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Cyclodextrins Based Rotaxanes and Polyrotaxanes: The Potential Hauler in Drug Delivery and Therapeutics

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ABSTRACT

This article deals with cyclodextrin (CD) based functional rotaxanes and polyrotaxanes for drug delivery. First, we present an overview of polyrotaxane preparations for biomaterials applications. Then we bring to light the studies and strategies implied for augmented drug delivery using CD-based polyrotaxanes. The concept for enhancing multivalent interaction of a ligand-mobile polyrotaxane with receptor proteins; which is significantly related to receptor-mediated drug delivery and the efficiency of ligand-conjugated polyrotaxanes is also demonstrated in the design of inhibitors which are recognized by intestinal transporters in mammalian tissues but neither absorbed into the tissue nor exhibiting any toxicity. This concept is of more practical applications, the studies on cytocleavable polyrotaxanes for gene delivery is the modern therapy. The dynamic motion of polyrotaxanes would contribute significantly to forming a polyplex with DNA to be delivered into target cells. Moreover, dissociating the polyrotaxane structure in intracellular environments is an effective way to release DNA for transfection at the nucleus. Furthermore, polyrotaxanes are also useful in manifestation of Niemann-pick type C disease. In present article, efforts have been taken with the intention to get thorough information of polyrotaxanes conjugation with drug/ligands along with a comprehensive review of diverse approaches adopted for drug delivery till date.

***Keywords*:** Cyclodextrins, Polyrotaxanes, siRNA, Niemann-pick type C disease, Gene silencing.

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INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six (α-cyclodextrin), seven (β-cyclodextrin), eight (γ-cyclodextrin) or more glucopyranose units linked by α-(1,4) bonds (Figure 1). They are also known as cycloamyloses, cyclomaltoses and Schardinger dextrins. They are produced as a result of intramolecular transglycosylation reaction from degradation of starch by CD glucanotransferase enzyme[1, 2]. Nowadays CDs have become interesting molecules because of their ability form inclusion complexes with wide variety of compounds. The X-ray study of these molecules showed that the secondary hydroxyl groups are situated on the wider edge of the ring and the primary hydroxyl groups on the other edge and the non polar hydrogens and the ether like oxygens are at inside of the torus like molecules[2]. As a result the CDs are hydrophilic from outside which can dissolve in water and non-polar cavity which is hydrophobic matrix thus can entrap drug molecules.[1]

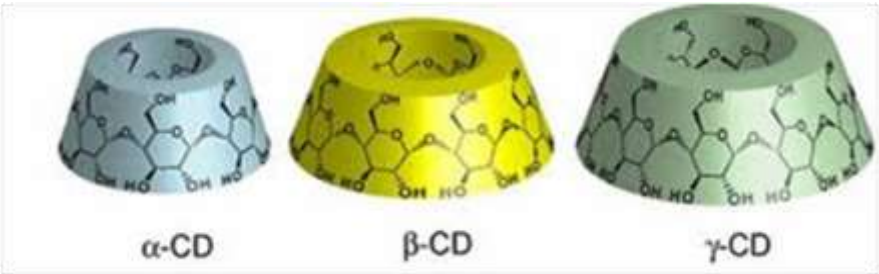

Figure 1: 3-D molecular structures of α, β and γ cyclodextrins.

CDs have been approved by the FDA as food additives and as drug formulations and PEG is a worldwide used water-soluble polymer for conjugating biologically active agents such as drugs and proteins. It is thus easily established that biodegradable polyrotaxanes consisting of α-CD molecules and a PEG chain capped with bulky end-groups via biodegradable linkages have much potential in drug delivery[3].

The most striking property of CDs is that they are being able to form solid inclusion complexes (host-guest complexes) with a very wide range of solid, liquid and gaseous compounds by a molecular complexation. In these complexes a guest molecule is held within the cavity of the CD host molecule. Complex formation is a dimensional fit between host cavity and guest molecule[4].The cavity of CD molecule is lipophilic thus providing a microenvironment into which appropriately sized non-polar moieties can enter to form inclusion complexes[5]. No covalent bonds are broken or formed during formation of the inclusion complex[6]. The main driving force of complex formation is the release of enthalpy-rich water molecules from the cavity. Water molecules are displaced by more hydrophobic guest molecules present in the

solution to attain an apolar–apolar association and decrease of CD ring strain resulting in a more stable lower energy state[7]. The binding of guest molecules within the host CD is not fixed or permanent but rather is a dynamic equilibrium. Binding strength depends on how well the 'host-guest' complex fits together and on specific local interactions between surface atoms. Complexes can be formed either in solution or in the crystalline state and water is typically the solvent of choice. Inclusion complexation can be accomplished in a co-solvent system and in the presence of any non-aqueous solvent. CD architecture confers upon these molecules a wide range of chemical properties markedly different from those exhibited by non-cyclic carbohydrates in the same molecular weight range. Inclusion in CDs exerts a profound effect on the physicochemical properties of guest molecules as they are temporarily locked or caged within the host cavity giving rise to beneficial modifications of guest molecules, which are not achievable otherwise[8]. These properties are: solubility enhancement of highly insoluble guests, stabilisation of labile guests against the degradative effects of oxidation, visible or UV light and heat, control of volatility and sublimation, physical isolation of incompatible compounds, chromatographic separations, taste modification by masking off flavours, unpleasant odours and controlled release of drugs and flavours. Therefore, CDs are used in food[9], pharmaceuticals[10], cosmetics[11], environment protection[12], bioconversion[13], packing and the textile industry[1, 14].

The ability of a CD to form an inclusion complex with a guest molecule is a function of two key factors. The first is steric and depends on the relative size of the CD to the size of the guest molecule or certain key functional groups within the guest. If the guest is the wrong size, it will not fit properly into the CD cavity. The second critical factor is the thermodynamic interaction between the different components of the system (CD, guest, solvent). For a complex to form there must be a favourable net energetic driving force that pulls the guest into the CD[1].

Through modification, the applications of CDs are expanded. CDs are modified through substituting various functional compounds on the primary and/or secondary face of the molecule. Modified CDs are useful as enzyme mimics since the substituted functional groups act in molecular recognition. This same property is used for targeted drug delivery and analytical chemistry as modified CDs display increased enantio selectivity over native CDs[1].

