

Introduction to Themed Issue in honour of Professor Christian Leumann

Participating journals: OBC, RSC Chem Biol, RSC Med Chem, RSC Adv, NJC

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In recent years, nucleic acid drugs have been positively validated in clinics, marking the arrival of a third therapeutic modality besides small molecules and biologics. Oligonucleotide (ON) therapeutics are different from conventional medicine as they can directly regulate the expression of genes of interest. They have very different properties compared to small molecule drugs as they usually cannot be administered orally, are subject to nuclease degradation, and due to their hydrophilic and anionic nature are rapidly distributed to the liver and kidneys. Immuno-response may also need to be considered. These are the hurdles that need to be overcome. While they require specialised formulations for delivery, usually through encapsulation in polyanionic nanoparticles, and extensive modification to circumvent nuclease degradation, the demand for ON therapeutics is expected to upsurge due to their versatility in targeting diseases. A major advantage of ON therapeutics is that they can address diseases that are classed undruggable due to off-target toxicity or unavailability of a protein inhibitor. A variety of medical conditions are aimed at, though cardiovascular diseases, central nervous system disorders (neurology), (emerging) infectious diseases, metabolic disorders, oncology, gastrointestinal diseases and muscular diseases seem to be the focus point in the therapeutic area.

Currently, there are very few approved drugs based on ONs and modified nucleotides. This has offered ON market players a wide scope to discover new molecular entities for

treatment of diseases that were conventionally thought of being undruggable. The estimated market value for 2024 is just over \$7B, and searching PubMed for “oligonucleotide therapeutics” yields ~40,000 results, demonstrating the potential of this therapeutic field. There are around 135 ON therapeutics currently in different stages of drug development, which may further add to the limited number of approved drugs presently available in the ON therapeutics market. This includes 38 FDA-approved cellular and gene therapy products, but only 11 in the EU. It should be noted that a few ON therapeutics have been withdrawn from the market, mostly due to commercial failure (Glybera) rather than for medicinal issues. Currently Novartis’ Zolgensma is the world’s most expensive drug at \$2.1M per dose as of 2023. This clearly demonstrates the urgent need for further fundamental research, combined with exploring market viability, to make these more widely accessible.

This is where Christian Leumann’s research efforts come into play. He rightly says “...in the past, we were very focused on basic research, and today we are translating our scientific findings more into added value for society. After all, that’s one of the tasks a university has.” We should, however, not neglect the basic research which lays the foundation for successful applications. Christian has made substantial contributions to the field of oligonucleotide therapeutics by developing the bicyclo–DNA analogues as early as 1993;^{1,2} the follow-on second generation tricyclo–DNA analogues followed soon thereafter in 1997.^{3, 4} Importantly, Christian’s bicyclo–DNA was one of the very first examples of nucleic acids based on conformational restriction and structural pre-organization.^{5,6} The reduced flexibility allowed to increase thermal stability by minimizing the entropic cost for duplex formation. The reduced torsional flexibility, increased nuclease stability and lack of RNaseH activity had a major impact in how we see and design nucleotide analogues for improved efficacy in oligonucleotide therapy and antisense technology.^{7,8} Noteworthy, the α -anomer of bc–DNA is now being explored for commercial applications by *Alpha Anomeric* making use of its unprecedented pharmacological properties.⁹ Further, tc–DNA is being produced by *Synthena*, which was co-founded by Christian Leumann and Luis Garcia.¹⁰ It can well be argued that this research formed the basis for the development of locked nucleic acids (LNA), which today constitutes a major modification used in antisense technology. Further to

contributions to the field of therapeutics, Christian developed a variety of novel approaches to modify oligonucleotides with insightful chemistries. For instance, at the forefront of unnatural base pairs and genetic alphabet expansion strategies, Christian introduced the bipyridine and related aromatic analogues into oligonucleotides.¹¹⁻¹³ This impressive research effort allowed to refine the understanding of the relative contributions of hydrogen bonding and stacking interactions to duplex stability.¹⁴

This virtual special themed issue, which is supported by several flagship RSC journals, clearly demonstrates the breadth of nucleoside analogues that are either still in development, or are already being in advanced stage of pharmacological testing. The research and review articles are all rooted in the field of chemically modified nucleic acids. A review article by Aurélie Goyenvale and colleagues summarizes recent progress made in the development of antisense oligonucleotide drugs with a strong emphasis on the contributions of Christian Leumann's laboratory and mainly on the tricyclo-DNA system. In the context of antisense oligonucleotides, the article by Punit Seth, Stephen Hanessian and colleagues presents an alternative chemical strategy to further constrain the rotational freedom of phosphodiester linkages. Particularly, two adjacent phosphate moieties of LNA nucleotides were connected via C15-long alkyl chains thus introducing a second layer of conformational restraint. Besides alterations to the sugar moiety, many therapeutic oligonucleotides contain phosphorothioate (PS) modifications. A limitation of this type of modification is the occurrence of desulfurization during synthesis. In a contribution of this collection, Satoshi Obika and co-workers have investigated the origin of this process and suggest that peroxides present in THF contribute to desulfurization by oxidizing phosphotriesters to the corresponding phosphodiester. The presence of these peroxides should thus be suppressed as much as possible. Finally, in peptide nucleic acids (PNA) the entire sugar-phosphate backbone is replaced by a neutral peptide-like scaffold. PNA has found numerous applications in therapy and diagnostics but adjustment and optimization of its properties is still an important and active field of research. In a manuscript by Miguel López-Tena and Nicolas Winssinger, the effect of additional charge (positive and negative) on duplex formation between PNA oligomers and complementary PNA and DNA sequences was thoroughly investigated.

