Abstract

Phosphorus is a biogenic element and a key element in modern organic and medicinal chemistry. The non-metallic catalysis based on chiral phosphates is a fast-evolving field similarly to phosphorus-based prodrugs. Phosphorus is also widely used in the industry, e.g. in pesticides or food additives. Many of the compounds named above bear a chiral centre on the phosphorus atom. Thus, it is critical to determine their structure and stereochemistry because it controls their physico-chemical properties. For structural and stereochemical analysis, NMR spectroscopy is a standard method, usually based on ^{1}H, ^{13}C, ^{15}N, ^{19}F or ^{31}P isotopes. ^{31}P NMR spectroscopy can also be ideal for reaction monitoring as ^{31}P spectra are less complicated than ^{1}H, and ^{31}P is more sensitive than ^{13}C nucleus.

In this work, I used ^{31}P NMR spectroscopy to monitor the photo-initiated fragmentation of phosphate-based self-immolative (SI) linkers. The linkers are decomposed in a cascade of chemical reactions via self-immolation based on intramolecular cyclisation resulting in the drug release. I studied a series of newly designed prodrug linkers bearing two cargos. The ^{31}P NMR reaction monitoring with *in situ* irradiation enabled us to observe the reaction course and capture even metastable reaction species in real time. We doubtlessly distinguished which cargo was released preferentially and found structure-activity relationships. This ultimately led to the design of new classes of SI likers suitable for the release of amine-containing drugs, which usually face difficulties in cell-membrane delivery. Using the prodrugs based on the SI process, we successfully released a variety of amine-cargos. I also identified an alternative decomposition pathway of amine-containing cargos with the P-NH-R motif, which could be misinterpreted as the amine-cargo release. However, a careful analysis of the NMR data revealed an alternative decomposition, and I initiated a search for reaction conditions avoiding this undesired decomposition.

During the SI studies, we found no clear trend between the ^{31}P NMR parameters and the molecular geometry, e.g., *J*-couplings vs. the number of chemical bonds between the interacting nuclei, as is usual in ^{1}H NMR spectroscopy. For instance, the two-bond $^{2}J_{C\text{-}P}$-couplings are often smaller than the three-bond $^{3}J_{C\text{-}P}$-couplings, etc. Therefore, I decided to investigate how the ^{31}P NMR parameters can contribute to the stereochemical determination.

For the ^{31}P NMR studies, model phosphorus-stereogenic small molecules were designed and prepared as pure diastereoisomers. The ^{31}P NMR parameters were used to study the relative configuration and conformation of model compounds. We complemented the ^{31}P structural analysis with quantum-chemical calculations and a thorough conformational sampling. ^{13}C–^{31}P *J*-coupling analysis unequivocally assigned the relative configuration of rigid molecules, while ^{31}P-based analysis of residual dipolar couplings (RDC) did not provide unambiguous results. This may be caused by insufficient conformational sampling based on low-energy structures. Thus, we applied molecular docking, generating a new ensemble

of conformers in the presence of an alignment medium. This approach improved the results for mildly flexible molecules but did not help for rigid compounds. This indicates that the rigid molecules do not adopt significantly different conformation after interaction with the alignment medium compared to the mildly flexible molecules. For more flexible molecules, we employed a molecular dynamics method with the use of NMR orientational constraints - MDOC. MDOC significantly improved the assignment of the relative configuration, determining even flexible molecules. However, highly flexible molecules with low motion restriction remain a problem which requires attention and further development in NMR methodology.