CDs are extensively used oral formulations via inclusion complexes formation for drugs having the following advantages like:

- Improved solubility, dissolution rates and enhanced stability and bioavailability.
- To alter the drug target site and the rate of release.
- To prevent the disagreeable odour or taste of the formulation.

- To reduce the GI side effects caused by certain drugs.
- To inhibit the drug-drug interactions or additional drug interactions.
- To modify the oil and liquid drugs into microcrystalline or amorphous powders[15].

The following shall be added as the disadvantages of the CDs that are as follows:

- Application of high doses shall be not being recommended.
- Due to biotransformation of CDs by intestinal bacteria may cause gastric discomfort and diarrhoea[14].

CDs can be used as building blocks for the construction for of supramolecular complexes. Since they have the ability to bond covalently or non-covalently specifically to other CDs. Thus these CDs can be architected into CD catenanes, rotaxanes, polyrotaxanes and tubes can be built or constructed[1]. These complexes can be formed by a variety of techniques that depend on the properties of the active material, the equilibrium kinetics, the other formulation ingredients and processes and the final dosage form desired[1]. Certain important characteristics features of CDs have been listed in Table 1[16].

Table 1: Characteristics features of diverse cyclodextrins.

Characteristics	α-Cyclodextrin	β-Cyclodextrin	γ-Cyclodextrin
Water solubility (g per 100 cm^3) at 25°C	14.5	1.85	23.2
Molar mass (g mol^{-1})	972	1135	1297
Internal diameter (10^{-10}m)	4.9	6.2	7.9
External diameter(10^{-10}m)	14.6	15.4	17.5
Cavity volume	174	262	472
Depth-height cone (10^{-10} m)	7.9-8	7.9-8	7.9-8
Crystal water (m %)	10.2	13.2-14.5	8.13-17.7

Polyrotaxanes (polypseudorotaxanes) are polymeric architectures with cyclic compounds threaded in polymeric chains[17]. These new class of supramolecular materials that have been evaluated in cell culture systems for their gene delivery[18-21] and drug release[21, 22] characteristics. Since the first synthesis of α-CD/poly(ethylene oxide) (PEO) polyrotaxanes[23-26] many efforts have focused on developing new generations of these macromolecular assemblies using different stoppers and CD monomers[19,21,22,26-39]. The common characteristic of a polyrotaxane architecture is the threading of macrocyclic host molecules onto a polymer chain of compatible dimensions via host-guest hydrophobic interactions, followed by capping the of the polymeric chain with bulky molecules to form a molecular necklace type structure[23, 40].

The pioneer report on constructing a inclusion complex (polypseudorotaxane) using CD was published by Ogata and his team in 1976[22]. They prepared several inclusion complexes

consisting of an aromatic or aliphatic diamine with β-CD and then applied the complex to polycondensation reactions with an acid chloride such as isophthaloyl and terephthaloyl dichloride to prepare CD included polyamides. In1990, Harada and Kamachi reported a very straightforward method to prepare an inclusion complex (pseudopolyrotaxane) between α-CD and a linear polymeric chain such as polyethylene glycol (PEG) of various molecular weights in aqueous conditions[36]. They found that one can obtain pseudopolyrotaxanes as a precipitate within a short period of time if the saturated aqueous solution of α-CD is directly mixed with aqueous solutions of PEG at room temperature. This unexpected finding led to explore quite a new paradigm of the world of polyrotaxane as functional polymers. At almost the same period of time, Wenz and his team reported the first polyrotaxane synthesis from α-CD and a polyamine[37]. Harada and Kamachi have analyzed not only the formation of a variety of pseudopolyrotaxanes, but also the preparation of polyrotaxanes and their related architectures, and their significant efforts contributed much to the progress in understanding polyrotaxanes using CDs[3,38].

The solubility, the pharmacokinetic and pharmacodynamic properties of pharmacologically active moieties can be changed by using novel drug delivery systems or by modifying the molecular structure and/or physiological parameters depending on the route of administration. The interaction between CD, drug, and dosage form influences the kinetics of key processes of a drug delivery, including dissolution and absorption. Thus, the choice of the best drug delivery system will aim to enhance drug bioavailability and reduce their toxicity. In the complexation processes, the different interactions between chemistry species (molecules, ions, radicals) that do not involve covalent bonds are mostly of the host-guest type. CDs seem to be the most important ones in this domain. Also, the incorporation of CDs into a drug dosage form may affect the activity of basic pharmaceutical ingredients and may modify the properties of the whole drug formulation. The complexation can influence the drug release, but the process does not affect the drug intrinsic ability to permeate lipophilic biomembranes. However, CD shave been used as permeation enhancers in topical formulations[41] and to increase the permeability of water-insoluble drugs by making the drug available at the biological membrane surface (skin, mucosa, or the eye cornea). For water-soluble drugs, CDs increase drug permeability by direct action on membrane and enhance drug absorption and/or bioavailability[42-49]. The drug release can be modified by using CDs and their derivatives[15]. Polyrotaxanes can be architected as injectable hydrogels, drug-polyrotaxane conjugates and nanoparticles for drug and/or gene delivery[17].

METHODS OF PREPARATION

Polyrotaxanes are unique complex molecules which are the derivatives of CDs. These molecules can usually synthesized by template-directed approach that depends on molecular recognition and self assembly processes. Another general method of synthesis for polyrotaxanes is the threading followed by stoppering technique. In this approach several macromolecules are threaded onto oligomeric or polymeric axles carrying recognition sites at prescribed intervals along the axles to form pseudorotaxnes, then both the ends of the axles are stoppered with bulky groups. Although this approach is relatively simple, it does not provide complete control over the number of threaded macrocycles, that is, the rings or beads are often not threaded onto all of the available recognition sites on the axles[50]. Common preparation of polyrotaxanes for use in living body by this method is that pseudopolyrotaxanes with amino acid derivatives as bulky end-groups via a condensation reaction, and a chemical modification of CD molecules in polyrotaxanes for improving water-solubility and/or for functionalizing by conjugation of biologically-active agents. Basically, the preparation of polyrotaxanes for biological applications consists of the following two steps: the preparation of a pseudopolyrotaxane by mixing α-CD and PEG-bis(amine) (PEG-BA) in water, and a subsequent capping reaction with amino acid derivatives such as N-benzyloxycarbonyl-L-tyrosine(Z-Tyr) In the first step, of course, pseudopolyrotaxanes consisting of α-CD and PEG–BA are commonly prepared according to the method reported by Harada and his co-workers as mentioned above. In the second step, Z-Tyr is allowed to react with the terminal amino groups of PEG–BA in pseudopolyrotaxanes by using a variety of suitable condensing agents in DMF or MeOH. When biologically labile linkages such as disulfide bond are introduced into the polyrotaxane, an SS-introduced PEG–BA is employed in place of PEG–BA mentioned in the above protocol[3].

