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MOLECULES

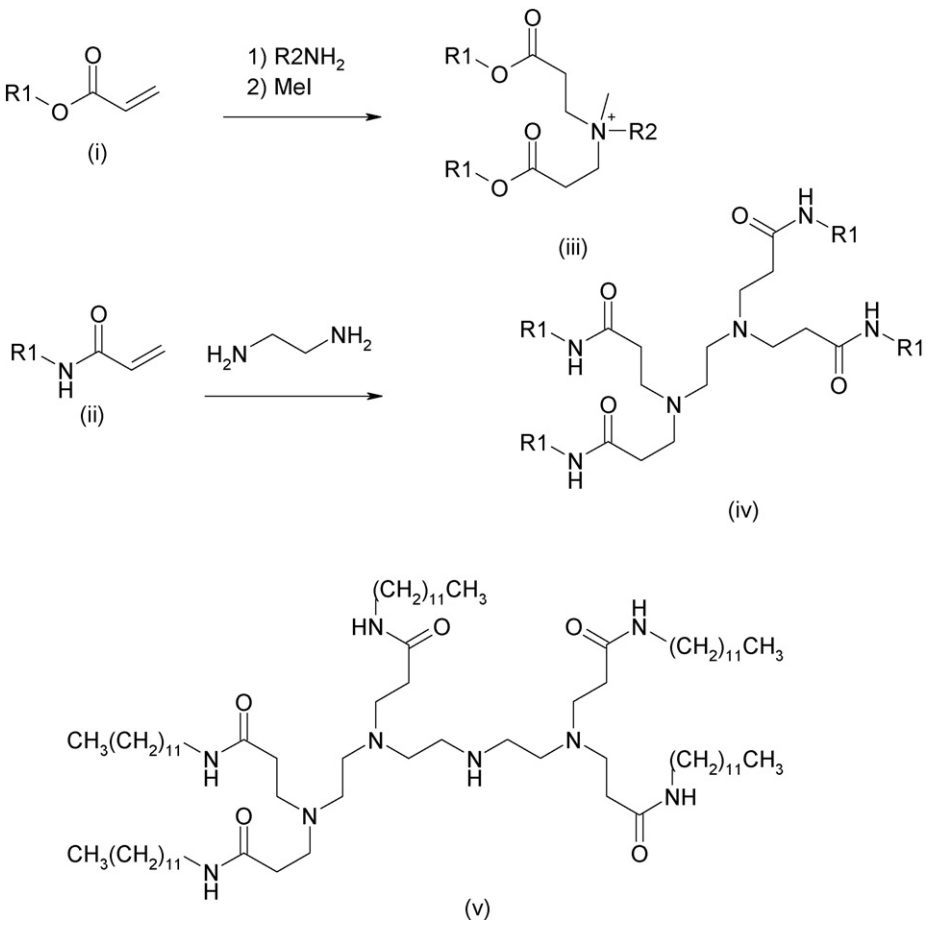
The use of technologies for the discovery of novel chemotherapeutic agents

Delivery of RNAi therapeutics from a combinatorial library of lipid-like materials

Gene expression can be modulated, silenced in the case of reduction, through the application of RNA interference (RNAi) technology. This modulation has the potential to create a new class of therapeutics that addresses previously untreatable diseases [1]. *In vivo* delivery of these putative RNAi therapeutics is, however, challenging. Certain viruses, such as respiratory syncytial virus, can be inhibited locally after administration of naked siRNA [2] and systemic delivery of siRNA to the liver of non-human primates was demonstrated using a lipid formulation [3]. Despite these examples, there are relatively few reports demonstrating the efficacy of systemically delivered siRNA in primates [3]. Research to date has focused on cationic lipid delivery systems, but the development of safe and effective methods both *in vitro* and *in vivo* has proven to be challenging. The delivery of siRNA has been mediated, for example, by direct conjugation of delivery agents to the RNA moiety [4] or peptide-based delivery systems [5]. A barrier to the exploration of new delivery materials is the synthesis of the delivery materials itself. Conventional lipid synthesis typically requires individually optimized, multiple-step synthesis, including protection and deprotection strategms, use and removal of catalysts, solvent exchanges and purification [6]. The optimization of each (multiple) synthetic step has, therefore, limited throughput and so limits the ability to generate libraries of substantial size and diversity. Recent work has attempted to develop chemical methods capable of the rapid, parallel generation of lipid-like molecules [7]. Library compounds were synthesized through a conjugate addition of alkylacrylates (i) or alkylacrylamides (ii) to primary or secondary amines, generating compounds of general structure (iii) and (iv). The advantage of using this type of chemistry is that, unlike many traditional lipid synthesis chemistries, this chemistry allows for reaction in the absence of solvent or catalysts and results only in lipidoid product, thereby eliminating the need for protection and deprotection steps and purification. Using this methodology, several libraries totalling more than 1200 lipidoid members were synthesised. Library members were then screened for their ability to deliver siRNA to a HeLa cell line that stably expresses both firefly (Photinus pyralis) and Renilla (Renilla reniformis) luciferase. Efficacy of siRNA delivery by lipidoids was determined by treating cells with siRNA–lipidoid complexes, prepared using a firefly luciferase-targeting siRNA and then measuring the ratio of firefly to Renilla luciferase expression. A number of compounds were found to enhance the delivery of siRNA to HeLa cells.

