown that rifampicin is concentrated in the hydrophobic bilayer of the synthetic positively charged lipid, dimethyldioctadecylammonium bromide. This provides high ATD loading of liposomes (> 75%) and, as a consequence, enhances the antibacterial activity of rifampicin against *M. tuberculosis*.[18, 19]

In *in vivo* experiments, ATDs are introduced into laboratory animals by various administration routes: intravenous, subcutaneous, inhalational and oral (Table 1). In the case of intravenous administration, the concentration of ATD-loaded liposomes after 2 – 6 h is several times higher in the liver and the spleen than in blood plasma and lungs. It was noted that the more rigid the lipid molecules forming the liposome, the greater the amount of ATDs that gets into the internal organs.[20] The use of liposomes increases the mean residence time of ATDs in blood plasma and in the lungs to 2 – 5 days (free ATDs are eliminated from blood plasma and internal organs within 1 – 2 days) and enhances the antibacterial action of ATDs.[20 – 23] An anti-tuberculosis drug encapsulated in liposomes (20 mg of ATD per kg of mouse body weight) induces a considerable (2 – 3-fold) decrease in the production of gamma-interferon (IFNγ) needed for macrophage activation in comparison with the effect caused by administration of free drug substance.

This means that ATD incorporation into liposomes enhances the antibacterial action.[20] Lipids are converted to solid lipid particles (using the emulsion solvent diffusion process), the *in vivo* and *in vitro* effects of which are similar to those of liposomes (see Table 1).[24 – 26]

Despite the above-described benefits, liposome-based ATD delivery systems have quite a few drawbacks. Upon oral administration, liposomes are affected by lipases in the digestive tract. When liposomes get into the blood stream, serum proteins (opsonins) bind to their surface and thus give a signal that is recognized by the phagocytic system, and the liposomes are eliminated from blood stream. The rate of liposome elimination depends on their properties: the greater the particle diameter and charge, the shorter the liposome circulation time.[10] The intravenous injection of liposomes is also restricted by their predominant uptake by liver and spleen macrophages

Table 1. Liposome-based passive anti-tuberculosis drug delivery systems.

Drug	Delivery system	Model (route of administration)	Observed effects	Ref.
Rifabutin	PC/phosphatidylglycerol, dimyristoylphosphatidylcholine/ dimyristoylphosphatidylglycerol, DPPC/dipalmitoylphosphatidylglycerol (7 : 3 mol.), multilamellar vesicles	Mice (intravenously)	ATD accumulation mainly in liver and spleen; clear-cut antimicrobial effect of ATD entrapped in liposomes with a rigid structure	20
Amikacin	PC/CL/distearoylphosphatidylglycerol (2 : 1 : 0.1 mol.)	Humans (intravenously)	Enhancement of the antimicrobial action of ATD in lungs	21
Pyrazinamide	DPPC/CL (7 : 2 mol.), multilamellar vesicles	Mice (subcutaneously)	The same	22
Rifampicin, isoniazid	PC/CL (2 : 1.5 mol.) multilamellar liposomes	Guinea pigs (by inhalation)	Increase in the mean residence time of ATD in lungs to 5 days; increase in the ATD circulation time in blood plasma to 2 days	23
Rifampicin, isoniazid, pyrazinamide	Stearic acid	Guinea pigs (by inhalation), mice (orally)	Longer mean residence time of ATD in blood plasma, liver, lungs, spleen (the drug is detectable after 5 – 10 days); 10 – 20-fold increase in the average retention time of ATD in blood plasma; 5 – 30-fold increase in the overall ATD concentration in blood plasma; and a 10 – 30-fold increase in the bioavailability	24, 25

rather than alveolar macrophages. In the case of inhalational administration, it is important to adjust the optimal liposome size for entering the pulmonary alveoli and for the uptake by the alveolar macrophages. Particles of diameter >15 μm are swallowed and get into the digestive tract, while <0.1 μm particles are mainly accumulated in the upper respiratory passages, trachea and bronchi, and only the particles measuring 0.5 – 2 μm are able to penetrate into the pulmonary alveoli.[27] Thus, ATD delivery systems based on liposomes require further development, first of all, regarding lung targeting of ATD-loaded liposomes.

Like liposomes, matrices made of natural or synthetic polymers are widely used for encapsulation of ATDs. The major effects of the use of polymers as carriers are a decrease in the toxicity of drug substances and more efficient macrophage uptake (as a result of immune stimulation of macrophages or enhancement of electrostatic interactions); also stable particles with high drug contents can be prepared.[28 – 31] The encapsulation of drug molecules into polymer networks occurs by means of various types of interaction (hydrophobic, electrostatic or hydrogen bonding). Polymer particles are highly diversified. Polymeric nano- and microspheres are solid matrices with different pore size, which depends on the degree of chemical cross-linking of polymer molecules. Drug substances can be incorporated into the pores *via* dissolution, adsorption, dispersion or covalent attachment or can occur on the particle surface *via* chemical bonding or adsorption. Polymeric micro- or nanocapsules are particles in which the drug occurs in a solution enclosed in a polymer shell. Polymeric nanomicelles are particles formed by amphiphilic copolymers; in this case, drug substances can be encapsulated in the hydrophobic (or hydrophilic) core, with the particle being coated by a hydrophilic (hydrophobic) shell.[4]

The polymers used for encapsulating anti-tuberculosis drugs include chitosan, gelatine, poly(butyl cyanoacrylate), dextrins, polyamidoamine dendrimers, polyanhydrides, *etc*. However, in these cases, too, some problems could not be avoided. The incorporation into poly(butyl cyanoacrylate) particles increases the toxicity of moxifloxacin towards macrophage cells, although the obtained composition has a more pronounced antibacterial effect *in vivo* than the free drug substance.[32, 33] Polyamidoamine dendrimers are toxic and induce hemolysis.[2] Preparation procedures have been developed, but no *in vivo* data are available for ATD delivery systems based on polymeric micelles, *e,g*., copolymers such as poly(ε-caprolactone-co-glycolide)-PEG-poly(ε-caprolactone-co-glycolide), PEG-poly(lactic acid) or PEG-poly(aspartic acid).[34 – 37]

The polymers most often loaded with ATDs are poly(lactide-co-glycolide) (PLG) and alginate, which have been approved by the US Food and Drug Administration.[4] Isoniazid, rifampicin and pyrazinamide encapsulated in PLG microspheres are detected in blood plasma during 3 – 5 days (mice, oral administration); in the case of pyrazinamide, the maximum concentration (C_{max}) increases approximately 3-fold.[38] In internal organs (lungs, liver, spleen and intestines), ATDs are detected after 7 – 12 days in concentrations higher than the minimum inhibitory concentration. The relative bioavailability of ATDs increases by an order of magnitude.[38, 39]

The subcutaneous administration of ATD-loaded PLG particles is more efficient than the oral administration regarding the time of drug release. Indeed, after a single-shot subcutaneous administration to laboratory mice, rifampicin is released from the hardened PLG microparticles in the internal organs (lungs, liver, spleen) within 42 days,[40] while isoniazid is released within 49 days.[41] This result is very important, because this slow release mitigates the adverse effects of ATDs and increases the bioavailability. The incorporation of both agents in the PLG microparticles ensures the release of isoniazid within 49 days in blood plasma and internal organs, and the release of rifampicin within 21 day in lungs and liver and within 49 days in spleen. Moreover, upon the subcutaneous administration of PLG particles loaded with a combination of isoniazid and rifampicin, a dose – effect dependence was detected. Increase in the doses of both ATDs encapsulated in PLG particles weakens their antibacterial effect (in lungs and spleen) down to the level of a mixture of ATDs administered in the same amounts.[42] The pronounced advantage of the use of PLG microspheres is not only prolonged drug release, but also the absence of hepatotoxicity.[40 – 42] The drawbacks of these systems are low ATD entrapment efficiency (not more than 20%), high consumption of the polymer and a cumbersome administration into the body.

In order to eliminate these drawbacks, ATDs are incorporated into PLG nanoparticles rather than microparticles. For isoniazid, rifampicin and pyrazinamide, the entrapment efficiency into PLG nanoparticles increases in comparison with that for microparticles and amounts to 55% – 70%.[43 – 45] After oral administration of rifampicin-, isoniazid- and pyrazinamide-loaded PLG nanoparticles to guinea pigs or laboratory mice, the ATD circulation time in blood plasma increases from 1 – 2 to 6 – 13 days and that in lungs, liver and spleen increases to 7 – 14 days. In the blood plasma of mice, C_{max} of the ATDs does not change, while in the case of guinea pigs, it increases by factors of 2 for pyrazinamide and 3 for isoniazid.[43, 44] These effects are comparable with the ATD effects in PLG microparticles. Anti-tuberculosis drugs introduced in PLG nanoparticles, like in microparticles, do not possess hepatotoxicity, and their relative bioavailability increases, on average, by 1 – 2 orders of magnitude.[45]

The use of PLG nanoparticles as carriers for ATDs ensures high entrapment efficiency of the drug substances into particles, lower polymer consumption and longer occurrence of the ATDs in blood plasma. However PLG contains organic solvent impurities, which have a toxic effect on the body; the most popular substitute for PLG is alginate.

Rifampicin, isoniazid and pyrazinamide are incorporated in alginate microparticles with entrapment efficiency of 20% – 90%,[46, 47] and the *in vivo* effects of ATDs in alginate and PLG particles have much in common. The time of drug circulation in blood

Table 2. Anti-tuberculosis drug delivery systems.

System	Particle composition	Drug	Drawbacks	Benefits	Ref.
Composites	Mesoporous silicon/PLG	Rifampicin	Complicated preparation technique	Prolonged release of ATD *in vitro*	49
	Ethylcellulose/chitosan	Rifabutin	The same	The same	50
Metal complexes	Cu^{II}-PEG-saccharose-ATD, Cu^{II}-PEG-glucose-ATD	Isoniazid	Toxicity of complexes	Enhancement of the antimicrobial action and increase in ATD binding to cell membrane *in vitro*	51
	Cu^{II}-PEG-ATD	Amikacin	The same	The same	52
	Cu^{II}-PEG-ATD	Rifampicin	"	"	53
	Cu^{II}-ATD	Capreomycin	"	"	54
Nano(micro)-suspensions and emulsions	Pluronic F68, phospholipon 90, sodium cholate	Clofazimine	Low load of ATD particles	Particle stability, reproducibility of procedures	6
	Gelatine/papain/chitosan/ Tween	Rifampicin, isoniazid, pyrazinamide	Complicated adjustment of component ratio	Prolonged drug release from the particles *in vitro*	55
	Sefsol 218, Tweens	Rifampicin	–	High particle stability, high ATD entrapment efficiency	56
Niosomes	Span 85/CL, spans/CL	Rifampicin	Complicated preparation procedures	High ATD concentration in blood plasma and internal organs *in vivo*	57 – 59

Note. Here and in Table 3 a dash means the absence of the data.

plasma increases to 4 – 5 days upon their oral administration to guinea pigs[46] and to 9 – 14 days upon inhalation.[47] In internal organs (lungs, liver, spleen, intestines), ATDs are detected during the period of up to 9 – 15 days in concentrations above the minimum inhibitory concentration.[46, 47] Moreover, alginate particles, like PLG, do not possess hepatotoxicity, and the relative bioavailability of the encapsulated ATDs increases by an order of magnitude in comparison with that for free drugs.[46] After 8 weeks of weekly administration of ATDs in alginate microparticles (8 doses of ATDs in microparticles are equivalent to 57 doses of free ATD), no bacilli were found in lungs, liver or spleen.[46] The inhalation of 3 doses of ATDs in alginate microparticles is equivalent to 45 doses of orally administered free ATDs.[47] Similar effects were observed for the rifampicin-, isoniazid- and pyrazinamide-loaded alginate – chitosan conjugate microparticles.[48] The major drawback of alginate particles (similarly to PLG) is the presence of impurities of toxic organic solvents; therefore, they are sparingly used for ATD delivery.