Purification of the polyrotaxanes is one of the important aspects in preparing biomaterials. In the course of the polyrotaxane preparations mentioned above, contamination from unthreaded CD and other chemicals is highly problematic and is to be completely removed. Usually, purification of polyrotaxanes by reprecipitation and dialysis in DMSO and water is very promising in removing these undesirable contaminants. Also, GPC measurements in DMSO or in suitable aqueous solutions are helpful to verify the purification of polyrotaxanes. Finally, chemical composition such as the number of threading CD molecules in the obtained polyrotaxane is calculated from the ratio of peak integrations for both C(1) protonsin α-CD around 4.8 ppm and methylene protonsin PEG around 3.5ppm in the NMR spectrum measured in D_2O/NaOD[5].

Water solubility of polyrotaxanes is a critical issue when considering their medical and pharmaceutical applications, such as drug delivery. In general, polyrotaxanes prepared following the above-mentioned methods are poorly soluble in water, illustrated by the fact that the insolubility of pseudopolyrotaxanes in water enabled their isolation with ease. In order to endow intact polyrotaxanes with water solubility, a variation of the chemical modifications which have been previously reported for CD chemistry[38] is applicable. In particular, charged functional groups such as amino and carboxyl groups can be introduced at the hydroxyl groups of CDs through suitable spacers, since such chemical modifications are useful not only for improving water solubility but also for further functionalizing polyrotaxanes with biologically-active moieties in the following step. For the introduction of carboxyl groups, CD-containing polyrotaxanes are allowed to react with dicarboxylic acid anhydrides such as succinic anhydride in pyridine. A variety of biologically active agents can be introduced at these groups in the polyrotaxanes via condensation reactions. The dialysis against water, GPC and NMR measurements in water are conventional steps to ascertain purification and chemical compositions[3].

Clipping approach is the one in which the macrocycles are formed from acyclic precursors in the presence of templating recognition sites on the dumbbells, has provided[39,51-53] a versatile means for the construction of some lower-order rotaxanes[3,50]. There are different methods of synthesis of polyrotaxanes like entering, slipping as shown below in Figure 2

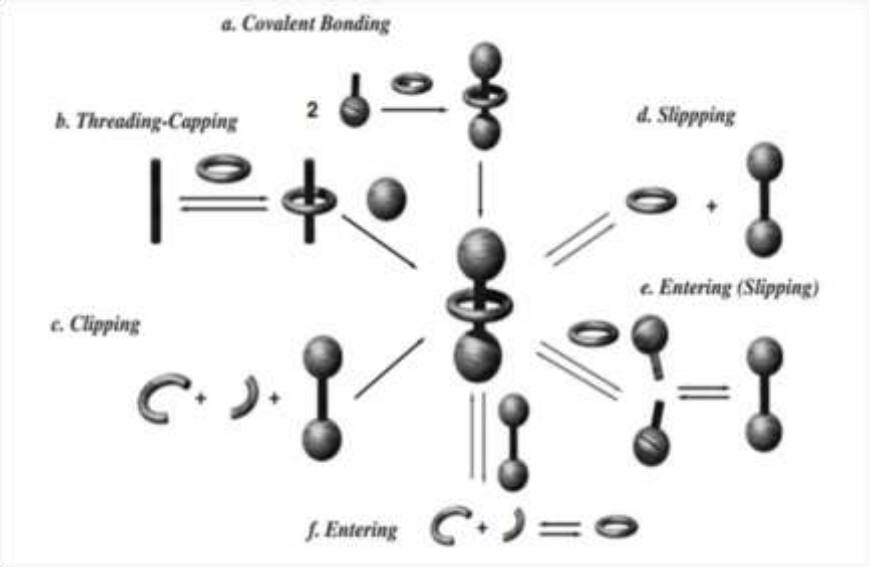

Figure 2: Schematic representation of different methods of synthesis of polyrotaxanes

POLYROTAXANES APPLICATIONS

In Multivalent Ligand Receptor Interactions

Control of the attaching of biologically active agents or ligands to receptor sites of proteins on the plasma membranes of cells is an important factor for modulating receptor-mediated cellular metabolism as well as endocytosis for drug delivery. One of the crucial aspects in this event is how effectively and specifically the attaching on membrane proteins using very low quantities of the agents or ligands can be achieved. In this perspective, a "multivalent interaction" using a functional polymer has been proposed and extensively studied over the last decades[54]. The term of multivalency is defined as a way to bind simultaneously multiple copies of ligands with receptor sites of proteins. This approach is believed to be promising for enhancing the attaching constant of ligand-receptor interaction, and is expected to exploit significant improvements of such applications as targeting drugs, drug mediated drug delivery and tissue regenerations.

A variety of functional polymers has been designed and demonstrated to be a tool of multivalent ligand-immobilized polymers. However, the binding constant using such polymers was not as enhanced as expected. Such unsatisfying results using a functional polymer were mainly attributed to a spatial mismatch between the ligand-polymers and receptor sites of proteins. Increasing the number of ligands in the polymer eventually causes an excessively large density of ligands, and this excess density is thermodynamically unfavourable to multivalent interactions between the ligands and receptors. From these perspectives, the multivalent interactions using polyrotaxanes is enhanced. As seen above as well as in Figure 3, the most amazing features observed in polyrotaxanes is the freely mobile nature of cyclic compounds threaded onto a linear polymeric chain capped with bulky end-groups. Thus, we can hope that polyrotaxanes are advantageous in deriving thermodynamic benefits for enhancing multivalent interaction with biological systems[3].Certain examples for drug delivery system for protein drugs with use of polypseudorotaxanes and polyrotaxanes are quoted in the Table 2 below.

Table 2: Examples of drug delivery systems for macromolecules and protein drugs with use of polypseudorotaxanes and polyrotaxanes.

