European Journal of Medicinal Chemistry 45 (2010) 326–334

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Designing dendrimers for ocular drug delivery

Grégory Spataro [a,b], François Malecaze [c,**], Cédric-Olivier Turrin [a,b], Vincent Soler [c], Carine Duhayon [a,b], Pierre-Paul Elena [d,**], Jean-Pierre Majoral [a,b,**], Anne-Marie Caminade [a,b,*]

[a] CNRS, LCC (Laboratoire de Chimie de Coordination), 205 route de Narbonne, F-31077 Toulouse, France
[b] Université de Toulouse, UPS, INPT; LCC, F-31077 Toulouse, France
[c] Hôpital Purpan, Service d'Ophthalmologie, Place Dr Baylac, 31059 Toulouse Cedex, France
[d] Iris Pharma, Les Nertières, 06610 La Gaude, France

ARTICLE INFO

Article history:
Received 22 July 2009
Received in revised form
7 September 2009
Accepted 8 October 2009
Available online 14 October 2009

Keywords:
Dendrimers
Drug delivery
In vivo assays
Carboxylic acids
Ammonium salts
Carteolol

ABSTRACT

New series of phosphorus-containing dendrimers, having one quaternary ammonium salt as core and carboxylic acid terminal groups have been synthesized from generation 0 (3 carboxylic acid terminal groups) to generation 2 (12 carboxylic acid terminal groups). These dendrimers react with the neutral form of carteolol (an ocular anti-hypertensive drug used to treat glaucoma) to afford ion pair (saline) species. The solubility in water of these charged dendrimers depends on the generation considered: generation 0 (3 carteolol) is well soluble, whereas generation 1 (6 carteolol) and generation 2 (12 carteolol) are poorly soluble. These dendrimers have been tested in vivo, as vehicle for ocular drug delivery of carteolol to rabbits.

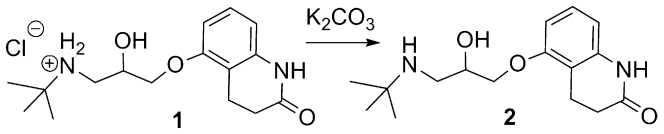
1. Introduction

Dendrimers constitute nowadays a ubiquitous type of precisely defined polymers [1], potentially usable in numerous applications. Their branched layered architectures displaying a high number of controlled terminal groups is in particular very promising for biomedical applications [2–6], and more precisely as drug carriers [7–9]. Physical entrapment in or of the dendritic skeleton (depending on the respective size and ratio of the drug and the dendrimer), or chemical conjugation are studied to use dendrimers as drug delivery vehicles. However, most of these studies concern *in vitro* delivery, and only very few *in vivo* drug delivery studies using dendrimers are available to date [10]. The first important point to be verified for such purpose concerns the water solubility and the biocompatibility [11]. PAMAM (polyamidoamine) [12–14] dendrimers are certainly the most widely used type of dendrimers for biological purposes, including drug delivery. However, there exists other types of biocompatible dendrimers [15–17], and we have shown in several occasions that the phosphorus-containing dendrimers we synthesize [18,19] are useful for biological purposes, in particular as transfection agents [20–22], anti-prion [23–25] (including *in vivo*) and anti HIV [26,27] agents, against Alzheimer [28] diseases, as imaging agents (including *in vivo*) [29–31], and for the activation and multiplication of human monocytes and innate immune Natural Killer (NK) cells [32–36].

The biocompatibility of drug delivery systems is particularly relevant when ocular delivery is concerned. Indeed, eyes have a quasi impermeable corneal surface epithelium, which necessitates a long residence time to increase the efficiency and the bioavailability of the instilled drug, to deliver it in the inner eye structure. The corneal surface is also susceptible to bacterial, fungal, and viral infections and inflammations, as well as to mechanical injuries. Furthermore, lachrymal drainage poses problems to obtain sufficiently high therapeutic drug concentration inside the eye, notably when treating disorders such as diabetic retinopathy or glaucoma, to name as a few [37]. The most common method for improving the bioavailability of a drug consists in increasing the viscosity by adding water-soluble polymers to enhance the bioadhesion of the solutions instilled [38]. However, such galenical formulations may induce a temporarily disturbed vision, particularly for people suffering of "dry eye" disorder [39]. Thus,

* Corresponding author. CNRS, LCC (Laboratoire de Chimie de Coordination), 205 route de Narbonne, F-31077 Toulouse, France. Fax: +33 5 61 55 30 03.
** Corresponding authors.
E-mail addresses: malecaze.fr@chu-toulouse.fr (F. Malecaze), pierre-paul.elena@iris-pharma.com (P.-P Elena), majoral@lcc-toulouse.fr (J.-P. Majoral), caminade@lcc-toulouse.fr (A.-M. Caminade).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.10.017



Scheme 1. Synthesis of neutral Carteolol **2**.

penetrating the ocular surface still presents a challenge for chemotherapy, and it appears tempting to use dendrimers instead of polymers in the formulation. Indeed, dendrimers have multiple extremities, which may increase the bioadhesion, but they have also very distinct properties compared to polymers, in particular a very low intrinsic viscosity [40,41], and a perfectly defined structure. However, to the best of our knowledge, only one paper has previously reported the use of dendrimers (PAMAM) for the *in vivo* ocular delivery of a drug (pilocarpine nitrate) [42].

In this paper we report the synthesis of a new series of phosphorus-containing dendrimers, and attempts to use them for the ocular delivery of a drug to treat glaucoma [43] and ocular hypertension, which are among the most frequent and severe ocular diseases, susceptible to degenerate to blindness [44]. These diseases require a very constraining life treatment, with instillations all the 3 or 4 h. Increase of the residence time of the drug could decrease the number of daily takings. We choose to test the well-known drug carteolol [45], which is a β-blocker and an ocular antihypertensive agent.