Systems such as composite materials, emulsions, ATD complexes with metals have been studied and described in less detail. Composite materials are manufactured from polymers, for example, PLG, and low-molecular-weight (mesoporous silicon) and high-molecular-weight (ethylcellulose) compounds.[49, 50] The key effect detected for systems of this type is the time of ATD release *in vitro*. It has been found that particles based on metal complexes of these agents inhibit the growth of the *M. tuberculosis* culture,[51 – 54] but no decrease in the drug toxicity is noted. Niosomes and surfactant-, polymer-, protein- and lipid-based emulsion systems[55 – 59] can also encapsulate ATDs; however, the obtained experimental data are rather contradictory (Table 2). To date, no positive results of *in vivo* and *in vitro* experiments are available and the methods for investigation of such systems should be considerably improved.

A promising process for the fabrication of new dosage forms is the supercritical fluid antisolvent precipitation technique (with supercritical CO_2 as the antisolvent) for the synthesis of micronized forms of levofloxacin and moxifloxacin — reserve fluoroquinolone anti-tuberculosis drugs.[60 – 62] Levofloxacin has been prepared as microparticles differing in size (1 – 10 μm) and morphology (thin plates, parallelepipeds, irregularly shaped aggregated particles) without a change in the chemical structure of the drug substance. The particle shape and solubility (at concentrations above 6 mg ml^{-1}) in model media (pH 7.5 and 4.0) depend on the type of the solvent used for micronization. The use of chlorinated hydrocarbons (chloroform, dichloromethane and 1,2-dichloroethane) gives rise to plate-like particles; precipitation from methanol, dimethyl sulfoxide or dimethylformamide affords microparticles shaped as oblong parallelepipeds. The highest dissolution rate in acid and neutral media is observed for the particles micronized in chloroform (water-immiscible solvent unable to form hydrogen bonds) and DMF (water-miscible solvent able to form hydrogen bonds). Apparently, the crystallization mechanisms for the drug substance are different for different solvents and, as a consequence, the micronized samples have different morphology,

which can affect the solubility.[60,61] Similar phenomena were detected for supercritical antisolvent precipitation micronization of moxifloxacin.[62]

Despite the presence of numerous publications, any and all ATD delivery systems are still at the laboratory research level. There are several patented inventions, discussed in a review,[2] but they do not refer to ATD delivery systems. The fact that many agents are inefficient against intracellular bacteria is due to very low accessibility of the focus of infection caused by interaction of mycobacteria with macrophages. Novel ideas are needed to boost the development of efficient carriers for ATDs and their further advancement to the pharmaceutical industry. From this standpoint, a promising idea is to design delivery systems targeting the macrophage receptors.

3. Interaction of alveolar macrophages with *Mycobacterium Tuberculosis*, macrophage receptors

Mycobacteria get into the human body through the respiratory passages and, in the most optimistic scenario, they are killed by mucociliary clearance. If the clearance system is impaired, the innate immune system starts to act. In the alveolar space, *M. tuberculosis* can be phagocytized by alveolar macrophages, epithelial cells, dendritic cells and neutrophiles.[63,64]

In the phagocytosis of mycobacteria by alveolar macrophages, the contacts between them are promoted by hydrophobicity of the *M. tuberculosis* cell wall and the presence of molecules functioning as ligands for macrophage receptors on their surface. The phagocytosis is accompanied by the formation of phagolysosomes, in which destruction of the pathogen cells takes place. Phagolysosomes are formed *via* fusion of phagosomes that encompass mycobacteria with lysosomes containing hydrolytic enzymes. Apart from the formation of phagolysosomes, other impacts damaging *M. tuberculosis* include the synthesis of reactive oxygen species by the membrane NADPH oxidase (NADPH is nicotinamide adenine dinucleotide phosphate) and reactive nitrogen species by the inducible NO-synthase. The ability of the alveolar macrophages to suppress the growth of mycobacteria is controlled by cytokines such as tumour necrosis factor-α (TNFα) and INFγ produced by T-lymphocytes. However, in some cases, the pathogenic *M. tuberculosis* cells survive and replicate within the macrophage cells, and migrate from lungs to regional lymph nodes, internal organs and central nervous system *via* lymphatic circulation; this gives rise to extrapulmonary tuberculosis.[10]

The ability of *M. tuberculosis* to survive inside the macrophage cells is associated with several factors. The internals of mycobacterial cells are reliably protected by the thick cell wall composed of lipids, glycolipids, mycolic acids, peptidoglycans, carbohydrates and proteins. The cell wall of *M. tuberculosis* creates a diffusion barrier for hydrophilic molecules and reactive oxygen and nitrogen species, and its high hydrophobicity maintains the stability against lysosomal enzymes of the alveolar macrophage cells.[10] Mycobacteria give off ammonia, which increases pH inside the phagosomes and prevents them from fusion with lysosomes (pH ~5) containing hydrolytic enzymes. Complexes of the cord factor with the sulfatides of the *M. tuberculosis* cell wall lead to neutralization of membranotropic cationic peptides, thus providing destruction of phagosomes. Mycosides and factors with the catalase and peroxidase activities inactivate oxygen free radicals. Endotoxins such as 5-mycoloyl-D-arabinose and its analogues suppress the respiration of the mitochondria of alveolar macrophage cells. Non-specific toxins — phthienoic and phthionic acids — disrupt the ATP synthesis, which results in energy deficiency of macrophage cells and subsequently in their apoptosis and cytolysis. Mycobateria induce a decrease in the calcium ion concentration within the cells, thus preventing maturing of phagosomes and disrupting phosphorylation of the proteins required for transcription of proinflammatory cytokines and reactive oxygen and nitrogen species. The *M. tuberculosis* bacteria release two metal ion-containing forms of superoxide dismutase (SOD) — Fe-SOD and Cu,Zn-SOD, which protect mycobacteria from reactive oxygen species and can inhibit NO-synthases, INFγ production and apoptosis of infected macrophage (non-aggressive phagocytosis).[9,65]

The alveolar macrophages with encapsulated mycobacteria represent the major components of granulomas — groups of cells accumulated at the site of infection. Granulomas consist of infected macrophage cells (including foamy macrophages), epithelioid cells, T- and B-lymphocytes, extracellular matrix components and so on.[4,9] Upon interaction with mycobacteria, macrophages produce cytokines TNFα and interleukins (IL1, IL6, IL10) until *M. tuberculosis* is completely destroyed. In the case of incomplete phagocytosis, cytokines act on T-lymphocytes, which migrate and gather around the macrophages, giving rise to granulomas.[64] Mycobateria internalize lipids of the host cell, thus forming macrophages with a highly vacuolated cytoplasm, which contain a large excess of various lipids.[10,13,14] *M. tuberculosis* bacteria cause destructive changes of the cytoskeleton of macrophage cells, with the subsequent cell fusion giving rise to multinuclear giant Pirogov – Langhans cells.[64] Latent tuberculosis in which mycobacteria reside in granulomas can last for years. The action of granulomas is dual: on the one hand, they isolate the pathogens and prevent them from spreading over the body, but, on the other hand, they are 'biological shelters' for *M. tuberculosis*,[10,65] because the mycobacteria in granulomas get nutrients for their vital activity.

As noted above, mycobacteria interact with alveolar macrophages, since the latter possess receptors recognizing pathogen cell wall components (pattern-recognition receptors, PRR). The pattern-recognition receptors of alveolar macrophages represent a large group of compounds of various nature, comprising toll-like receptors (TLR), complement receptors, (CR), scavenger receptors (SR) and Ca^{2+}-containing C-type lectin-like receptors. These receptors recognize cell wall components (lipoproteins), CpG-DNA (CpG are phosphate-separated cytosine and guanine islands), phosphatidylinositol mannoside, mannose and so on. The receptors activate signalling pathways of alveolar macrophages, thus inducing secretion of anti-inflam-

matory cytokines, chemokines and antimicrobial molecules, killing the bacilli or inducing the formation of granulomas.[63, 66–68]

The key role in the interaction of macrophages with bacteria is played by toll-like receptors, which are transmembrane glycoproteins comprising ten family members located either on the cell membrane (TLR1, TLR2, TLR4, TLR5, TLR6, TLR10) or on the endoplastic reticulum membrane (TLR3, TLR7, TLR8, TLR9). The recognition of mycobacteria by alveolar macrophages is accomplished with the receptors TLR2 and TLR4, the ligands of which on *M. tuberculosis* surface are arabinomannan and galactomannan. The recognition occurs apparently *via* the interaction with the surface active proteins A and D of the lung surfactant, which play an important role in antimicrobial protection through bacterial agglutination.[69]

Among the macrophage receptors responsible for the recognition of foreign bacteria, complement receptors 1, 3, 4 (CR1, CR3, CR4; complement factor C3 receptors) are significant. The mediated reactions that are excited by C3 factor derivatives located on the surface of mycobacteria play an important role.[70]

The class A scavenger receptors occur on the surface of macrophage cells and comprise three representatives: SR-AI, SR-AII and the macrophage receptor with collagenous structure (MARCO). These membrane glycoproteins are sulfolipids and can bind modified low density lipoproteins and other polyanions of *M. tuberculosis* cell wall components. The receptor molecules contain a collagen-like domain with a polyanion binding site, a cysteine rich domain and an α-helical domain.[71]

Macrophages are prospective targets for therapeutic agents containing carbohydrates due to the presence of C-type lectins. The receptors are located in the cytoplasmic membrane and recognize carbohydrates of microbial origin, which are often related to post-translational modification of proteins. Receptor molecules contain one or several carbohydrate recognition domains and are classified into three groups. The first group includes the mannose receptor CD 206 and receptor DEC-205, which are type I transmembrane proteins containing several carbohydrate recognition domains. The second group of receptors consists of type II transmembrane proteins containing carbohydrate recognition domains. These are dectins and dendritic cell-specific intercellular-adhesion-molecule-3-grabbing non-integrin (DC-SIGN). The third group (soluble forms) includes mannose-binding lectin (MBL).[66, 72]

Dectins 1 and 2 play an important role in the innate immune system and interact with molecules of cell wall of fungal infectious agents. Dectin 1 binds β-glucans, while dectin 2 binds carbohydrates with high mannose content and acts as a functional receptor for α-mannans. Apart from the carbohydrate recognition domain, dectin 1 molecule contains an immunoreceptor tyrosine-based activation motif (ITAM), which triggers cell activation. The physiological role of dectins is to stimulate the formation of reactive oxygen species and to activate the intracellular NF-κB signalling pathway and secretion of anti-inflammatory cytokines.[73] The dectin 2 family includes macrophage-inducible C-type lectin (Mincle) recognizing mycobacteria, the surface of which bears trehalose-6′6′-dimycolate (better known as cord factor).[74, 75]

The transmembrane protein DC-SIGN interacts with mycobacteria and contributes to regulation of adaptive immunity. The DC-SIGN molecules contain one carbohydrate recognition domain and form tetramers on the surface of dendritic cells.[72] Mannose-binding lectin is an oligomeric protein that binds the array of carbohydrate structures on the pathogen surfaces. The mannose-binding lectin molecules form trimers, which are, in their turn, combined into hexamers.[76]

The macrophage mannose receptor (CD 206) is a transmembrane protein, the N-terminal part of which is provided with a short (45 amino acid residues) cytoplasmic moiety. Mannose receptors are expressed by most macrophages, the highest expression level being inherent in the hepatic endothelial cells, renal mesangial cells, smooth muscle cells and eye retina pigment epithelial cells.[77, 78] The expression of mannose receptors is stimulated by vitamin D and interleukins 4, 10 and 13 and suppressed by INFγ and TNFα. The extracellular part of the receptor, which is responsible for binding to mannose residues, consists of a short NH_2-terminal cysteine rich domain (CysRD), fibronectin II repeat (FRII) and C-type lectin-like domain (CTLD).[66]