Drug	Polypseudorotaxanes or Polyrotaxanes	Application	Reference
FITC-dextran (model compound)	Polypseudorotaxane hydrogel	Sustained release carrier	(87,74, 88)
PEGylated insulin, PEGylated lysozyme	Polypseudorotaxane	Sustained release carrier, stabilizer to peptidase	(89, 90)
Insulin	Polypseudorotaxane hydrogel	Sustained release carrier	(91)
Glucose oxidase	Polypseudorotaxane nanosphere	Stabilizer to heating	(92)
Lysozyme	Polypseudorotaxane hydrogel	Sustained release carrier	(93, 94)
BSA	Polypseudorotaxane hydrogel	Sustained release carrier	(95, 96, 75)

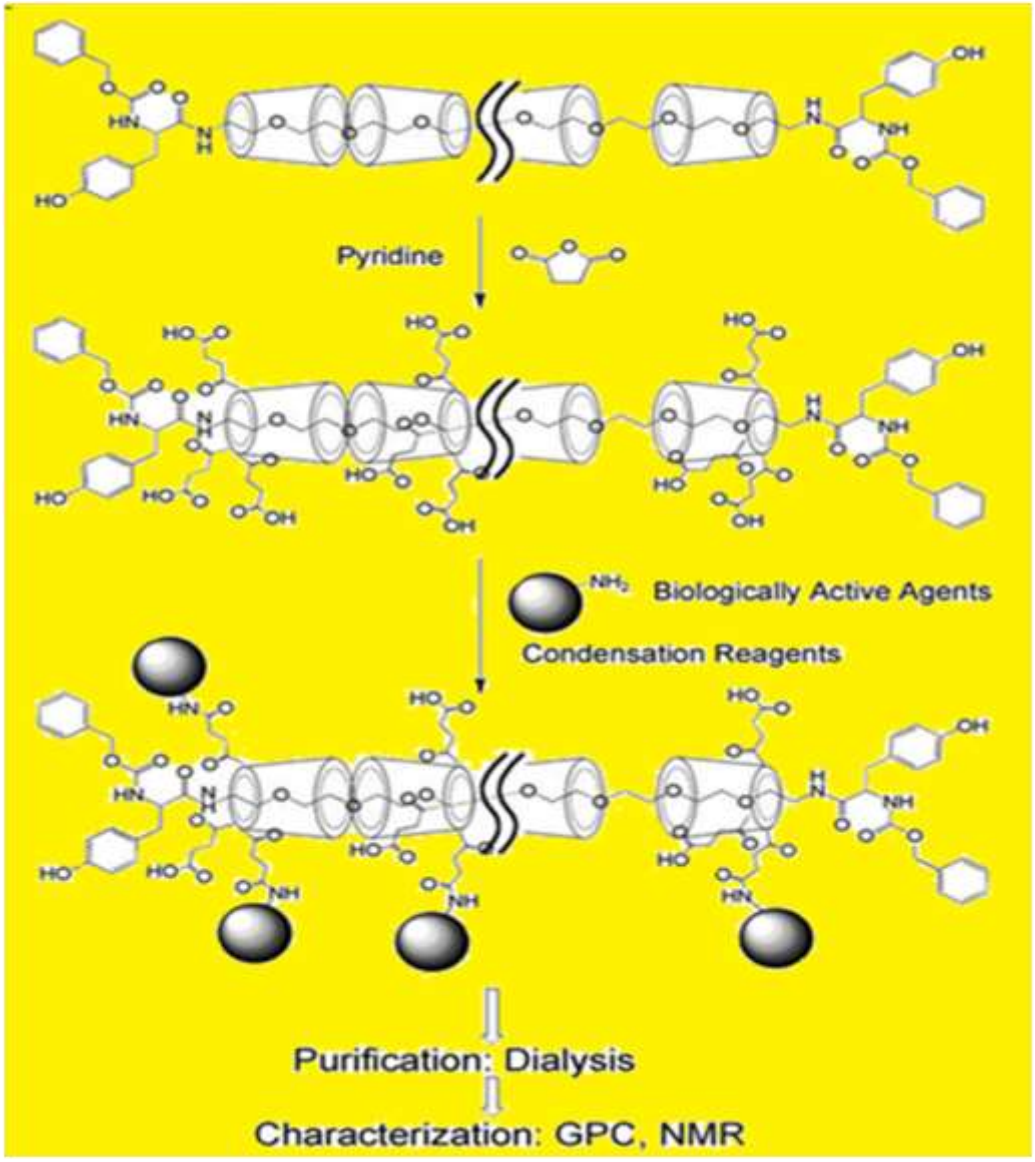

Figure 3: Synthetic scheme of synthesis of functional polyrotaxanes, in which carboxyl groups were introduced into hydroxyl groups of CDs[3]

Freely mobile ligands conjugated to the cyclic compounds in polyrotaxanes would effectively bind to receptor proteins in a multivalent manner, which is based on the enthalpic gain due to enhanced opportunity of the binding for increasing internal energy of the bond molecules via their excellent mobility close to low molecular-weight compounds. Although the multivalent event is entropically unfavourable, the enthalpic gain would over compensate it in comparison with conventional ligand–polymer conjugates. This has been proven that polyrotaxanes show pronounced effect on ligand receptor multivalent interactions by use of saccharide-conjugated polyrotaxanes with a lecithin, a model receptor protein A series of the maltose–conjugated polyrotaxanes with different numbers of threading α-CD molecules was prepared from an

inclusion complex consisting of α-CD and an α, ω-diamino-PEG with an average molecular weight of 20, 000 Da[3].

As Anticancer Drug Carriers

Most anticancer drugs are hydrophobic molecules with serious toxicity to normal tissues and cells. The focus of anticancer drug delivery is to enhance the solubility of anticancer drugs in aqueous medium and target to tumors. Hydrogels are widely used carriers for anticancer drug release. With the spontaneous self-assembly of polyrotaxanes in aqueous medium, the polyrotaxane crystals act as physical cross linkers to form supramolecular hydrogels: this type of hydrogel is injectable. The drug release study of the injectable hydrogels using a high molecular-weight model drug, as well as small anticancer drugs, exhibited long sustaining drug release. The polyrotaxane hydrogel exhibited a promising perspective for anticancer drug delivery, and it could be used for *in situ* cancers therapy. Drug-carriers conjugate is an important form to deliver anticancer drugs. Anticancer drugs were conjugated on the polyrotaxanes via stimuli-sensitive linkages with the combination of cleavable bonds in the end-caps of the polyrotaxanes[17, 55].

