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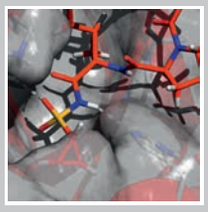

International Year of the Periodic Table 2019: Elements Important for Life Sciences

Division of Medicinal Chemistry and Chemical Biology
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Phosphorus – Friend of the Medicinal Chemist

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Phosphorus is the 11th most abundant element in earth's crust and accounts for 2–4% of the dry weight of most cells. It was discovered by Hennig Brandt in 1669. The name of the element is derived from the Greek 'phosphoros', meaning 'bringer of light'.

As an element, phosphorus exists in many allotropic forms and is divided into the white, red and black families. Phosphorus exists as a single stable isotope ^{31}P with the mass 30.97376. It sits in the 15th group, the third period and belongs to the p-block of the Periodic Table. The electron configuration of phosphorus in the ground state is [Ne] $3s^2 3p^3$. The three unpaired electrons together with the vacant, low energy 3d orbitals are responsible for the frequent occurrence of the oxidation states III and V in phosphorus compounds.

The chemistry of phosphorus is highly versatile, adopting a broad range of coordination numbers and geometries with most elements excluding the noble gases. The chemical reactivity and stability of phosphorus compounds strongly depend on the substituents with which it is associated. Thus, blessed with plentiful electrons, orbitals and oxidation states, phosphorus has a rich chemistry that entertains inorganic-, coordination-, synthetic- and medicinal chemists[1] alike. When nucleoside chemists call at number 15 of the Periodic Table, they are invariably thinking of *phosphates*.

Phosphates and their anhydrides are ubiquitous in living systems, as well as being a major reservoir of energy. Nature selected phosphates as the interconnecting components in the monomers of life – the nucleosides of DNA and RNA. As eloquently explained by Westheimer,[2] only the phosphodiester group meets all of the conditions for connectors of DNA and RNA nucleosides that can be speedily assembled and disassembled by biocatalysts. Nucleoside linkages are exceptionally stable to hydrolysis and temperatures; they have negative charges to resist nucleophiles, to render them poor leaving groups, to hold them firmly inside a membrane-encompassed cell and to make them water soluble. Indeed, it was the phosphodiesters of foreign DNA and RNA that focused the attention of pharma-industry chemists charged with the development of life-saving drugs against some of the most serious threats to human health: DNA and RNA viruses.

Acyclovir (**A**) was discovered in 1974 by intensive screening (Fig. 1).[3] It inhibits viral DNA polymerase and became the gold standard for the treatment of infections of Herpes simplex virus 1 and 2. Although the drug lacks the all-important phosphate, it was foremost in the minds of the chemists during drug design. Since phosphate-bearing groups on drugs are metabolically labile, acyclovir was developed as a pro-drug that is phosphorylated in three steps by viral and cellular kinases inside infected cells. Acyclovir is on the WHO list of essential medicines and was the subject of the Nobel Prize in Physiology or Medicine in 1988.

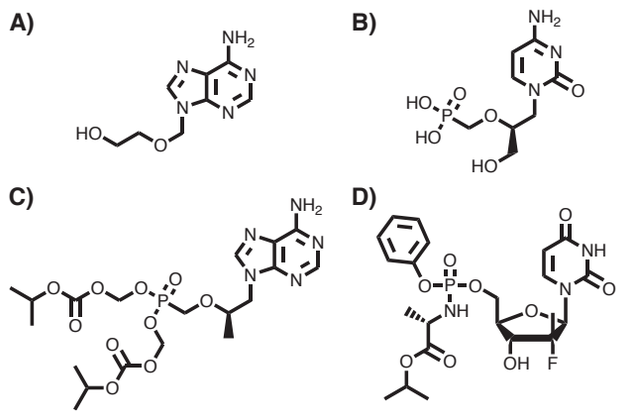


Fig. 1. Antiviral nucleoside pro-drug analogs.

The phosphate problem of nucleoside analogs was elegantly solved by Holý, with the introduction of a phosphonate bioisostere group. Nucleoside phosphonate analogs are metabolically-stable but are accepted by cellular kinases for phosphorylation. They were shown by De Clercq to have potent antiviral activity against several deadly viruses, including human immune deficiency virus (HIV) and hepatitis B virus (HBV).[4] Cidofovir (**B**) was the first acyclic phosphonate nucleoside to gain regulatory approval in 1996, intravenously-administered to AIDS patients to treat cytomegalovirus retinitis.

This was followed by approval of two adenosine analogs: adefovir dipivoxil is a phosphate ester pro-drug which exhibits activity against retroviruses such as HIV; it was superseded by tenofovir disoproxil (**C**), an orally-administered analog of adefovir which was approved for the treatment of HIV and HBV infections.

Over time, the phosphorus has been decorated with more sophisticated ligands. Sofosbuvir (**D**) is a highly effective phosphoramidate-containing inhibitor of RNA-dependent RNA polymerase from the Shinazi group.[5] It was approved in 2013 as a new treatment for hepatitis C virus (HCV) infections. Without doubt, new nucleoside structures with novel phosphorus-containing cores will continue to be developed as antiviral drugs.