The structure of the dendrimers has been engineered in order to fulfill two criteria. The first one concerns the interaction with carteolol: carboxylic acid terminal groups should be suitable for an electrostatic association with the amino group of carteolol (via hydrogen transfer from carboxylic acid to amine). The second criterion concerns the limitation of chemical entities in the formulation: benzalkonium chloride $[PhCH_2NMe_2R]^+Cl^-$ is soften used as preservative [46]; we thought that having a quaternary ammonium group as core of dendrimers could replace benzalkonium chloride.

2. Results and discussion

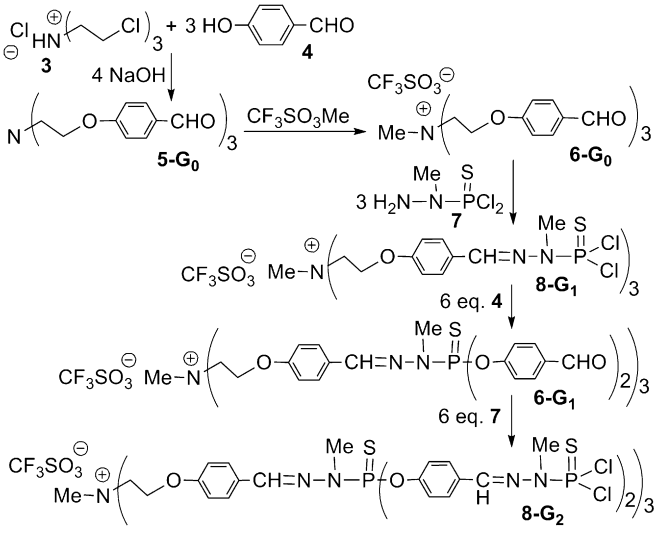
As indicated in the introduction, the interaction between a dendrimer and the drug to be delivered can be either covalent or not. The first case is frequently inefficient because the grafting of the drug is often chemically difficult, and may totally modify the properties of the drug [47], unless a prodrug is grafted. On the other hand, a purely physical entrapment might be not enough strong. An alternative option is to enhance the stability of the drug/vehicle system through the formation of the so-called catanionic systems [48]. The latter are mixtures of oppositely charged surfactants or hydrotrope entities, the resulting ion pair being stabilised by lipophilic interactions among others. Such mixtures generate a growing interest, particularly for pharmaceutical applications [49,50]. They are frequently obtained by mixing a positively charged entity such as an ammonium salt with a negatively charged entity such as a carboxylic acid salt. However, in this case the catanionic mixture is obtained together with a salt, frequently sodium chloride. A more elegant method consists in using an amine and a carboxylic acid to generate the catanionic mixture without any by-product [51]. We have already applied successfully this procedure to carboxylic acid terminated phosphorus-containing dendrimers and long chained amino sugars, leading to the first catanionic dendrimers [26,27]. These results inspired us to adapt this strategy to carteolol, which bears a free amine group, in order to form ion pair (saline) dendritic systems. The location of counter-ions in solutions of charged dendrimers is still a matter of debate, but recent simulations suggest that charged dendrimers have a high local counter-ions concentration, necessitated by charge neutrality [52].

2.1. Syntheses

The presence of a secondary amine in carteolol makes it perfectly suitable for ion pair interactions with dendrimers ended by carboxylic acids. Neutral carteolol **2** is obtained by reacting the hydrochloride derivative **1** (the commercially available form of the drug) with K_2CO_3 (Scheme 1).

As indicated in the introduction, in order to limit the number of chemical entities in the formulation, we decided to design a new family of dendrimers, possessing an analogue of benzalkonium chloride as core and carboxylic acid terminal groups for the interaction with carteolol. We chose tris(2-chloroethyl)amine hydrochloride **3** as analogue of benzalkonium chloride, and suitable as core of the dendrimer. In order to be able to apply the method of synthesis of dendrimers that we classically use [18,19], we need to graft benzaldehyde functions to **3**. The reaction of 3 equivalents of hydroxybenzaldehyde **4** with NaOH in ethanol, followed by the reaction with **3** affords compound **5-G$_0$**, issued from both the grafting of hydroxybenzaldehyde and the neutralization of the ammonium salt. The next step for the classical synthesis of our dendrimers [18,19] should be the condensation with the phosphorhydrazide **7** ($H_2NNMeP(S)Cl_2$) but the presence of an amine at the core of the dendrimer could induce unwanted side reactions, such as a partial oligomerization of compound **7**. To avoid this problem, we decided to alkylate the tertiary amine, using methyl triflate to afford compound **6-G$_0$** (Scheme 2). The alkylation is shown by the presence of a new singlet (methyl group) in ^1H and ^{13}C NMR at 3.38 and 51.4 ppm, respectively.

Compound **6-G$_0$** constitutes the real core of the new series of dendrimers that we will now describe. The next step is the condensation reaction with the phosphorhydrazide **7**, affording the first generation dendrimer **8-G$_1$**, as shown by the total disappearance of the signals corresponding to the aldehydes by ^1H and ^{13}C NMR. Substitution reactions of chlorine atoms by hydroxybenzaldehyde **4** give the other first generation dendrimer **6-G$_1$**. This reaction is monitored by ^{31}P NMR, which displays first the appearance of an intermediate singlet at $\delta = 70$ ppm,



Scheme 2. Synthesis of dendrimers having a quaternary ammonium as core.