The anticancer drugs were smartly released, triggered by the *in vivo* environments of tumors to fulfill targeting release. Because of the large amount of the hydroxyl groups on polyrotaxanes, high drug loading content was easily achieved in the conjugates. Besides high drug-loading content, targeting efficiency is more important for anticancer drug delivery. Many researchers have found that the targeting design of anticancer delivery systems is efficient *in vitro*; however, the *in vivo* targeting efficiency is very poor. It is attributed to the complicated *in vivo* obstacles, which diminishes the targeting recognition. It is well known that the receptors on the cell membranes have the conformation recognition capability, in the traditional ligand-receptor targeting mode, the ligands are usually immobilized on the carriers, which limit their mobility to form multivalent interactions with the receptors on cell membranes[3], and it is an important factor to decrease the targeting efficiency. In polyrotaxane–drug conjugates, both anticancer drugs and ligands were immobilized on CDs via the functional hydroxyl groups. As the modified CDs could slide along and rotate around the PEG chains, this flexible motion avoided the mismatch of ligand-receptor interaction and the multivalent interactions between the conjugates and cell membranes could be formed. This active recognition mode provides a promising strategy to enhance targeting efficiency. The polyrotaxanes for drug/gene delivery were only developed in the last two decades and there is a long way to go. Currently, most of the research highlights the fundamental aspects of the design of carrier architectures and *in vitro* studies. The preliminary success of the *in vivo* investigations of the polyrotaxanes for drug delivery has evoked more and

more solicitudes to biomaterials scientists and pharmacists due to its great potential significance in cancer therapy[17].

Another new approach of constructing supramolecular nanoparticles generated by self assembly polyrotaxanes for antitumor drug delivery has been reported by use of cinnamic-acid-modified PEG chains that were threaded in α-CDs to form polyrotaxanes. The polyrotaxanes self-assembled supramolecular nanoparticles. The morphology of the nanoparticles was changed from nanovesicle to micelle after the antitumor drug, doxorubicin, was loaded. The release profile of the drug-loaded nanoparticles was investigated, and it was found that the sustaining release time could last for 32 hours. The drug-loaded nanoparticles were co-cultured with mouse 4T1 breast cancer cells with a drug concentration of 10μg/ml; the cell survival rate was 3.3% after 72-hour incubation. In an *in vivo* study of breast cancer in a mouse model, the drug-loaded nanoparticles were injected in the tail veins of mice with a dose of 5 mg/kg body weight. The tumour inhibition rate of drug-loaded nanoparticles was 53%, which was better than that of doxorubicin hydrochloride. The cardiac toxicity of doxorubicin was also decreased greatly after the encapsulation into supramolecular polyrotaxane nanoparticles[19].

For Manifestation of Niemann-Pick Type C Disease

Niemann-Pick type C is biochemically, genetically and clinically distinct from Niemann-Pick Types A or and B. In Types A and B, there is complete or partial deficiency of an enzyme called acid sphingomyelinase. In Niemann–Pick type C, the protein product of the major mutated gene NPC1 is not an enzyme but appears to function as a transporter in the endosomal-lysosomal system, which moves large water-insoluble molecules through the cell. The protein coded by the NPC2 gene more closely resembles an enzyme structurally but seems to act in cooperation with the NPC1 protein in transporting molecules in the cell. The disruption of this transport system results in the accumulation of cholesterol and glycolipids in lysosomes.

Cholesterol and glycolipids have varied roles in the cell. Cholesterol is a major component of cell plasma membranes, which define the cell as a whole and its organelles. It is also the basic building block of steroid hormones, including neurosteroids. In Niemann-Pick type C, large amounts of free or unesterfied cholesterol accumulates in lysosomes, and leads to relative deficiency of this molecule in multiple membranes and for steroid synthesis. The accumulation of glycosphingolipids in the nervous system has been linked to structural changes, namely ectopic dendritogenesis and meganeurite formation, and has been targeted therapeutically. Several theories have attempted to link the accumulation of cholesterol and glycolipids in the lysosomes with the malfunction of the NPC-1 protein[56]. The defect manifests itself in the form of

neurological, hepatic, and/or pulmonary symptoms that increase in intensity over time. These can involve enlargement of organs, dysarthria, dysphagia, ataxia, and other neurological symptoms including epilepsy and dementia. NPC typically manifests in children and is ultimately fatal, even with current treatment regimens[38].

Treatment of Niemann-Pick C (NPC) type 2-deficient fibroblasts with the polyrotaxane derivatives produced substantial reductions in sterol accumulation, which can be observed by diminished filipin staining in these cells, suggesting that Pluronic-based polyrotaxanes may be promising vehicles for delivery of HP-β-CD to cells with abnormal cholesterol accumulation[40].

Gene Delivery

Gene delivery using cationic polymers is one of the greatest challenges for inventing non-viral gene carrier systems, instead of virus-based vector systems which are risky due to genomic integration concerns, and have low usefulness due to single use limitations. Cationic polymers can form a polyplex in some buffer conditions through an electrostatic interaction with phosphate anions of pDNA. However, low transfection efficiency still remains a bottleneck, preventing the use of cationic polymers in clinical applications. It was assumed that the polyplex stability in the blood circulation as well as the pDNA release from the polyplex in target cells was an important factor to enhance the transfection activity of nonviral gene carriers[57].

In order to enhance the pDNA release from the gene carrier, many research groups have focused on the introduction of disulfide linkages to into the main chain of polycations, because the cleavage of disulfide linkages decreases the molecular weight of polycations induce the dissociation of the polyplex (the release of pDNA) in the cytosolic milieu and/or in the nuclei[14, 53, 58, 59]. However, an excess amount of the disulfide linkages can result in the over stabilization of the polyplex against the counter-polyanion exchange reaction[10]. In order to overcome these problems, biocleavable polyrotaxane have been designed, in which dimethylaminoethyl-modified α-CDs (DMAE-α-CDs) are threaded onto a poly(ethylene glycol) (PEG) chain capped with benzyloxycarbonyl-L-tyrosine (Z-L-Tyr) via disulfide linkages (DMAE-SS-PRX). The supramolecular structure of the DMAE-SS-PRX led to polyplex formation and to pDNA release, which was induced by the cleavage of only two SS linkages at both terminals, and which might be due to the dissociation of the PRX into α-CDs and PEG thus optimizing the design for effective gene carrier[3, 57].