Development of other chemically modified oligonucleotide therapeutics is reported in several contributions of this collection. For instance, an article by Tom Brown and colleagues describes an alternative cleavable linker moiety connecting oligonucleotides to drug payloads. As a proof-of-principle, the microtubule destabilizing reagent combretastatin A-4 (CA4) drug was attached to a DNA sequence and, after transfection into cells, was efficiently released, eventually causing cell death. In another article by Karolina Bartosik and Ronald Micura, progress towards the fabrication of chemically modified caps, a key element of therapeutic mRNAs, is described. This facilitated synthetic route mainly involves a ligation of an RNA fragment containing the central triphosphate unit to the rest of the mRNA sequence via the click reaction. Besides antisense and mRNA, improving the CRISPR/Cas9 treatment modality is the topic of several manuscripts of this themed collection. Indeed, Jean-Louis Reymond and co-workers strive to improve the cellular delivery of larger CRISPR/Cas9 DNA plasmids, while Dae-Ro Ahn and colleagues have investigated the effect of the presence of modified N^6 -methyladenosine nucleotides on the DNA cleavage activity.

In addition to oligonucleotides, nucleosides can also serve as important and potent therapeutics. A prominent example is highlighted in the contribution by Radim Nencka and co-workers, where potent inhibitors of the methyltransferase nsp14 of the coronavirus that caused the SARS CoV-2 pandemics. The inhibitors are based on a scaffold of adenosine 5'-carboxamide and display IC_{50} values in the low nanomolar range. Another contribution by Mahesh Lakshman *et al.* explores a novel synthetic approach for the modification of position of N^6 -aryl-purines. This Pd-catalyzed arylation method grants access to a vast chemical diversity of purine nucleoside analogues.

Besides therapeutics, nucleic acids are useful biopolymers for the development of powerful diagnostic tools and nanomaterials. In a contribution by Robert Häner and colleagues, the light harvesting capacity of DNA-chromophore conjugates was investigated. By inserting phenanthrene units at both internal and terminal locations of DNA oligonucleotides, nanospheres could be constructed that displayed potent light harvesting capacities. In the manuscript by Hans-Achim Wagenknecht and colleagues, nuclease-resistant molecular beacons are presented. This detection system consists of

the mirror image of naturally occurring DNA, i.e. L-DNA, and is equipped with a fluorophore-quencher pair at both extremities. At increased temperatures, the molecular beacon switches to an open form which coincides with the emission of fluorescence. This system could serve as a potential cellular thermometer.

Most therapeutic oligonucleotides are produced by chemical synthesis where suitable phosphoramidite building blocks are attached on a solid-support bound nucleosides in an iterative manner. Alternatively, synthetic oligonucleotides can be obtained by the polymerase-catalysed insertion of chemically modified nucleotides. A review article by Yusuke Takezawa and Mitsuhiko Shionoya summarizes recent progress made in enzymatic synthesis of base-modified nucleotides for the installation of artificial metal base pairs. One research contribution by John Chaput and colleagues reports an improved synthesis of a TNA (threose nucleic acid) nucleoside triphosphate equipped with a modified nucleobase. This route does not require any lengthy HPLC purification and grants an easier access to enzymatic TNA synthesis. Another article by Yitzhak Tor and co-workers reports the combined use of polymerases and ligases to produce RNA oligonucleotides containing base-modified adenosine analogs. This chemoenzymatic approach successfully yielded modified hairpins that are substrates for the endoribonuclease MazF. The construction of such hairpin analogues will facilitate structural studies and real-time monitoring of enzymatic reactions. In a last contribution by Marcel Hollenstein and colleagues dealing with enzymatic nucleic acid synthesis, a small subset library of nucleotides modified with photosensitizers was prepared and shown to be compatible with various DNA polymerases. These nucleotides will be useful for the identification of modified aptamers to improve specificity of photodynamic therapy (PDT). Aptamers is also the topic of the contribution by Juewen Liu and colleagues who raised aptamers against tris(hydroxymethyl)aminomethane (Tris). Besides identifying Tris-binding aptamers, the authors propose a list of buffer components compatible with aptamer selection.

In future, where can ON therapeutics make a difference? Over 4,000 human diseases are known to be monogenic (caused by a single genetic mutation) accounting for up to 40% of the work of hospital based paediatric practice; in cancer, the global burden is expected

to grow to >20 million new cases and 13 million deaths by 2030. Therapies generally target the symptoms to aid quality of life, stabilise the disease and extend the life expectancy; the survival rate for patients with metastasis cancers still remains low. As for inherited genetic diseases such as Huntington's disease (HD), there is currently no cure. Viral infections should also not be underestimated. Basic as well as applied research is still needed to address these issues. We extend our gratitude to all authors who could contribute here, but equally to the many researchers who continue to develop and improve the applicability of ON therapies, which is clearly one of the key future technologies to cure diseases.

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