The CysRD domain consists of 147 amino acids, contains 6 cysteine residues and binds glycoproteins bearing a terminal 4-sulfo-*N*-acetylglucosamine, for example, lutropin and thyrotrophic hormone. It was shown that sulfated *N*-acetylglucosamine also forms strong hydrogen bonds with the cysteiene groups of mannose receptors. The C-lectin-like domain consists of eight carbohydrate recognition domains (CRD). The CRD domains bind sugars (*N*-acetylglucosamine, fucose, mannose) and contain two β-helices and two antiparallel β-sheets, and also calcium cations (Ca^{2+}) for carbohydrate binding. The mannose and fucose residues possess higher affinity to the mannose receptor than *N*-acetylglucosamine.[66, 68]

To conclude this Section, we would like to emphasize the important role of the spatial arrangement of the carbohydrate recognition domains in receptor molecules. The linear arrangement of carbohydrate recognition domains in the polypeptide chain of the mannose receptor is convenient for recognition of the terminal mannose molecules. The carbohydrate recognition domains of the DC-SIGN tetramer have a higher affinity to mannose residues in branched structures with a small distance between mannose residues. The trimers of mannose-binding lectin molecules combined into hexamers are suitable for binding ligands with high density of mannose residues.[72]

Specific features of the tuberculosis infection impose restrictions on the ATDs being developed. The uptake of drug substance particles by macrophages can be enhanced by not only control of their physicochemical properties (size, charge, presence of surface modification agents), but also the application of ligands specific to various types of macrophage receptors. The alveolar macrophages containing myco-

bacteria in phagosomes are able to phagocytize drug-loaded particles in such a way that the *M. tuberculosis* cells and ADT-loaded particles are not co-localized.[9] The next stages are the formation of phagolysosomes (in which the mycobacteria and the particles occur together) and slow particle degradation with release of ATD, which kills *M. tuberculosis* bacilli located in the phagolysosomes. The simplest structure is inherent in ligands for macrophage mannose receptors, out of which mannose residues have the highest receptor affinity. Moreover, mannose is an inexpensive and readily accessible reagent. Thus, active targeting of macrophage mannose receptors can be considered as an efficient strategy for the design of targeted ATD delivery systems.

4. Mannosylated carriers for anti-tuberculosis drug delivery

4.1. Mannosylated liposomes

There are three methods for the preparation of mannosylated liposomes:[72]

1) synthesis of mannose – lipid conjugates followed by formation of liposomes,

2) covalent binding of mannose derivatives to liposomes,

3) adsorption of mannose derivatives on the liposome surface.

These methods are rather simple and convenient, they do not require much time or complex equipment. According to the first method, a mixture of lipids with mannose (or its derivative) is dissolved in an organic solvent. Then the solvent is evaporated in a vacuum until a dry lipid film is formed; the film is treated with an aqueous solution, which gives a liposomal dispersion. If necessary, the dispersion is disintegrated by extrusion or sonication.[72, 79] The hydration and/or disintegration are accompanied by spontaneous formation of modified liposomes in which the mannose residues are hydrophilic molecular moieties pointing towards the aqueous phase.

In the second case, the covalent modification of liposomes is accomplished *via* reactions between their surface groups and modifying agents. When the direct chemical reaction between liposomes and modifying agents is impossible, bifunctional agents are used, which react with both the liposome surface groups and the groups of modifying agents. For example, mannosylation of the surface of liposomes consisting of phosphatidylethanolamine with *p*-aminophenyl-α-D-mannopyranoside is conducted using glutardialdehyde.

According to the third option, liposomes are merely incubated with mannose or its derivatives.[72] Methyl-α-D-mannopyranoside is embedded by its methyl group into the hydrophobic layer of lipid particles composed of cholesterol myristate and the palmitic acid – tripalmitin system.[80]

Only few papers describing the effects of mannosylated liposomes *in vitro* and *in vivo* have been reported to date. However, despite the scantiness of data, researchers have managed to cover and disclose a variety of aspects of the observed phenomena. The key conclusion is that macrophage interaction with mannosylated liposomes occurs *via* receptor-mediated endocytosis. This means that the macrophage uptake of mannosylated liposome particles is determined by interaction of mannose residues on the liposome surface with the macrophage mannose receptors. The modification of lipid particles or liposomes with man-

Table 3. Effects of mannosylated liposomes in *in vitro* experiments.

Liposome composition (component ratio, mol.)	Properties of the particles [a]	Drug	Macrophage cell line	Observed effects	Ref.
PC/dioleylphosphatidylcholine/CL/dicetyl phosphate (3.5 : 3.5 : 2 : 1 : 1 [b])	1 μm, −70 mV	Ciprofloxacin	NR8383	Enhancement of the macrophage uptake	11
PC/CL/dicetyl phosphate (7 : 2 : 1 : *n*,[b] where *n* = 0.25, 0.5, 1, 2)	1 μm, −55 mV	–	NR8383	Enhancement of the macrophage uptake with increasing degree of mannosylation	12
Stearylamine/soya lecithin/tristearin	389 nm, −11.7 mV	Rifabutin	J774	Enhancement of the macrophage uptake, longer time of ATD release	81
PC/CL/dicetyl phosphate (7 : 2 : 1 : 1 [b])	1 μm	–	NR8383	Increase in the macrophage uptake of the particles	17
Soybean oil/PC/cholesten-5-yloxy-*N*-{4-[(1-imino-2-D-thiomannosylethyl)amino]butyl}formamide (75 : 30 : *n*, where *n* = 2.5, 5.0, 7.5)	80 – 90 nm, −1 to 7 mV	–	Macrophages from the lungs of laboratory mice	Dependences of the macrophage uptake of mannnosylated liposomes on the degree of modification, temperature and incubation time were revealed	82
1,2-Distearyl-*sn*-glycero-3-phosphocholine/CL/cholesten-5-yloxy-*N*-{4-[(1-imino-2-D-thiomannosylethyl)amino]butyl}formamide (60 : 35 : *n*, where *n* = 0, 2.5, 5, 7.5)	90 – 125 nm, −9 to 15 mV	–	Macrophages from the lungs of laboratory rats	The same	83

[a] The size and ζ-potential characterizing the electrical double layer are presented; [b] the last numeral is the amount of mannose.

nose gives rise to negatively charged particles of mainly micrometre size (Table 3).

Nimje *et al.*[81] obtained rifabutin-loaded mannosylated solid lipid particles. The decrease in the ATD entrapment efficiency for mannosylated particles (82.6%) in comparison with that for non-modified particles (87.2%) can be considered to be an undesirable consequence of using this technique. Mannose reacts with the amino groups on the surface of solid lipid particles at pH 4.0, where the rifabutin solubility is the highest, which results in the loss of the drug substance adsorbed on the particle surface. It is worth noting that despite this decrease, the ATD entrapment efficiency into mannosylated solid lipid particles still remains at a high level. As a benefit, one can consider the slower *in vitro* release of ATD (in the phosphate buffered salt solution, 65% of rifabutin is released within 120 h at 37 °C) from mannosylated lipid particles than from non-modified particles (89% of ATD).

A principal and very important result of mannose modification of liposomes is the enhancement of their uptake by macrophages *in vitro* (see Table 3). The ratio of fluorescence signals of modified and non-modified fluorescein isothiocyanate (FITC)-labelled lipid particles in macrophage cells (J774) after 6 h of incubation is 5.81.[81] A twofold enhancement of the macrophage (NR8383) uptake of particles upon mannosylation has been observed by Chono *et al.*[11,17] for liposomes of various composition. An increase in the degree of mannosylation of liposome microparticles [(0, 2.4, 4.8, 9.1 and 16.7) mol.% mannose] or lipid-based nanoemulsions (2.5, 5.0, 7.5 mol of mannose residues per particles) results in increasing uptake of the particles by macrophages.[12,82] It has been assumed[11,12,17,81,82] that particle mannosylation enhances the uptake because of interaction with mannose receptors on the macrophage cell surface (receptor-mediated endocytosis).

The regulation of the interaction of macrophage mannose receptors with mannosylated liposomes *via* the receptor-mediated endocytosis was confirmed by inhibition experiments. The amount of mannosylated liposomes taken up by macrophages decreases in the presence of mannan or mannose. It can be stated with confidence than mannan (mannose) behaves as a competitive inhibitor by binding to the macrophage mannose receptors instead of modified liposomes.[12,83] The internalization of particles of mannosylated lipid emulsions by macrophages is weakened upon macrophage pretreatment with iodoacetate, which is in line with the above data. Treatment of a mixture of macrophages and mannosylated emulsions with ethylenediaminetetraacetate (EDTA) or acetate buffer (pH 4.0) reduces the particle association with the cell surface; hence, the interaction of mannose receptors with mannosylated particles is controlled by not only mannose receptors, but also calcium cations.[82]

The amount of mannosylated liposomes taken up by macrophages is determined by the properties of the medium, that is, it increases with increasing concentration of liposomes in a mixture of alveolar macrophages, increasing time of co-incubation and with temperature rise (in the 0–200 μmol litre^{-1} range of liposome concentrations at temperatures of 4 and 37 °C for 0–2 h).[83] Mannosylated emulsions based on lipids interact with macrophages in a similar way.[82]

It was found[84] that the PC/CL/*N*-glutarylphosphatidylethanolamine (55 : 45 : 11.2 mol.) liposomes bind equal amounts of lectins (*Ricinus communis* agglutinin and *Canavalia ensiformis* lectin) provided that the amounts of sugar residues per liposome are equal. Binding of modified liposomes to lectins is inhibited by mannan and azelofein (compounds containing mannose and galactose terminal residues). This attests to the presence of some specific interactions of the mannosylated liposome surface with lectins, which are considered as models of mannose receptors.

According to the results of *in vivo* experiments,[83] the alveolar macrophage uptake of mannosylated particles of a lipid-based emulsion is enhanced with increasing degree of modification (2.5–7.5 moles of mannose per particle). The addition of mannan (5 mg per kg of body weight for laboratory mice) induces a decrease in the uptake of mannosylated particles (competitive inhibition) located within the macrophage cells; the uptake of non-modified particles is not affected by mannan. These two experimental facts, as well as *in vitro* studies,[11,82] confirm that macrophages interact with mannosylated lipid particles *in vivo* by the receptor-mediated endocytosis mechanism. As the density of mannose residues on the liposome surface increases, the number of binding sites between the liposome and macrophage increases and, hence, the uptake of the particles grows. Targeting of the alveolar macrophage cells by mannosylated liposomes is confirmed by the fact that the liposomes are virtually absent in type II alveolar cells. Similarly, the presence of lung surfactant *in vitro* (0.1 mg ml^{-1}, 37 °C) does not affect the macrophage uptake of mannosylated particles.[83]

When ATDs are encapsulated in mannosylated lipid particles, the following advantages appear: targeted ATD delivery to the damaged organ and prolonged release in the blood stream.[81] Free rifabutin is utilized in 48 h from the blood serum of laboratory rats, in 78 h from solid lipid particles and in 96 h from mannosylated particles (upon single-short intravenous administration of 4.2 mg rifabutin per kg of body weight). This effect has been attributed to slower rifabutin diffusion from particles (mannose coating of the particles retards the diffusion), resulting in a slower change in the drug level in blood serum. A study of rifabutin biodistribution among the organs demonstrated that upon administration of free or lipid particle-encapsulated ATDs, the highest drug concentration is reached in spleen, while in the case of ATDs incorporated into mannosylated solid lipid particles, the highest concentration is in lungs (receptor-mediated endocytosis) and in liver. The size of ATD-loaded mannosylated particles (approximately 400 nm) is optimal for their retention in phagocytes, whereas rifabutin-loaded non-modified particles (250 nm) are small and, hence, their concentration in lungs is lower.