Interestingly there are also CD based polyrotaxanes which carry the drugs to the gene which can be described as the supramolecular structures formed between CDs (CDs) and polymers have inspired interesting developments of novel supramolecular biomaterials. This review will update

the recent progress in studies on supramolecular structures based on CDs and block copolymers, followed by the design and synthesis of CD-based supramolecular hydrogels and biodegradable polyrotaxanes for potential controlled drug delivery, and CD-containing cationic polymers and cationic polyrotaxanes for gene delivery. Supramolecular hydrogels based on the self-assembly of the inclusion complexes between CDs with biodegradable block copolymers could be used as promising injectable drug delivery systems for sustained controlled release of macromolecular drugs. Biodegradable polyrotaxanes with drug-conjugated CDs threaded on a polymer chain with degradable end-caps could be interesting supramolecular prodrugs for controlled and targeting delivery of drugs. CD containing cationic polymers as gene carriers showed reduced cytotoxicity than non-CD-containing polymer counterparts. More importantly, the polyplexes of CD-containing cationic polymers with DNA could be pegylated through a supramolecular process using inclusion complexation between the CD moieties and a modified PEO. Finally, new cationic polyrotaxanes composed of multiple oligo ethylenimine-grafted CDs threaded and end-capped on a block copolymer chain were designed and synthesized as a new class of polymeric gene delivery vectors, where the chain interlocked cationic cyclic units formed an integrated supramolecular entity to function as a macromolecular gene vector. The development of the supramolecular biomaterials through inclusion complexation has opened up a new approach for designing novel drug and gene delivery systems, which may have many advantages over the systems based on the conventional polymeric materials[60].

In siRNA Delivery

Nowadays there is fast growing acceptance of vast therapeutic potential of RNAi in aspects of disorders like neurological, cancer and cardiovascular diseases. Advantages such as high specificity and the ability to act “upstream” from conventional chemotherapeutic agents gives them the ability to potentially target any protein and evade drug resistance. Safe delivery of siRNA (small interfering RNAs) to affected cells may offer target specific treatment of genetic diseases[61-64], however, the clinical success of this approach is limited by the absence of efficient, low toxicity delivery vehicles. Viral and non-viral vectors have been studied for this purpose, but they both suffer from several key limitations. Although efficient and persistent, viral vectors are challenged by issues such as large-scale production, immunogenicity, and safety, whereas non-viral vectors are limited primarily by lack of efficiency. The scalability and modest host immunogenicity relative to viral vectors has garnered considerable attention for non-viral gene delivery approaches. Many different non-viral delivery vehicles have been explored for siRNA delivery, including nanoparticle complexes based on CD-oligomers[65-69], cationic lipids[70],

lipid/Ca^{2+} mixtures[71], Au or PLGA[72]. All these vehicles are capable of forming nanoparticles that are smaller than 200 nm in diameter and can efficiently deliver siRNA into target cells. They can also protect the nucleic acid from enzymatic degradation and enhance the cell permeability of the siRNA cargo; however, most of these vehicles have undesirable toxicity, immunogenicity and/or serum stability profiles. Use of clinically approved biodegradable and bio-compatible materials for constructing new non-viral delivery vehicles can potentially address these problems[73].

RNA interference has broad therapeutic potential due to its high specificity and ability to potentially evade drug resistance. Three cationic α-CD: poly(ethylene glycol) polyrotaxanes derived from polymer axle different sizes (MW 2000, 3400 and 10000) have been synthesized for delivering siRNA. These polyrotaxanes are able to condense siRNA into positively charged particles that are < 200 nm in diameter, enabling their facile internalization into mammalian cells. Thus it can be proposed that the cationic polyrotaxanes display a size activity relationship, but the higher molecular weight polyrotaxanes (PEG 3400 and 10000) are able to condense and deliver siRNA better than the lower molecular weight material (PEG2000)[73].

In Gene Silencing

RNA interference (RNAi) has gained considerable attention for its potential application in the treatment of diseases such as varied neurological, viral, cancer and heart diseases. Small interfering RNAs (siRNAs) are[70, 74, 75] nucleotide long fragments that are incorporated into the RNA-interference silencing complex (RISC) in the cytoplasm of the cell[61]. A multifunctional protein present inside the RISC known as Argonaut then unwinds the siRNA, leading to cleavage of the sense strand[61]. The antisense strand is retained within the activated RISC where it helps to selectively bind and degrade its complementary mRNA target. Due to its catalytic nature, appropriately designed siRNA can theoretically silence any gene in the body via cleavage of multiple mRNA strands, thus making it a promising tool for therapy[61]. The primary advantage of RNAi over conventional chemotherapy is its high specificity and the action of siRNA "upstream" from most chemotherapeutic agents, thereby conferring the ability to potentially evade drug resistance by targeting virtually any transcript to knock down protein expression of the selected sequence[66, 76].

Despite advances in the identification of various gene targets and therapeutic siRNAs, the clinical success of RNAi is greatly impeded by the lack of a robust, safe, efficient, and manufacturable delivery vector[77]. Many viral and non-viral vectors have been studied for this purpose, but they all suffer from key limitations. Immunogenicity and safety issues hamper the broad utilization of otherwise efficient and persistent viral vectors[63]. Non-viral vectors, on the

other hand, are primarily limited by their lack of efficiency; however, they are generally safer, far less immunogenic and scalable. Such features make non-viral gene delivery an attractive option for gene therapy[60, 66].

A variety of non-viral vectors have been studied for siRNA delivery, including CD oligomers[63, 69]cationic lipids[70, 71, 78] lipid/calcium based formulations[79] and gold[80] or PLGA[81] nanoparticles. All these vehicles are capable of forming nanoparticles that are smaller than 200 nm and can efficiently deliver siRNA into target cells. These particles can also protect the genetic material from degradation and enhance their cell permeability. However, most of these particles face serum stability and acute toxicity challenges. Development of biodegradable, low toxicity materials for use as siRNA vehicles is one means to address these problems[60, 82].