From phosphate-containing drugs of the past and present, to drugs of the future. The idea in the late eighties that a single-stranded oligodeoxynucleotide could be applied as a drug to inhibit translation of a messenger RNA[6] was considered highly improbable in most sectors of the pharma industry and academia alike. A large piece of that skepticism was the presence of almost two dozen phosphodiester groups in an oligonucleotide backbone. How could such a molecule be manufactured at scale? How could such a molecule resist the ubiquitous nucleases in living systems? How could such a molecule be delivered into human cells?

Two decades later, clinical programs were looking promising, and in 2016, nusinersen was approved as a personalized treatment of spinal muscular atrophy (SMA).[7] Nusinersen is an 18-nucleotide derivative of RNA assembled with Swiss chemistry.[8] It binds sequence-specifically to an intronic sequence of the SMN2 pre-mRNA in the central nervous system of young SMA patients. There, it modifies mRNA splicing to produce functional

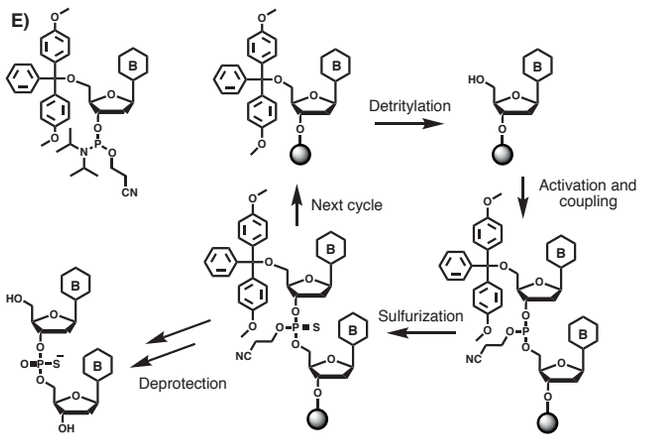
SMN protein, thereby offering a life-saving solution to SMA patients. It is a phenomenal drug.

Key to the success of the oligonucleotide therapeutics field is twenty years of medicinal chemistry, during which the phosphate group presented chemists with two major headaches: robust synthesis at scale and unfavorable ADME (absorption, distribution, metabolism and excretion) properties.

First, the synthesis. Phosphodiester chemistry for oligonucleotides began with Khorana, who reported the synthesis of 12-mer oligodeoxy-ribonucleotides in 1972.[9] In 1976, Letsinger described the use of P(III) chemistry in which a phosphite triester is efficiently oxidized in step-wise fashion to the corresponding phosphotriester.[10] The major breakthrough came five years later when Beaucage and Caruthers introduced nucleoside phosphoramidites,[11] which have been used in the solid phase synthesis of structurally-modified oligonucleotide drugs ever since (Scheme 1). Optimization of this synthon was performed in several laboratories and culminated in the 2-cyanoethyl-N,N-diisopropylphosphoramidite building block (**E**), which eventually enabled the synthesis of longer oligonucleotides on ever larger solid phase synthesizers.

Second, the pharmacology. The field of oligonucleotides teetered on the edge of failure because of a seemingly unsolvable problem attributed to the phosphodiesters: transport of the oligonucleotide into cells. The solution to this issue arrived unexpectedly when tackling the sensitivity of oligonucleotides to nuclease-mediated degradation *in vivo*. As with the phosphonate analogs, it was a remarkably simple but innovative solution, that represented the most important advance in oligonucleotide medicinal chemistry – the phosphorothioate linkage.[12] The slow rates of hydrolysis of phosphorothioate diesters suggested to F. Eckstein that their inclusion into DNA offered a means of protecting the oligonucleotides against nucleases, while maintaining the essential properties of the oligonucleotide. Crucially, the substitution of a non-bridging oxygen for sulfur required only minor changes to the solid phase synthesis protocol, since the very same phosphoramidite building blocks could be easily combined with sulfurization to P(V), instead of oxygenation. Serendipitously, the sulfurization of oligonucleotides endowed them with two additional useful properties for their use as single-stranded drugs: i) the ability to cross cellular membranes *in vivo*, albeit by mechanisms that are still poorly understood; and ii) a moderately weak non-specific binding to serum proteins that protects them from rapid renal excretion. The fly in the soup was that sulfurization at phosphorus generates a chiral center, producing a huge population of phosphorothioate diastereoisomers. Although this has not slowed the development of the field, it is nevertheless an irritation to oligonucleotide chemists.

Nusinersen has been followed by a new wave of oligonucleotide drugs with advanced chemistries, all of which are improving the lives of rare disease patients: Inotersen and Patisiran (the first approved siRNA) for hereditary ATTR (transthyretin amyloidosis), and Givosiran for acute hepatic porphyrrias. The power of oligonucleotide-based therapies has been hammered home in recent weeks by FDA approval of a new oligonucleotide drug: milasen. This antisense drug – reported broadly across mainstream and social media – was developed for a single child patient in a matter of *months* in order to save her from fatal Batten disease.[13] This startling departure from conventional drug discovery and development may herald a radical new way for society to take care of some its rare disease patients.



Scheme 1. Phosphoramidite and phosphorothioate functionalities for the solid phase of oligonucleotide drugs (shaded circle is solid support; B is nucleobase).

Taken together, society owes a great deal to number 15 of the Periodic Table.

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