The targeting of mannosylated liposomes is confirmed by comparison of the pharmacokinetic param-

eters of modified and non-modified particles (in the absence of ATDs). The bronchial wash of laboratory rats (intranasal introduction of liposomes in a quantity of 5 μmol of lipids per kg of body weight) showed enhanced accumulation of liposomes upon modification. The total concentration of mannosylated liposomes (over 24 h) is 2.2 times higher and the maximum concentration is 3 times higher than these values for non-modified particles.[17] Similar data have been obtained in a study of pharmacokinetics after pulmonary administration of mannosylated liposomes.[12] Mannosylated liposomes are eliminated from the respiratory tract within 24 h after administration.[12,17]

Similar results have been obtained in *in vivo* experiments with drug-loaded liposomes (intratracheal administration of ciprofloxacin in liposomes to laboratory rats, 166 μg of the ATD per kg of weight). The C_{max} of ciprofloxacin in alveolar macrophages is twice higher when it is introduced in mannosylated liposomes (~900 μg of ATD in 1 ml) than in the case of non-mannosylated liposomes (~400 μg of ATD in 1 ml) or free drug (<100 μg of ATD in 1 ml). The total concentration of ciprofloxacin (over 24 h) in alveolar macrophage cells is 20 times higher when it is encapsulated in mannosylated liposomes than in the free state. Unfortunately, like the free drug, encapsulated ciprofloxacin is not detected in the alveolar macrophages 24 h after getting into the body.[11]

As a drawback of mannosylated liposomes for the targeted drug delivery, one can mention the absence of prolonged drug release. From this standpoint, the use of solid lipid particles is more preferred. The use of mannosylated liposomes for the ATD delivery can be justified for increasing the overall and maximum ATD concentrations in lungs after administration by various methods.

It is noteworthy that mannose is more beneficial for liposome modification than, for example, fucose or galactose (other ligands of macrophage mannose receptors). Galactosylated liposomes are accumulated in hepatocytes (*in vivo* experiments in mice) in much higher amounts than mannosylated liposomes.[84,85] Fucosylated liposomes are mainly accumulated in the non-parenchymal liver cells, which have a large number of fucose receptors.[85]

4.2. Mannosylated polymers

There is much less information concerning the *in vivo* and *in vitro* effects of mannosylated polymers than mannosylated liposomes. The amount of nanoparticles of mannosylated polystyrene–PEG ($PS_{1.5k}$-β-PEG_{5k}) block copolymer that *in vitro* bind to macrophage cells (J774E) depends on the degree of mannose modification of their surface. The highest (three times as high as that for non-modified particles) binding of mannosylated block copolymer particles to macrophage cells is observed when the degree of mannose modification is 9%. The enhancement of particle binding to macrophages with increasing degree of mannosylation is caused by the multivalent interactions between the polymer and mannose receptors on the macrophage cell surface (receptor-mediated endocytosis). By analogy with liposomes, macrophage binding to a mannosylated polymer is also inhibited by sugars. The amount of mannosylated nanoparticles bound to macrophage cells decreases by 69% in the presence of dextran (60–80 kDa), which functions as a competitive inhibitor. The decrease in the amount of particles bound to macrophages on further increase (>9%) of the degree of particle surface mannosylation may be attributed to the fact that the formation of too many receptor–mannose contacts increases the rigidity of cell membrane or leads to overstimulation of intracellular signalling pathways mediating endocytosis.[86]

A study of interaction of isoniazid-loaded mannosylated gelatine microspheres with macrophage cells (*ex vivo*) showed an increase in the ATD concentration within the cells (the uptake is twice larger for modified particles than for non-mannosylated ones) and inhibition of the macrophage uptake of the modified microspheres by mannose (0.05 mol litre^{-1} of mannose decreases the intracellular ATD concentration 8-fold). Free isoniazid is metabolized more rapidly within macrophage cells (~20% of ATD lost in 24 h) than that encapsulated into mannosylated gelatine microspheres (~5% of ATD lost). In *in vivo* experiments, the maximum ATD concentration in blood plasma decreased and the time required to reach this concentration increased when isoniazid-loaded mannosylated particles were used (201 ng ml^{-1}, 8 h), as compared with non-modified particles (318 ng ml^{-1}, 2 h).[7]

Mannosylated 5th-generation polypropyleneimine dendrimers interact with rifampicin molecules *via* hydrophobic and electrostatic interactions and hydrogen bonding, which provides incorporation of ATD molecules into the polymer network (the rifampicin entrapment efficiency is moderate, ~37%). The solubilizing ability of dendrimers decreases by approximately an order of magnitude upon mannosylation, because the aldehyde groups of mannose bind to the charged amino groups to give Schiff bases, thus decreasing the electrostatic component and creating steric hindrance to the access of the drug substance into the dendrimer cavity.[87]

The mannosylation of polypropyleneimine dendrimers decreases the hemolysis of red blood cells as a result of inhibition of their interaction with quaternary ammonium ions, which are present in non-mannosylated dendrimer molecules. Mannosylated polymer particles are non-toxic [determined by the MTT assay; MTT is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] due to shielding of positively charged groups by mannose residues. Mannosylation of the particles provides prolonged release of rifampicin at pH 7.4 (100% of ATD is released in 90 h). The tertiary nitrogen atoms within the dendrimer cavities are highly basic (pK 9.5) and cause deprotonation of rifampicin molecules (pK for ionogenic groups are 1.7 and 7.9). The quaternary nitrogen atoms of dendrimers form ion pairs with deprotonated groups of ATD, while mannose residues create steric restrictions preventing rifampicin molecules from coming out of dendrimer cavities.[87]

Mannosylation procedures for structurally sophisticated polymers [chitosan, poly(ethylene glycol), cyclodextrins] widely used in medicine have been well

developed. This can be regarded as an important step forward in view of the complexity of not only the chemical reactions involved, but also selection of modification conditions.

4.3. Chitosan

Chitosan is a natural polysaccharide — a biodegradable, biocompatible and non-toxic polymer. The application of chitosan as a carrier for ATDs is related to its ability to decrease the toxicity of antibacterial agents and enhance the uptake of ATD-loaded particles by macrophages (owing to immune stimulation).[88] Chitosan mannosylation occurs through the reactions of chitosan amino groups with mannose or its derivatives. Three types of functional groups accessible for modification are present in the chitosan molecule: primary amino groups and primary and secondary hydroxyl groups. The mannosylation is performed at the amino groups by means of reactions presented below. Most often, these are nucleophilic addition reactions.

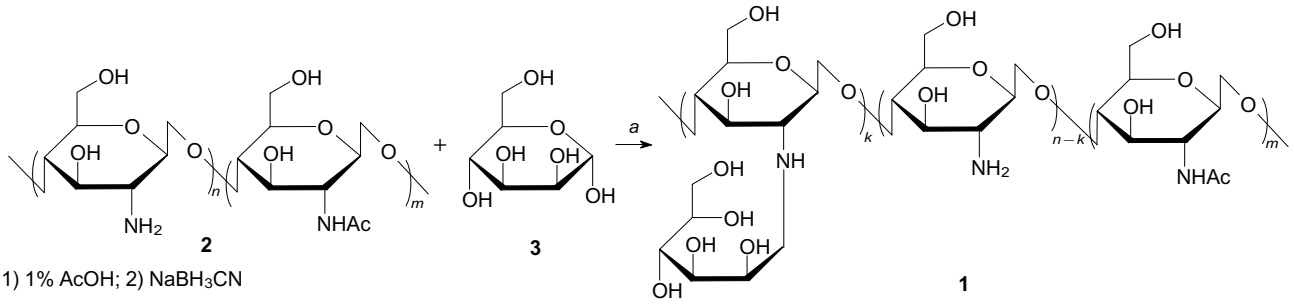
The simplest route to mannosylated chitosan **1** is the direct reaction of chitosan (**2**) with α-D-mannose (**3**). The reaction is accompanied by mannose ring opening and gives the Schiff base upon the reaction of the sugar aldehyde group with the primary amino groups of chitosan; the Schiff base is then reduced with sodium cyanoborohydride (Scheme 1).

The chitosan reaction with mannose takes place in acid medium (1% acetic acid, pH 5.5). The reaction of chitosan with a mannose derivative such as 1-α-formylmethyl-α-D-mannopyranoside (**4**) and with mannobiose (**5**) proceed in a similar way (stirring of reactants in an acid medium at room temperature with the subsequent addition of sodium cyanoborohydride) (Scheme 2).

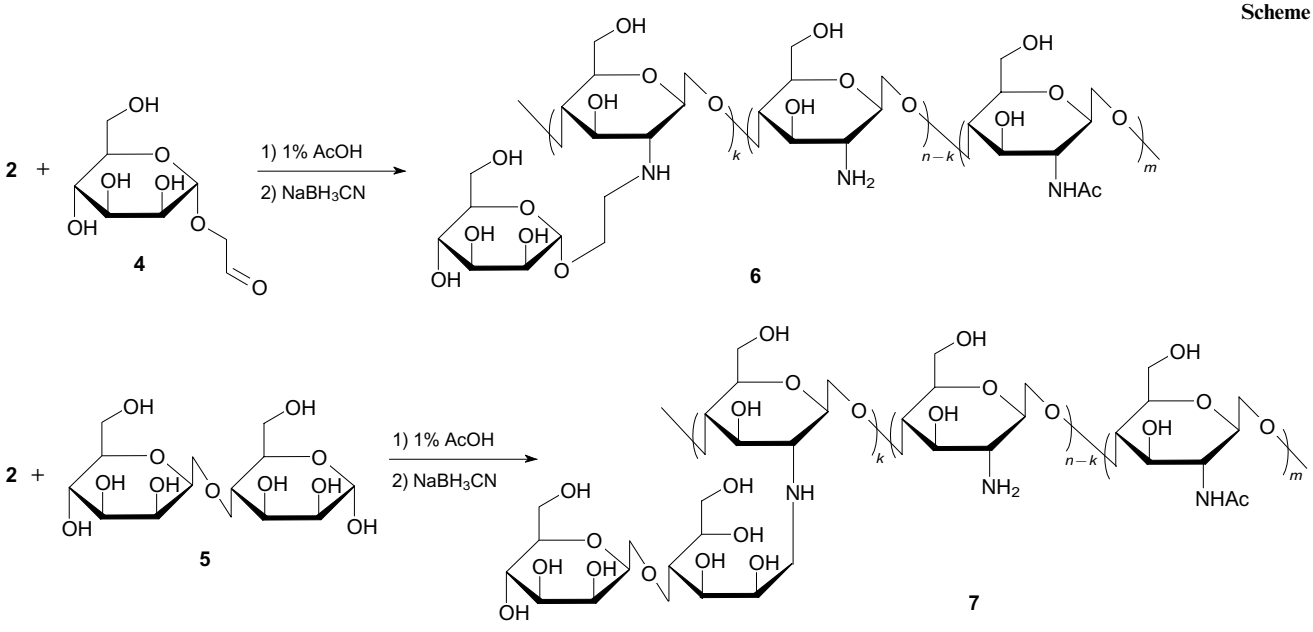
These reactions tend to give products **6** and **7** in moderate yields (< 50%). The mannosylated chitosan derivatives are purified from low-molecular-weight components by dialysis and characterized by elemental analysis, thermogravimetry and NMR and IR spectroscopy.[89–94]

The intravenous injection of rifampicin encapsulated in mannosylated chitosan particles to laboratory rats (12 mg of ATD per kg of weight) results in an ATD concentration in liver after 2 h being 20% higher that that observed upon the injection of rifampicin encapsulated in non-mannosylated particles and 50% higher than that detected for free ATD. The same values for spleen are 10% and 15%. The half-life of the agent in the blood stream increases from 1.69 h (for free rifampicin) to 40.42 h (ATD in mannosylated chitosan particles); the maximum concentra-