A family of branched polyrotaxanes (bPRTx+), threaded with multiple cationic α-CDs (α-CDs) onto a multi-armed poly(ethylene glycol) (PEG) core, were synthesized and studied as gene silencing vectors. These bPRTx+ formed stable, positively charged complexes with diameters of 150-250 nm at N/P ratios as low as 2.5. The bPRTx+ materials were shown to have gene silencing efficiencies comparable to those of Lipofectamine 2000 (L2k) and bPEI, while displaying similar toxicity profiles. The unique structure of these polyrotaxanes allows them to effectively condense and complex siRNA into nanoparticles at much lower N/P ratios than L2k or bPEI. These findings suggest that bPRTx+ may be useful materials for gene therapy applications[60]. Table 3 gives a list of examples of drug delivery systems for gene and siRNA with use of polyrotaxanes and pseudopolyrotaxanes as mentioned below.

Table 3: Examples of drug delivery systems for gene and siRNA with use of polypseudorotaxanes and polyrotaxanes.

Drug	Polypseudorotaxanes or Polyrotaxanes	Application	Reference
siRNA (against luciferase)	Polyrotaxanes of CDs and ionenes	Effective siRNA transfer carrier	(97)
pDNA (luciferase gene)	Polypseudorotaxane of γ-CD and PEI	Safe gene transfer carrier	(36)
siRNA (against GFP)	Multi-armed cationic polyrotaxane	Effective siRNA transfer carrier	(60)
pDNA (luciferase gene), siRNA (against luciferase)	Polyrotaxane having disulfide linkages	Bio cleavable gene and siRNA transfer carrier	(97,98, 99)
siRNA (against GFP)	Cationic polyrotaxane	Effective and safe siRNA transfer carrier	(73)
pDNA (luciferase gene)	Oligoethyleneimine-grafted polyrotaxane	Effective and safe gene transfer carrier	(100, 101,102103)
pDNA (luciferase gene)	Polypseudorotaxane of PEG-appended α-CDE/pDNA polyplex	Sustained release gene carrier	(104,105)

pDNA (luciferase gene)	Polypseudorotaxane of pDNA polyplex with α-CD	Sustained release gene carrier	(106)
pDNA (LacZ gene)	β-CD/PEG-PPG-PEG nanoparticle encapsulating PEI/pDNA polyplex	Effective and safe gene transfer carrier	(107)
pDNA (luciferase gene, GFP gene, p53 gene)	Folate-terminated polyrotaxanes modified with PEI	Tumour selective gene carrier	(108)

For Gold Nanoparticles Drug Delivery

β-CD based rotaxane functionalized gold nanoparticles were developed by introducing azobenzene-substituted β-CD (CDAS) onto the surface of gold nanoparticles via a ligand-exchange reaction. The key step in the synthetic strategy is the preparation of self-included CDAS in water as an intermediate. In this process, an aqueous solution is beneficial to the formation of the gold nanoparticles rotaxane hybrid, whereas non-self-included CDAS functionalized gold nanoparticles can be produced in a DMF/water mixture solution. The combination of molecular machines and metal nanoparticles into single entities may lead to new applications in nanoscale digital information processing[83].

For Inhibitory Effect on Intestinal Transports

One of the possible applications directly related to multivalent ligand-receptor interactions will be the design of polymeric inhibitors which specifically bind receptors existing on cellular membranes to inhibit the uptake of biological substrates via the receptors. For example, there are many kinds of intestinal membrane transporters for the specific uptake of digested food (proteins and carbohydrates) as well as drugs such as antibiotics. The restriction of these uptakes is strongly required for patients suffering from chronic renal diseases. In this sense, it is useful, for improving their quality of life, to achieve temporal inhibition of the uptake using an inhibitor. In particular, researchers noted that such an inhibitor should be specifically recognized by a transporter without being absorbed to preclude kidney damage. From these points of view, it is obvious that ligand-conjugated polyrotaxane can be advantageous as a multivalent ligand–conjugated polymer. First they prepared dipeptide-conjugated polyrotaxanes and studied their inhibitory effect on digested peptide uptake by intestinal human peptide transporter (hPEPT1)[84]. Here, Val–Lys as a dipeptide was conjugated to α-CD threaded onto a PEG 4,000 in polyrotaxanes, and they examined the inhibitory effect of the polyrotaxanes on the uptake of a model dipeptide via hPEPT1 using hPEPT1-expressing HeLa cells. The uptake of the model dipeptide was significantly inhibited by the polyrotaxanes, and the inhibition was much greater than dipeptide-conjugated reference samples such as dextran and α-CD. Also, they found that the effect of the polyrotaxanes was significantly enhanced by preincubation with hPEPT1 expressing

cells (30 min before adding the model dipeptide), although the inhibitory effect of dipeptide-conjugated α-CD on the uptake was reduced by the preincubation. These results suggest that the supramolecular structure of the polyrotaxanes contributes to inhibiting the uptake via hPEPT1 in a multivalent manner. Furthermore, it was confirmed that the inhibitory effect is dependent upon the molecular weight of the PEG chain in the polyrotaxanes when the average molecular weight of PEG was changed between 4,000 and 100,000. At the same concentration of dipeptide, the highest molecular weight of PEG (MW: 100,000) showed the best inhibitory effect. After selecting the optimum molecular weight, dependence of hPEPT1 expression was also examined by changing the amount of the expressed hPEPT1 (10, 20, 60μg per 15 cm dish). When the amount of expressed hPEPT1 on HeLa cell surface was the highest (60μg per 15-cmdish), the dipeptide-conjugated polyrotaxane was found to exhibit the greatest inhibitory effect on the uptake of (3H) Gly-Sar. This result suggests that high expression level of hPEPT1 was essential for inducing multivalent interaction[85]. *In vivo* performance of biomaterials after *in vitro* studies is one of the important steps for proving their applicability. When they applied the dipeptide-conjugated polyrotaxanes to an *in vivo* model using mice, the result was not as striking as expected. They considered this failure is due to insufficient blockage of the binding sites of the hPEPT1 located on the intestinal membrane: it is not so easy to occupy fully all the binding sites of the transporter with dipeptide molecules conjugated to the polyrotaxane. It is also well known that the activity of hPEPT1 is governed by proton concentration at the membrane. In order to maintain the binding with hPEPT1 via the multivalent interaction and then decrease the surrounding proton concentration on the intestinal membrane to complete the inhibition of the uptake, a polyrotaxane conjugated with dipeptide as well as sodium carboxylate was prepared as a revised sample. This polyrotaxane was found to decrease the pH of the intestinal tract significantly and to cause the inhibition of the dipeptide uptake in mice[85].