In a further round of experiments, Factor VII, a blood clotting factor, was chosen as a target to assess targeted siRNA delivery to the liver. At least seven lipidoid formulations were identified that mediated significant reduction of serum Factor VII protein levels, with the largest reduction observed for compound (v). This work is of interest as the development of this library of lipid-like materials represents an expansion of the diversity and collection of intracellular delivery materials. A number of materials were identified with both *in vitro* and *in vivo* utility in mind. Further studies are warranted to investigate lipidoid-based delivery of RNA and other drugs and to extend this technology to the broader applications of RNAi therapy and drug delivery.

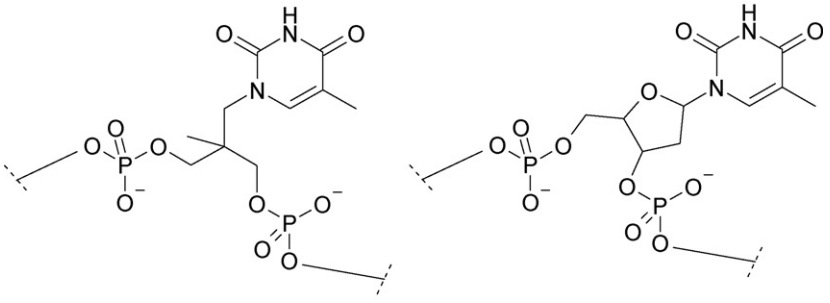
1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2009.01.001



methyl group, and the presence of a prochiral carbon in the linker that develops into a chiral center when the modified nucleotide is inserted in the sequences. The ability of the synthesised sequences to assume a quadruplex conformation has been evaluated by NMR, UV, and CD spectroscopies, as well as evaluation of the compounds for their thrombin inhibitor activity in Prothrombin Time and purified fibrinogen clotting assays. This work went on to synthesise oligonucleotide analogues using standard solid-phase DNA chemistry on a controlled pore glass support. The oligomers were detached from the support and deprotected by treatment with an aqueous ammonia solution (33%) at 55 °C overnight. The oligonucleotides were evaluated for any effect, following incubation with human plasma, on Prothrombin Time and to determine the concentration of oligonucleotide required to double the fibrogen clotting time. From this library, a number of active oligonucleotides were obtained. One of the most active oligonucleotides gave a Prothrombin Time of 24 s at a concentration of 2 μM and a doubling of clotting time was achieved by a concentration of 70 nM of the oligonucleotide in a fibrogen clotting assay. As the thrombin-binding aptamer could be considered one of the more promising anticoagulant drugs at the moment, the further elucidation of its structure–activity relationships is warranted to help understand better the nature of the thrombin-binding aptamer–thrombin recognition process.

New thrombin-binding aptamer analogues containing an acyclic nucleotide

The serine protease thrombin plays a central role in the blood clotting process, converting as it does soluble fibrinogen to fibrin, activating the factors V, VIII and XI, and stimulating platelet aggregation. There are many examples of molecules that bind to this protein allowing regulation of the clotting process, and so preventing any loss of blood and inappropriate clotting [8]. The development of anticoagulant strategies to inhibit thrombogenesis is of great interest and, among these, the most important are focused on direct or indirect inhibition of thrombin. One of these direct inhibitors that specifically binds to human thrombin (with an EC_{50} of 20 nM) is the thrombin-binding aptamer [9], a 15-mer oligonucleotide. The solution-state three-dimensional structure of the thrombin-binding aptamer has been determined by NMR methods [10]. On the basis of other NMR results produced by studying the folding of modified aptamers, it can be deduced that the tertiary structure of the thrombin-binding aptamer is intramolecular and that it adopts a chair-like conformation. Recent work has attempted to obtain new insights into the thrombin–thrombin-binding aptamer recognition [11]. This work studied the synthesis and the structural and biological characterisation of a new series of modified thrombin-binding aptamers in which the thymidines have been replaced, sequentially, by the acyclic nucleoside N^1-(3-hydroxy-2-hydroxymethyl-2-methylpropyl)-thymine to give (vi). Oligonucleotides incorporating acyclic nucleotides are of current interest, partly, because they are less susceptible to nuclease action in relation to their natural counterparts. The structural changes between the acyclic (vi) and the unmodified T residue (vii) cause a one bond shortening between the T base and one phosphate group. Additionally, the change causes the presence of a hydrophobic moiety, namely a



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