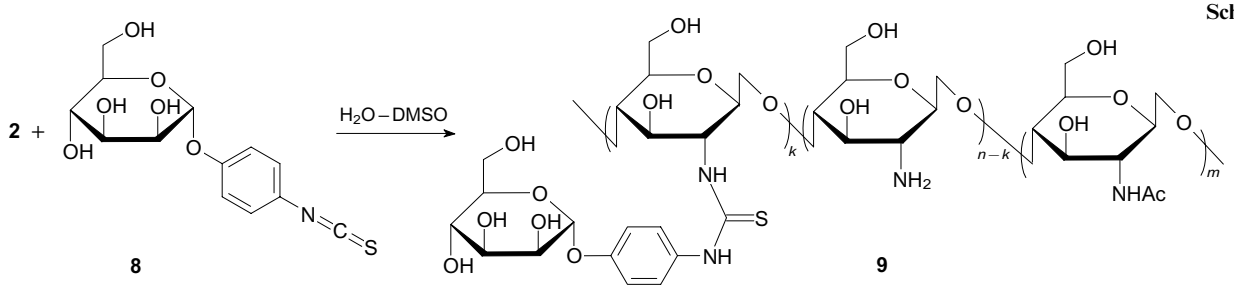
Scheme 1



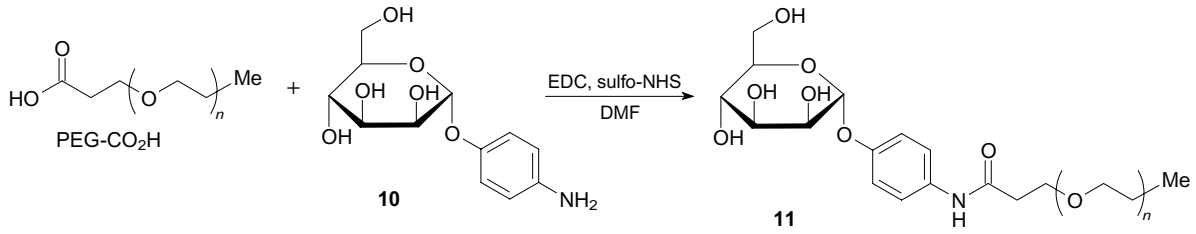
Scheme 2



Scheme 3



Scheme 4



tion, conversely, decreases from 279 to 5.4 μg ml^{-1} (see Ref. 89).

Chitosan modification with mannobiose (30% degree of modification) endows polymer **7** with water solubility at pH 6.5–9.0.[90] Mannosylation of 10%–20% of the chitosan amino groups with 1-α-formylmethylmannopyranoside **4** increases the survival of human liver carcinoma cells (HepG-2 and SMMC-7721 cell lines) when treated with mannosylated chitosan (100 μg ml^{-1}) by 5%–10% compared with the non-modified polymer.[91]

Freichels *et al.*[92] demonstrated the possibility of interaction of 3-*O*-(α-D-mannopyranosyl)-D-mannose- and α3,α6-mannotriose-functionalized chitosan particles with lectin (*Galanthus nivalis* agglutinin), which is considered as a model of mannose receptors.

The yield of mannosylated products can be increased by reacting chitosan molecules with thiocyanate ions, which are good nucleophiles for amino groups (Scheme 3).

The reaction with α-D-mannopyranosylphenyl isothiocyanate (**8**) takes place on mixing the reactants in a water–DMSO mixture.[93,94] It is noteworthy that this mannoside is unstable and expensive and, therefore, it is seldom used for modification of mannose. In the reaction of isothiocyanates with chitosan, the yield of product **9** reaches 100%.

4.4. Poly(ethylene glycol)

Poly(ethylene glycol) is not used in a pure state for drug delivery, but it is employed in combinations with low-molecular-weight compounds and polymers.[95,96]

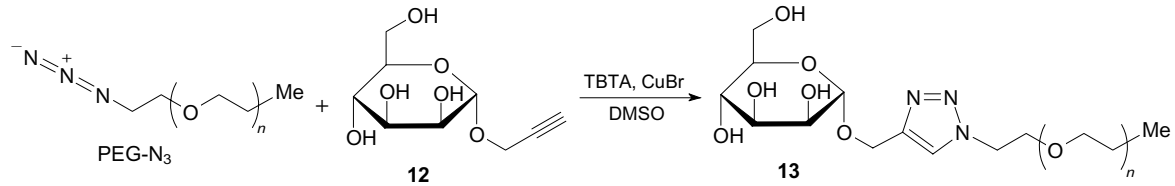
By mannose modification of PEG molecules, it is possible to achieve a stronger binding of PEG-containing particles to macrophages. For conjugation with mannose, PEG has to be activated by replacing the terminal OH group by a more reactive functional group. Examples of these groups are amine, ester, maleimide, aldehyde, sulfonic, thiol, *etc.* group. The modification of PEG involving the terminal amino groups can be accomplished using the same reagents **3**–**5** as for chitosan modification.[97,98]

The carbodiimide method (Scheme 4) is needed to activate the carboxyl group of the modified PEG molecule (PEG-CO_2H) towards the subsequent reaction with the amino group of the mannose derivative.[98] 3-Ethyl-1-(3-dimethylaminopropyl)carbodiimide (EDC) reacts with PEG-CO_2H in acid medium to give unstable *o*-acylisourea ether, which is converted to a more stable derivative *via* the reaction with *N*-hydroxysulfosuccinimide (sodium 1-hydroxy-2,5-dioxopyrrolidine-3-sulfonate, sulfo-NHS) in a neutral medium (DMF serves as the solvent for modifying agents). The amino ether sulfo-NHS reacts with *p*-aminophenyl-β-D-mannopyranoside (**10**) to give the peptide bond in product **11**.

The addition of mannose residues to PEG is often accomplished using the so-called click reaction, that is, 1,3-dipolar cycloaddition of an azide (*e.g.*, PEG molecule with an N_3 terminal group) to an alkyne [*e.g.*, α-D-propargylmannoside (**12**)] (Scheme 5). This reaction is catalyzed by copper(I) compounds; it is regioselective and gives the product in a high yield (>80%). Tris(1-benzyltriazol-4-ylmethyl)amine (TBTA) is used as the chelating agent to bind copper.[99]

The mannosylation of polyethyleneimine and poly(ethylene glycol) block copolymer (molecular weights of 25 and 2 kDa, respectively) on treatment

Scheme 5



with α-D-mannopyranosylphenyl isothiocyanate (**8**) promotes fast transfection (within 2 h) of complexes of this block copolymer and RNA (Raw264.7 macrophage cells having mannose receptors).[100]

The selection of the polymer structure is believed to be the key issue for attaining the optimal macrophage uptake of the particles. Chen *et al.*[101] optimized the PEG molecule size (5–40 kDa), modification degree of the carrier (1 to 4 mannose residues) and the distance between the mannose residues in the PEG molecule (39–89 Å). The best macrophage (J774.E) endocytosis behaviour was found for linear 12-kDa PEG particles modified by two mannose residues, which are located at a 56 Å distances. An increase in the PEG molecular weight to 40 kDa or a decrease to 5 kDa, an increase in the number of mannose residues in the PEG molecule to 4 or a decrease to 1 impair the particle phagocytosis. Probably, by varying the polymer chain length and the distances between the mannose residues, it is possible to adjust the spatial configuration for the mannose and PEG conjugate to make the interaction with mannose receptors on the macrophage cell surface most sterically favourable.

4.5. Cyclodextrins

Cyclodextrins (CDs) are cyclooligosaccharides consisting of 6, 7 or 8 α-(1→4)-D-glucopyranose units (α-, β- and γ-CD, respectively). The molecule is shaped as a truncated cone with a hydrophobic inner cavity and hydrophilic outer hydroxyl groups. On the one hand, the hydrophobic cavity usually serves for inclusion of hydrophobic molecules. On the other hand, owing to the hydrophilic shell, cyclodextrins are water-soluble. The combination of these two features makes CDs convenient carriers for pharmaceuticals. The first line ATDs (see above) possess two major drawbacks: low water solubility and toxicity. The inclusion of these drugs into the cyclodextrin hydrophobic cavity increases the targeting efficiency, decreases the toxicity and increases the solubility. Little is known about the use of cyclodextrins as ATD carriers. In particular, anti-tuberculosis activity was detected for their complexes with iron salts and a synthetic isoniazid analogue.[102, 103]

Mention should be made of the vigorously developing methods for the synthesis of cyclodextrin conjugates and complexes of fluoroquinolones. The application of these systems for encapsulation of fluoroquinolone type antibiotics has been described in detail in a review.[104] In addition, it has been shown that fluoroquinolone type ATDs (levofloxacin, moxifloxacin) form complexes with 2-hydroxypropyl-β-cyclodextrin in acid medium (pH 2–4) as a result of incorporation of the aromatic moiety of the fluoroquinolone molecule into the hydrophobic cavity of the CD molecule (host–guest complexes).[105] The most stable complexes are formed at equimolar amounts of levofloxacin and 2-hydroxypropyl-β-cyclodextrin at pH 2 (the dissociation constant K_{dis} is $\sim 9 \times 10^{-4}$ mol litre^{-1}); when pH of the medium increases to 4, the stability of the complex decreases ($K_{dis} = 1.0 \times 10^{-3}$ mol litre^{-1}). In the case of moxifloxacin, the complex formation is observed at pH 4 ($K_{dis} = 3.3 \times 10^{-3}$ mol litre^{-1}). At pH 7, the fluoroquinolone complexes with the polymer are not formed, apparently, due to the deprotonation of the carboxyl group, giving rise to the negative charge of the drug molecule. It is important that the fluoroquinolone complexes with 2-hydroxypropyl-β-cyclodextrin are not destroyed upon the transfer from acid medium to physiological conditions (pH 7).[105] Fluoroquinolones may also form complexes with hydrophobic CD derivatives, these complexes being more stable because of additional hydrophobic interactions.[106]

A method has been proposed for the synthesis of particles containing moxifloxacin (1 mass %–4 mass %) with CD and mannose based on the supercritical fluid antisolvent precipitation.[106] The pharmacokinetic parameters were studied *in vivo* upon the oral administration of particles (the particles contained 4% moxifloxacin in a CD–mannose mixture) to laboratory rats in comparison with the free drug substance. The pharmacokinetics of moxifloxacin in CD–mannose particles was found to considerably differ from that of free moxifloxacin (Fig. 1): the maximum drug concentration in blood stream is attained much later and the C_{max} value is lower than for the free drug substance (this decreases the toxic action of moxifloxacin). The absence of prolonged release of the drug in the blood stream should be mentioned as a drawback.

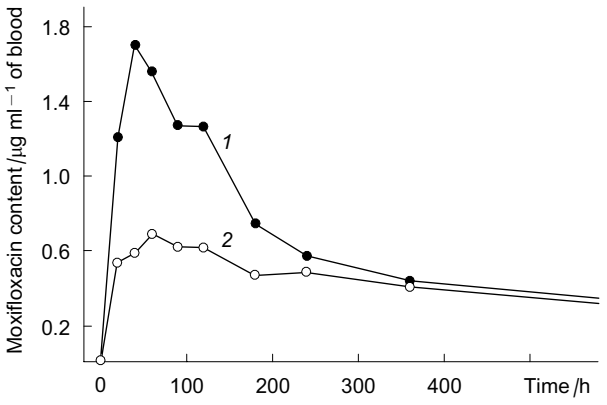


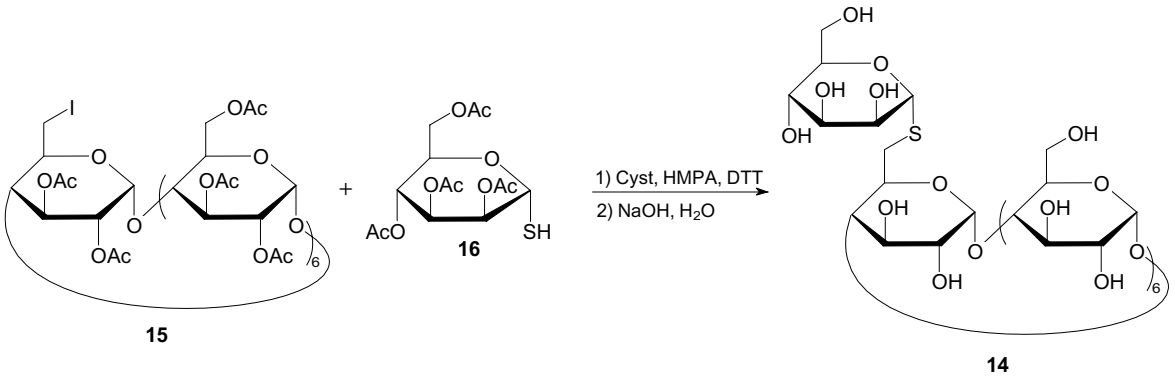
Figure 1. Variation of moxifloxacin concentration in the blood of laboratory rats upon oral administration of the free drug substance (control, curve *1*) and CD–mannose particles containing 4 mass % moxifloxacin (*2*).[106]
The solutions of test substances were administered orally using an enteral tube in a dose of 25 mg of moxifloxacin per kg of weight.