Presumably, the polyrotaxane can bind the intestinal membranes via multivalent interaction with hPEPT1 and effectively contribute to decreasing the local pH on the membrane, resulting in a significant inhibition of the uptake in mice. These results clearly indicate the efficacy of the ligand–conjugated polyrotaxanes as an inhibitor in intestinal uptake *in vivo*. Furthermore, researchers prepared 2-(N,N-dimethylamino) ethylcarbamoyl (DMAEC) polyrotaxanes and examined their inhibitory effect on the uptake of cations via carnitine/organic cation transporter, OCTN2, existing on the intestinal membrane and in tissues[82]. The DMAEC-polyrotaxanes were prepared by treating polyrotaxane with carbonyldiimidazole (CDI) in DMSO, followed by condensation with N,N-dimethylethylenediamine. The inhibitory effect was evaluated in terms of

the uptake of L-carnitine in OCTN2-transfected HEK293/PDZK1 cells. The DMAEC polyrotaxanes prepared from a PEG 20,000 were found to inhibit completely the uptake, although DMAEC-CD, the constituent molecule, and the polyrotaxanes with a shorter PEG 4,000 showed far less inhibition. This result indicates that DMAEC groups along the polyrotaxanes are actually recognized simultaneously with multiple copies of OCTN2 on the cell surfaces, resulting in the complete inhibition of L-carnitine uptake. Another interesting feature seen in the cationic polyrotaxanes in this study is their very low cytotoxicity. In general, cationic polymers such as polyarylamine (PAAm, Nitoo Boseki Co. Ltd., Japan; - $(CH_2CH(CH_2NH_2))n$-) have been known to show high cytotoxicity when applied to native tissues and cells, due to non-specific interaction with plasma proteins, sometimes leading to intracellular damage, including mitochondrial energy transfer. Indeed, the PAAm inhibited the uptake markedly, but showed very high cytotoxicity in terms of the MTT assay. The MTT assay used here measures the reduction of a tetrazolium compound (MTT) to an insoluble formazan product by the mitochondria of living cells[86]. Thus, this result suggests that the inhibition observed for PAAm is not due to the specific binding with OCTN2 but to a non-specific binding with plasma proteins, resulting in undesirable cytotoxicity through mitochondrial metabolism. However, our designed DMAEC-polyrotaxanes showed less cytotoxicity in term of the MTT assay, and the cell viability was almost the same as that of a control. Presumably, the lower density of DMAEC groups along the polyrotaxane structure is considered to prevent intracellular uptake (endocytosis) of the polyrotaxane accompanied by electrostatic interactions with plasma proteins. The mobility as well as low density of DMAEC groups along the polyrotaxane structure appears to prevent cytotoxicity. These results strongly suggest that the cationic polyrotaxanes which were designed are promising candidates for an effective inhibition of cation transport via OCTN2 with less cytotoxicity [3]. Table 4 covers examples of drug delivery system of low molecular weight drugs with use of polyrotaxanes and polypseudorotaxanes.

Table 4: Examples of drug delivery systems for low molecular weight drugs with use of polypseudorotaxanes and polyrotaxanes.

Drug	Polypseudorotaxanes or Polyrotaxanes	Application	Reference
Ascorbic acid	Polypseudorotaxanes	Stabilizer, absorption enhancer	(109)
Indomethacin	Polypseudorotaxanes	Sustained release carrier	(110)
Prednisolone	Polypseudorotaxanes	pH responsible and controlled release carrier	(111, 112)
Coenzyme Q10	Polypseudorotaxanes	Stabilizer, absorption enhancer	(113,114)
Cisplatin	Polypseudorotaxanes hydrogel	Sustained release carrier	(115)

Paclitaxel	Cationic polyrotaxane	Controlled release carrier	(116)
Salicylic acid	Polypseudorotaxanes	Stabilizer, absorption enhancer	(117)
Doxorubicin	Polypseudorotaxanes hydrogel	Sustained release carrier	(118, 55)
Doxorubicin	Polyrotaxane nanoparticles	Controlled release carrier	(119)
Doxorubicin	Polypseudorotaxanes nanoparticles	Sustained release carrier	(120)
Doxorubicin	Polypseudorotaxanes of pH sensitive micelle	Controlled release carrier	(121)
Doxorubicin	Polyrotaxane capped with quantum dot	Controlled release carrier	(75)
Doxorubicin, Camptothecin	Protamine-appended polypseudorotaxanes	Sustained release carrier	(122,123)
Ciclopirox, Tricinolone	Polypseudorotaxanes	Release enhancer of drug	(124)
Amphotericin-B	Polyrotaxane	Sustained release carrier	(125)
Theophylline	Hydroxypropylated polyrotaxane	Controlled release carrier	(125, 126)
Calcein (model compound)	Polypseudorotaxanes of mesoporous silica	pH responsible drug carrier	(127)

CONCLUSION

CD based polyrotaxanes and polypseudorotaxanes due to their low cytotoxicity, controllable size, and unique architecture, have inspired interesting exploitation as novel biomaterials. These polyrotaxanes can be produced by different simple, rapid and reproducible methods as mentioned in the article. The versatility of polyrotaxanes derivatives of CDs is having excellent contribution and applicability in drug delivery, therapeutics and many other areas of pharmaceutical field. CD based biodegradable polypseudorotaxane hydrogels could be used as promising injectable drug delivery systems for sustained and controlled drug release. Polyrotaxanes with drug or ligand-conjugated CDs threaded on polymer chain with biodegradable end group could be useful for controlled and multivalent targeting delivery. In addition, cationic polyrotaxanes consisting of multiple oligoethylenimine-grafted CDs threaded on a block copolymer chain were attractive non-viral gene carries due to the strong DNA-binding ability, low cytotoxicity, and high gene transfection efficiency. Cytocleavable end caps were also introduced in the polyrotaxane systems in order to ensure efficient endosomal escape for intracellular trafficking of DNA. Moreover, hydrolyzable polyrotaxane hydrogels with cross-linked α-CDs could be a desirable scaffold for cartilage and bone tissue engineering. Thus, in view of all these potential features and applications, CD based rotaxanes and polyrotaxanes are seemed to gain pristine accession in drug delivery and therapeutics.

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