The covalent modification of cyclodextrin with mannose may give a highly stable conjugate and, hence, ensure more efficient delivery of the ATDs accommodated in the macromolecular cavities to mycobacteria and granulomas. Three types of reactions are used for CD mannosylation: nucleophilic substitution, acylation and 1,3-dipolar cycloaddition.[107–110]

4.5.1. Nucleophilic substitution

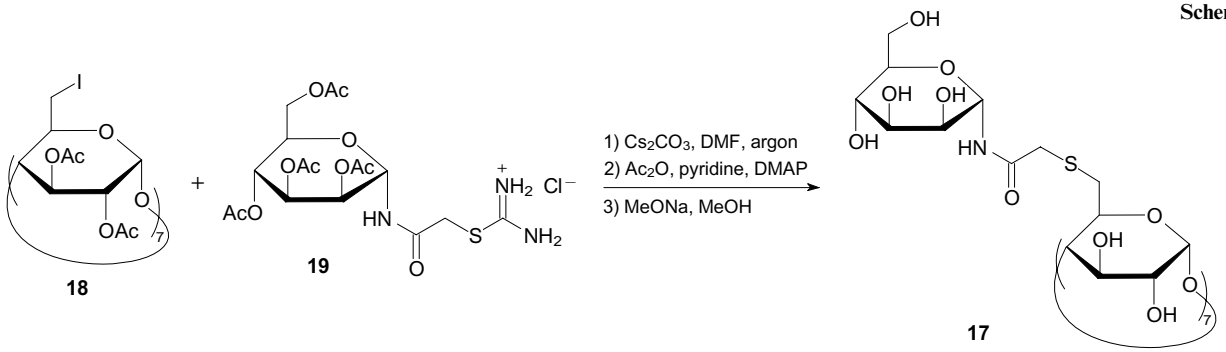
S-α-D-Mannopyranosyl-6-thiolcyclomaltoheptaose glycocluster (**14**) has been prepared from mono-iodo β-CD derivative, O-acylated 6-deoxy-6-iodocyclomalto-

Scheme 6



15 **16** **14**

Scheme 7



18 **19** **17**

heptaose (**15**) and O-acylated 1-mercapto-α-D-mannose (**16**)[108] (Scheme 6).

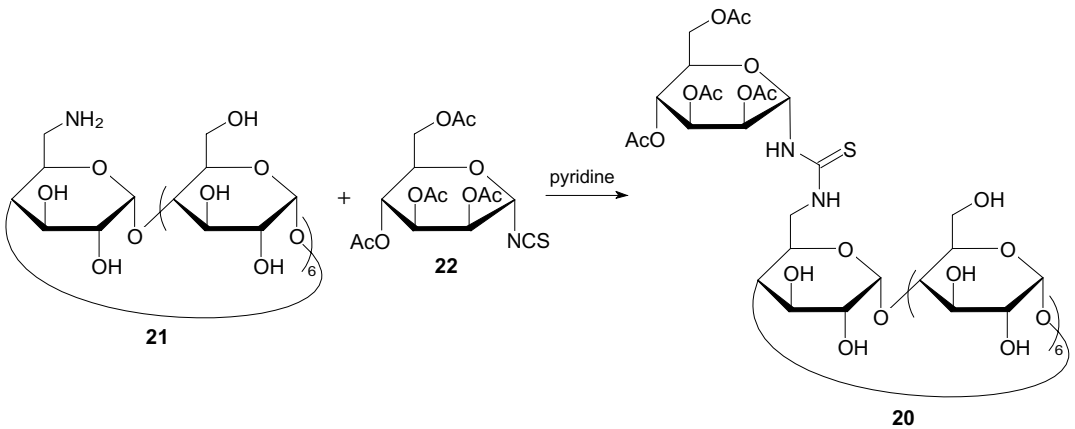
The reaction mixture was treated with cystamine (Cyst) in hexamethylphosphoramide (HMPA) in the presence of 1,4-dithiothreitol (DTT). This reaction gives an acylated monovalent conjugate in a yield >80%. The deacylation is carried out by treating the acyl derivative with sodium hydroxide in water (1 : 5 vol.). It is obvious that the degree of CD modification with mannose residues depends on the number of iodine atoms in the CD molecule.

A discussion of the procedures for preparation of glycocluster **17** by conjugation of heptaiodo-β-cyclodextrin **18** with α-D-mannopyranose isothiourea derivative **19** has been reported[109] (Scheme 7). The reaction occurs on mixing the components in the presence of Cs_2CO_3 in dry DMF under argon. After several days, acetic anhydride, pyridine and 4-dimethylaminopyridine (DMAP) are added to the resulting conjugates for their exhaustive acetylation. The reaction gives the glycoconjugate with mannose residues bound to cyclodextrin *via* a spacer, with the yield of the acylated product being >90%. Glycoconjugates in which the carbohydrate residues are linked to CD through spacers were shown to more strongly bind to lectins (analogues of mannose receptors) than the non-spacered derivatives. Deacetylation gives mannosylated β-CD **17**.[108, 109]

4.5.2. Acylation

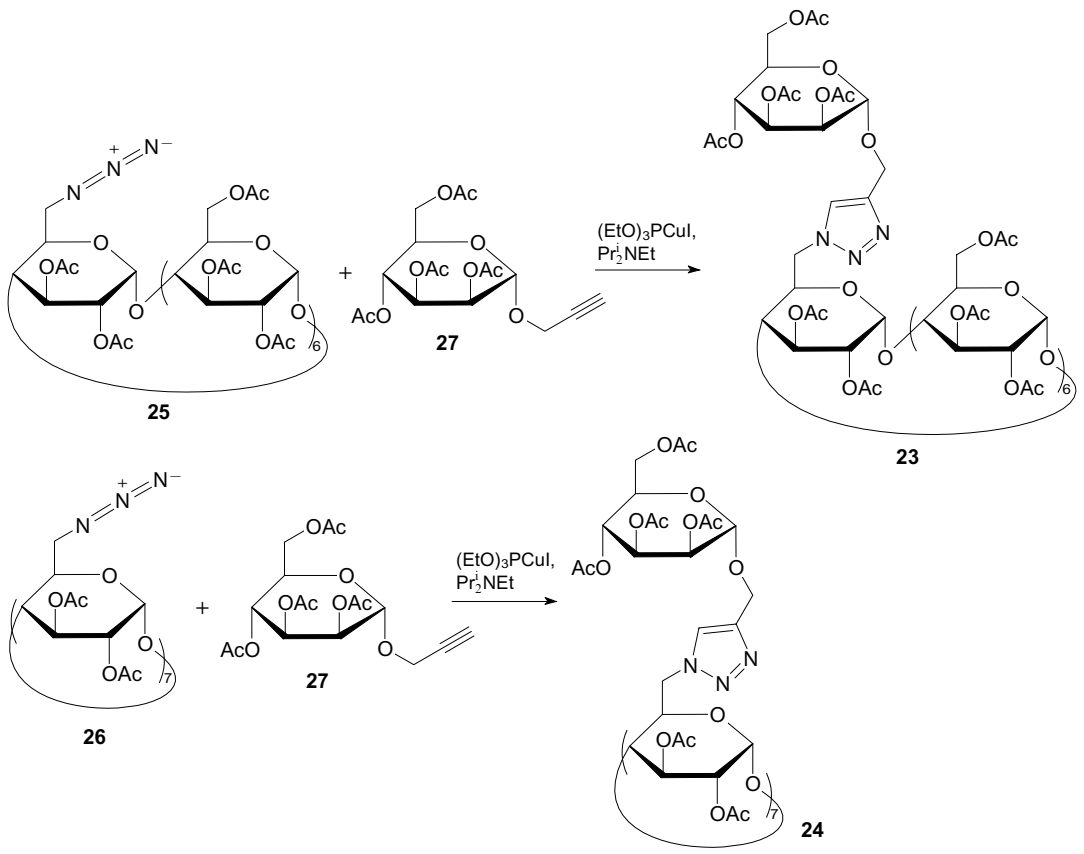
Glycoconjugates **20** based on CD and mannose can be obtained by acylation of cyclodextrin **21** containing amine and hydroxyl groups with isothiocyanate **22** in pyridine at room temperature (Scheme 8). The intro-

Scheme 8



21 **22** **20**

Scheme 9



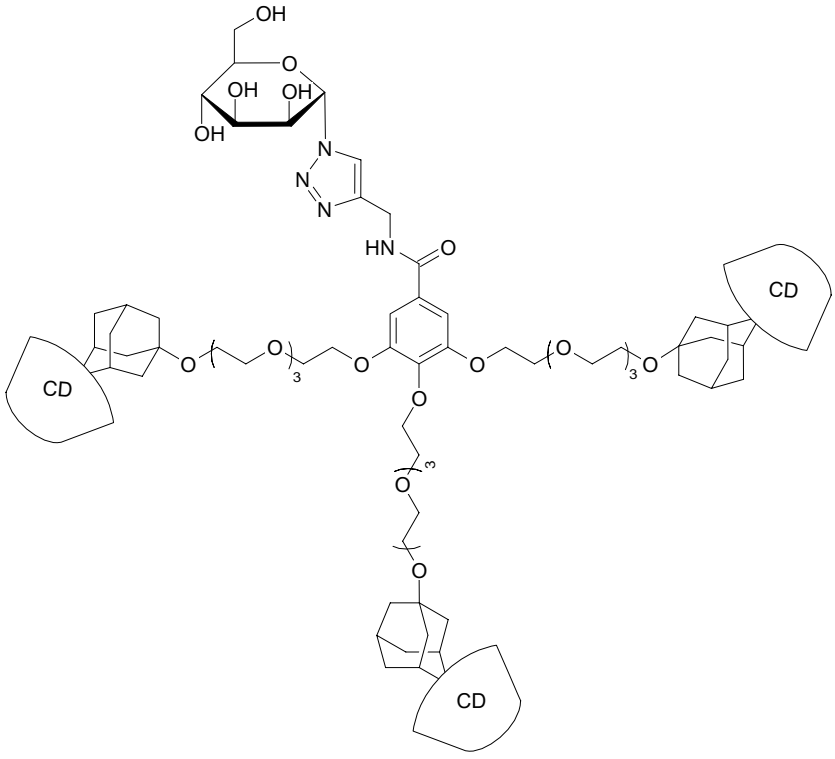


Figure 2. Inclusion complex of mannose conjugate with adamantane (**28**) involving cyclodextrin.

duction of a thiourea moiety into CD reduces the hemolytic effect of the macrocyclic molecule. High product yields (80% – 100%) are observed in the synthesis of monoglycoconjugates, while the synthesis of heptaglycoconjugates affords a mixture of anomers in a ~50% yield. By alkaline deacetylation, it is possible

to obtain the expected hydroxy CD derivative. The binding of glycoclusters synthesized using α-D-mannose and β-D-mannose to ConA lectin did not differ significantly.[107]

4.5.3. 1,3-Dipolar cycloaddition

The click reaction catalyzed by copper(I) salts proceeds readily and gives products of regiospecific addition in high yields. Mono- (**23**) and heptadentate (**24**) β-CD derivatives can be prepared by 1,3-dipolar cycloaddition of mono- (**25**) and heptaazides (**26**), respectively, to α-D-propargylmannoside **27** (> 80% product yields) (Scheme 9).

An unusual approach to the preparation of particles containing mannose and CD has been employed.[110] The CD molecules contain a hydrophobic cavity, which perfectly accommodates the hydrophobic adamantane molecule. The ability to be included in the hydrophobic CD cavity is also inherent in mannose conjugates with adamantane in which one mannose molecule and one to three adamantane molecules are connected by a linker (*e.g.*, compound **28**). The adamantyl moieties present in conjugate **28** molecule form inclusion complexes with CD *via* hydrophobic interactions (Fig. 2). Since these conjugates contain one to three adamantane molecules per mannose molecule, the inclusion complexes can correspondingly contain one to three CD molecules. These particles were shown to interact with ConA lectins, which indirectly proves their ability to bind also to mannose receptors.

The formation of non-covalent CD and mannose complexes with a binding constant of 1 mol litre^{-1} in a phosphate buffer solution (pH 7.0) has been reported.[111]

Thus, most of the data available from the literature are concerned with methods for chemical modification of CDs. Modified cyclodextrins can bind to lectins, which can be regarded as models of the macrophage mannose receptors. The above data indicate that ATD molecules could potentially be encapsulated into mannosylated cyclodextrins.

5. Conclusion

Currently, no efficient systems for targeted ATD delivery are present in the market of pharmaceuticals. A key cause for this situation is low accessibility of the focus of infection, since mycobacteria are mainly located in alveolar macrophages and granulomas. It is necessary to encapsulate drug substances into particles that are stable during storage, incorporate large amounts of ATD and ensure prolonged drug release and high *in vivo* uptake of the particles by alveolar macrophages. For targeted ATD delivery to alveolar macrophages, the systems of choice are those containing ligands (carbohydrates, proteins, lipids, peptidoglycans and so on) capable of binding to macrophage receptors.

The development of ATD carriers targeting the mannose receptors of alveolar macrophages can be promising for the following reasons:

(1) methods for the synthesis of complexes and conjugates of various carriers (polymers, liposomes, lipid particles) with mannose have been well developed; it is possible to obtain particles of specified composition, size and degree of modification;

(2) high efficiency of ATD delivery to macrophages using carriers containing mannose moieties (receptor-mediated endocytosis) has been demonstrated in *in vitro* experiments;

(3) mannose is commercially available (unlike many proteins, lipids or peptidoglycans);

(4) beneficial effects *in vivo* have been found for ATD-loaded mannosylated carriers (liposomes, solid lipid particles, chitosan), particularly, an increase in the overall and maximum ATD concentrations in lungs, a decrease in the ATD concentration in blood plasma (mitigation of toxic effects of drug substances), the absence of hepatotoxicity.

The most important fact is that the use of mannosylated carriers results in ATD accumulation in lungs rather than in liver or spleen, as in the case of ATD delivery with non-mannosylated liposomes or polymers. As a result of increasing ATD concentration in the focus of mycobacterial infection (lungs), one might expect a decrease in the duration of therapy; however, no such data have been reported.

The use of mannosylated carriers for targeted ATD delivery is faced with some problems. First of all, this is a small number of clinical trials; therefore, the published information should be considered only at the hypothesis level.

Liposome mannosylation results in the absence of prolonged ATD release, although this effect is present for the drug substances encapsulated in non-modified particles. The duration of ATD release increases in the case of mannosylated solid lipid particles, which should be preferred over liposomes for the targeted ATD delivery.

Synthetic polymers contain impurities toxic for living organisms and, for this reason, they are sparingly used for ATD delivery (despite the persistent interest of researchers[112, 113]). Natural non-toxic and biodegradable polymers such as gelatine, chitosan and cyclodextrins should be considered, in our opinion, to be more appropriate carriers for the design of delivery systems for these drugs. Chitosan becomes soluble at physiological pH upon mannosylation; ATD encapsulation into mannosylated chitosan particles increases their excretion half time. The agents encapsulated into gelatin particles showed good results *in vitro*, while mannosylated CDs are able to bind to lectins (modelling mannose receptors) and quite number of procedures are available for their modification to fabricate particles with specified properties.

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References

1. H.K.Mohajan. *Am. J. Infect. Dis.*, **3**, 18 (2015)
2. M.A.Sanzhakov, O.M.Ipatova, T.I.Torkhovskaya, V.N.Prozorovskii, E.G.Tikhonova, O.S.Druzhilovskaya, N.V.Medvedeva. *Ann. Russ. Acad. Med. Sci.*, **68** (8) (2013)
3. D.Pham, E.Fattal, N.Tsapis. *Int. J. Pharm.*, **478**, 517 (2015)

4. G.Griffiths, B.Nyström, S.B.Suraj, G.K.Khuller. *Nat. Rev.: Microbiol.*, **8**, 827 (2010)
5. *Tuberkulez Organov Dykhaniya: Rukovodstvo dlya Vrachei.* (Tuberculosis of the Respiratory System: a Guide for Physicians). (Ed. N.A.Brazhenko) (St. Petersburg: SpetsLit, 2011)
6. A.Sosnik, Á.M.Carcaboso, R.J.Glisoni, M.A.Moretton, D.A.Chiappetta. *Adv. Drug Deliv. Rev.*, **62**, 547 (2010)
7. S.Tiwari, A.P.Chaturvedi, Y.B.Tripathi, B.Mishra. *AAPS PharmSciTech*, **12**, 900 (2011)
8. R.G.Bellini, A.P.Guimaraes, M.A.C.Pacheco, D.M.Dias, V.R.Furtado, R.B.de Alencastro, B.A.C.Horta. *J. Mol. Graph. Model.*, **60**, 34 (2015)
9. A.Dube, Y.Lemmer, R.Hayeshi, M.Balogun, P.Labuschagne, H.Swai, L.Kalombo. *Expert Opin. Drug Deliv.*, **10**, 1725 (2013)
10. M.Pinheiro, M.Lúcio, J.L.Lima, S.Reis. *Nanomedicine (London)*, **6**, 1413 (2011)
11. S.Chono, T.Tanino, T.Seki, K.Morimoto. *J. Control. Release*, **127**, 50 (2008)
12. S.Chono, K.Kaneko, E.Yamamoto, K.Togami, K.Morimoto. *Drug Dev. Ind. Pharm.*, **36**, 102 (2010)
13. L.N.Zhuravleva. *Okhrana Materinstva i Detstva*. (2), 82 (2016)
14. V.V.Erokhin, L.N.Lepekha, M.V.Erokhina, O.V.Lovacheva. *Surfactantnaya Sistema pri Tuberkuleze Legkikh.* (Surfactant System (Surfactant System in Pulmonary Tuberculosis) (Moscow: Izd. FGBU 'TsNIIT' RAMN, 2013)
15. G.Chimote, R.Banerjee. *Colloids Surf. B*, **62**, 258 (2008)
16. M.Zaru, C.Sinico, A.De Logu, C.Caddeo, F.Lai, M.L.Manca, A.M.Fadda. *J. Liposome Res.*, **19**, 68 (2009)
17. S.Chono, T.Tanino, T.Seki, K.Morimoto. *J. Pharm. Pharmacol.*, **59**, 75 (2007)
18. S.P.Vyas, M.E.Kannan, S.Jain, V.Mishra, P.Singh. *Int. J. Pharm.*, **269**, 37 (2004)
19. L.Barbassa, E.M.Mamizuka, A.M.Carmona-Ribeiro. *BMC Biotechnol.*, **11**, 40 (2011)
20. M.M.Gaspar, A.Cruz, A.F.Penha, J.Reymao, A.C.Sousa, C.V.Eleuterio, S.A.Domingues, A.G.Fraga, A.Longatto Filho, M.E.M.Cruz, J.Pedrosa. *Int. J. Antimicrob. Agents*, **31**, 37 (2008)
21. P.R.Donald, F.A.Sirgel, A.Venter, E.Smith, D.P.Parkin, B.W.Val de Wal, D.A.Mitchison. *J. Antimicrob. Chemoter.*, **48**, 877 (2001)
22. M.S.El-Ridy, D.M.Mostafa, A.Shehab, E.A.Nasr, S.Abd El-Alim. *Int. J. Pharm.*, **330**, 82 (2007)
23. R.Pandey, S.Sharma, G.K.Khuller. *Int. J. Antimicrob. Agents*, **24**, 93 (2004)
24. R.Pandey, G.K.Khuller. *Tuberculosis*, **85**, 227 (2005)
25. R.Pandey, G.K.Khuller. *Tuberculosis*, **85**, 415 (2005)
26. E.Maretti, T.Rossi, M.Bondi, M.A.Croce, M.Hanuskova, E.Leo, F.Sacchetti, V.Iannuccelli. *Int. J. Pharm.*, **462**, 74 (2014)
27. S.Gill, R.Lobenberg, T.Ku, S.Azarmi, W.Roa, E.J.Prenner. *J. Biomed. Nanotechnol.*, **3**, 107 (2007)
28. R.Pandey, S.Sharma, Z.Ahmad. *Nanomedicine: NBM*, **7**, 259 (2011)
29. A.Misra, A.J.Hickey, C.Rossi, G.Borchard, H.Terada, K.Makino, P.B.Fourie, P.Colombo. *Tuberculosis*, **91**, 71 (2011)
30. P.Ahlin, J.Kristl, A.Kristl, F.Vrecer. *Int. J. Pharm.*, **239**, 113 (2002)
31. D.Huang, D.Li, T.Wang, H.Shen, P.Zhao, B.Liu, Y.You, Y.Ma, F.Yang, D.Wu, S.Wang. *Biomaterials*, **52**, 417 (2015)
32. K.O.Kisich, S.Gelperina, M.P.Higgins, S.Wilson, E.Shipulo, E.Oganesyan, L.Heifets. *Int. J. Pharm.*, **345**, 154 (2007)
33. E.V.Shipulo, I.I.Lyubimov, O.O.Maksimenko, L.V.Vanchugova, E.A.Oganesyan, P.G.Sveshnikov, S.F.Biketov, E.S.Severin, L.B.Heifets, S.E.Gel'perina. *Pharm. Chem. J.*, **42**, 145 (2008)
34. Z.Jiang, Y.You, D.J.Hao. *Polymer*, **48**, 4786 (2007)
35. L.Chen, Z.Xie, J.Hu, X.Chen, X.Jing. *J. Nanopart. Res.*, **9**, 777 (2007)
36. M.Silva, A.S.Lara, C.Q.F.Leite, E.I.Ferreira. *Arch. Pharm. Med. Chem.*, **334**, 189 (2001)
37. M.Silva, N.L.Ricelli, O.E.Seoud, S.C.Valentim, A.G.Ferreira, D.N.Sato, C.Q.F.Leite, E.I.Ferreira. *Arch. Pharm. Med. Chem.*, **339**, 283 (2006)
38. Q.Ain, S.Sharma, S.K.Garg, G.K.Khuller. *Int. J. Pharm.*, **239**, 37 (2002)
39. Q.Ain, S.Sharma, G.K.Khuller. *Antimicrob. Agents Chemoter.*, **47**, 3005 (2003)
40. M.Dutt, G.K.Khuller. *Indian J. Exp. Biol.*, **38**, 887 (2000)
41. M.Dutt, G.K.Khuller. *Int. J. Antimicrob. Agents*, **17**, 115 (2001)
42. M.Dutt, G.K.Khuller. *J. Antimicrob. Chemoter.*, **47**, 829 (2001)
43. A.Sharma, R.Pandey, S.Sharma, G.K.Khuller. *Int. J. Antimicrob. Agents*, **24**, 599 (2004)
44. R.Pandey, S.Sharma, G.K.Khuller. *Drug Deliv.*, **13**, 287 (2006)
45. R.Pandey, A.Sharma, A.Zahoor, S.Sharma, G.K.Khuller, B.Prasad. *J. Antimicrob. Chemoter.*, **52**, 981 (2003)
46. Q.Ain, S.Sharma, G.K.Khuller, S.K.Garg. *J. Antimicrob. Chemoter.*, **51**, 931 (2003)
47. A.Zahoor, S.Sharma, G.K.Khuller. *Int. J. Antimicrob. Agents*, **26**, 298 (2005)
48. R.Pandey, G.K.Khuller. *J. Antimicrob. Chemoter.*, **53**, 635 (2004)
49. W.Xu, X.Wei, K.Wei, X.Cao, S.Zhong. *Int. J. Pharm.*, **476**, 116 (2014)
50. H.Feng, L.Zhang, C.Zhu. *Colloids Surf. B*, **103**, 530 (2013)
51. T.Manning, S.Plummer, T.Baker, G.Wylie, A.C.Clingenpeel, D.Phillips. *Bioorg. Med. Chem. Lett.*, **25**, 4621 (2015)
52. T.Manning, H.Patel, G.Wylie, D.Phillips, J.Jarvis. *Bioorg. Med. Chem. Lett.*, **25**, 5825 (2015)
53. T.Manning, R.Mikula, G.Wylie, D.Phillips, J.Jarvis, F.Zhang. *Bioorg. Med. Chem. Lett.*, **25**, 451 (2015)
54. T.Manning, R.Mikula, H.Lee, A.Calvin, J.Darrah, G.Wylie, D.Phillips, B.J.Bythell. *Bioorg. Med. Chem. Lett.*, **24**, 976 (2014)
55. S.K.Mehta, G.Kaur, A.Verma. *Colloids Surf. A*, **375**, 219 (2011)
56. M.Ahmed, W.Ramadan, D.Rambhu, F.Shakeel. *Pharmazie*, **63**, 806 (2008)
57. C.P.Jain, S.P.Vyas. *J. Microencapsul.*, **12**, 401 (1995)
58. C.P.Jain, S.P.Vyas, V.K.Dixit. *Indian J. Pharm. Sci.*, **68**, 575 (2006)
59. A.R.Mullaicharam, R.S.R.Murthy. *J. Drug Deliv. Sci. Techhol.*, **14**, 99 (2004)
60. K.V.Sukhoverkov, E.V.Kudryashova, A.M.Egorov, K.V.Glasunova. *Acra Naturae*, (Special Issue), 155 (2016)
61. E.V.Kudryashova, I.M.Deygen, K.V.Sukhoverkov, L.Yu.Filatova, N.L.Klyachko, A.M.Vorobei, O.I.Pokrovskiy, K.B.Ustinovich, O.O.Parenago, E.N.Antonov, A.G.Dunaev, L.I.Krotova, V.K.Popov, A.M.Egorov. *Russ. J. Phys. Chem. B*, **10**, 1201 (2016)

62. E.V.Kudryashova, K.V.Sukhoverkov, I.M.Deygen, A.M.Vorobei, O.I.Pokrovskiy, O.O.Parenago, D.E.Presnov, A.M.Egorov. *Sverkhkritich. Flyuidy: Teor. Prakt.*, **11** (3), 71 (2016)
63. K.Dheda, S.K.Schwander, B.Zhu, R.N.Van Zyl-Smit, Y.Zhang. *Respirology*, **15**, 433 (2010)
64. S.A.Lyalikov, N.M.Tikhon. *Klinicheskaya Immunologiya i Allergologiya*. (Clinical Immunology and Allergology). (Minsk: Vyssheishaya Shkola, 2005)
65. N.K. Zenkov, A.V. Chechushkov, P.M. Kozhin, T.A.Kolpakova, E.B. Menshchikova. *Biol. Bull. Rev.*, **6**, 289 (2016)
66. P.R.Taylor, L.Martinez-Pomares, M.Stacey, H.-H.Lin, G.D.Brown, S.Gordon. *Annu. Rev. Immunol.*, **23**, 901 (2005)
67. A.Yonekawa, S.Saijo, Y.Hoshino, Y.Miyake, E.Ishikawa, M.Suzukawa, H.Inoue, M.Tanaka, M.Yoneyama, M.Oh-hora, K.Akashi, S.Yamasaki. *Immunity*, **41**, 402 (2014)
68. R.A.B.Ezekowitz, K.Sastry, P.Bailly, A.Warner. *J. Exp. Med.*, **172**, 1785 (1990)
69. E.V.Svirshchevskaya, V.S.Mitrofanov, R.I.Shenderova, N.M.Chuzhova. *Problemy Med. Mikolog.*, (7), 3 (2005)
70. M.A.Velasco-Velazquez, D.Barrera, A.Gonzalez-Arenas, C.Rosales, J.Agramonte-Hevia. *Microb. Pathogen.*, **35**, 125 (2003)
71. V.N.Kokryakov. *Ocherki o Vrozhdennom Immunitete*. (Essays on Innate Immunity). (St. Petersburg: Nauka, 2006)
72. J.M.Irache, H.H.Salman, C.Gamazo, S.Espuelas. *Exp. Opin. Drug Deliv.*, **5**, 703 (2008)
73. S.Saijo, Y.Iwakura. *Int. Immunol.*, **8**, 467 (2011)
74. G.D.Brown. *Nat. Immunol.*, **9**, 1099 (2008)
75. S.Yamasaki, M.Matsumoto, O.Takeuchi, T.Matsuzawa, E.Ishikawa, M.Sakuma, H.Tateno, J.Uno, J.Hirabayashi, Y.Mikami, K.Takeda, S.Akira, T.Saito. *J. Exp. Med.*, **206**, 2879 (2009)
76. E.C.van Asbeck, A.I.M.Hoepelman, J.Scharringa, B.L.Herpers, J.Verhoef. *BMC Microbiol.*, **8**, 229 (2008)
77. D.G.Nguyen, J.E.Hildreth. *Eur. J. Immunol.*, **33**, 483 (2003)
78. C.Leteux, W.Chai, R.W.Loveless, C.-T.Yuen, L.Uhlin-Hansen, Y.Combarnous, M.Jankovic, S.C.Maric, Z.Misulovin, M.C.Nussenzweig, T.Feizi. *J. Exp. Med.*, **191**, 1117 (2000)
79. V.P.Torchilin, V.Weissig. *Liposomes: A Practical Approach*. (New York: Oxford University Press, 2003)
80. E.Maretti, L.Costantino, C.Rustichelli, E.Leo, M.Antonietta-Croce, F.Buttini, E.Truzzi, V.Iannuccelli. *Int. J. Pharm.*, **528**, 440 (2017)
81. N.Nimje, A.Agarwal, G.K.Saraogi, N.Lariya, G.Rai, H.Agrawal, G.P.Agrawal. *J. Drug Target.*, **17**, 777 (2009)
82. W.Yeeprae, S.Kawakami, F.Yamashita, M.Hashida. *J. Control. Release*, **114**, 193 (2006)
83. W.Wijagkanalan, S.Kawakami, M.Takenaga, R.Igarashi, F.Yamashita, M.Hashida. *J. Control. Release*, **125**, 121 (2008)
84. A.Nag, P.C.Ghosh. *J. Drug Target.*, **6**, 427 (1999)
85. S.Kawakami, J.Wong, A.Sato, Y.Hattori, F.Yamashita, M.Hashida. *Biochim. Biophys. Acta*, **1524**, 258 (2000)
86. S.M.D'Addio, S.M.Baldassano, L.Shi, L.Cheung, D.H.Adamson, M.Bruzek, J.E.Anthony, D.L.Laskin, P.J.Sinko, R.K.Prud'homme. *J. Control. Release*, **168**, 41 (2013)
87. P.V.Kumar, A.Asthana, T.Dutta, N.K.Jain. *J. Drug Target.*, **14**, 546 (2006)
88. A.Grenha, S.Al-Qadi, B.Seijo, C.Remunán-López. *J. Drug Deliv. Sci. Technol.*, **20**, 33 (2010)
89. P.Chaubey, B.Mishra. *Carbohydr. Polym.*, **101**, 1101 (2014)
90. X.Zhou, B.Liu, X.Yu, X.Zha, X.Zhang, Yu Chen, X.Wang, Y.Jin, Y.Wu, Yue Chen, Y.Shan, Yan Chen, J.Liu, W.Kong, J.Shen. *J. Control. Release*, **121**, 200 (2007)
91. W.Yao, Y.Jiao, J.Luo, M.Du, L.Zong. *Int. J. Biol. Macromol.*, **50**, 821 (2012)
92. H.Freichels, M.Wagner, M.Okwieka, R.Meyer, V.Mailander, K.Landfester, A.Musyanovych. *J. Mater. Chem. B*, **1**, 4338 (2013)
93. G.S.Asthana, A.Asthana, D.V.Kohli, S.P.Vyas. *BioMed Res. Int.*, Art. ID 526391 (2014)
94. V.K.Mourya, N.N.Inamdar. *React. Funct. Polym.*, **68**, 1013 (2008)
95. S.Parveen, S.Sahoo. *Clin. Pharmacokinet.*, **45**, 965 (2006)
96. M.Rajan, V.Raj. *Int. J. Pharm. Pharm. Sci.*, **4**, 255 (2012)
97. M.Muthiah, H.Vu-Quang, Y.K.Kim, J.H.Rhee, S.H.Kang, S.Y.Jun, Y.J.Choi, Y.Y.Jeong, C.S.Cho, I.K.Park. *Carbohydr. Polym.*, **92**, 1586 (2013)
98. M.E.Giorgi, R.Agusti, R.M.de Lederkremer. *Beilstein J. Org. Chem.*, **10**, 1433 (2014)
99. S.-J.Richards, E.Fullam, G.S.Besra, M.I.Gibson. *J. Mater. Chem., B*, **2**, 1490 (2014)
100. N.J.Kim, D.Jiang, A.M.Jacobi, K.A.Lennox, S.D.Rose, M.A.Behlke, A.K.Salem. *Int. J. Pharm.*, **427**, 123 (2012)
101. P.Chen, X.Zhang, L.Jia, R.K.Prud'homme, Z.Szekely, P.J.Sinko. *J. Control. Release*, **194**, 341 (2014)
102. P.Dandawate, K.Vemuri, E.M.Khan, M.Sritharan, S.Padhye. *Carbohydr. Polym.*, **108**, 135 (2014)
103. P.Dandawate, K.Vemuri, K.V.Swamy, E.M.Khan, M.Sritharan, S.Padhye. *Bioorg. Med. Chem. Lett.*, **24**, 5070 (2014)
104. I.M.Le-Deygen, A.A.Skuredina, E.V.Kudryashova. *Russ. J. Bioorg. Chem.*, **43**, 487 (2017)
105. I.M.Le-Deygen, A.A.Scuredina, I.V.Uporov, E.V.Kudryashova. *Anal. Bioanal. Chem.*, **409**, 6451 (2017)
106. A.A.Skuredina, I.M.Le-Deygen, I.V.Uporov, E.V.Kudryashova. *Colloid. J.*, **79**, 688 (2017)
107. D.V.Titov, M.L.Gening, Yu.E.Tsvetkov, N.E.Nifantiev. *Russ. J. Bioorg. Chem.*, **39**, 451 (2013)
108. C.Lanceton-Pin, H.Driguez. *Tetrahedron Lett.*, **33**, 3125 (1992)
109. J.García-López, F.Santoyo-González, A.Vargas-Berenguel, J.J.Gimenez-Martinez. *Chem. – Eur. J.*, **5**, 1775 (1999)
110. U.Kauscher, B.Ravoo. *Beilstein J. Org. Chem.*, **8**, 1543 (2012)
111. F.Hacket, J.Coteron, H.Schneider, V.Kazachenko. *Can. J. Chem.*, **75**, 52 (1997)
112. W.Gao, Y.Chen, Y.Zhang, Q.Zhang, L.Zhang. *Adv. Drug Deliv. Rev.*, (2018) (in the press); doi: 10.1016/j.addr.2017.09.015
113. Y.Pei, Y.Yeo. *J. Control. Release*, **240**, 202 (2016)