Streszczenie w języku angielskim

Bioorthogonal chemistry is a new trend in chemistry that combines organic synthesis with cellular research. Bioorthogonal chemistry uses chemical reactions to study the metabolism of drugs and biomolecules in living organisms without affecting natural biochemical processes. In bioorthogonal chemistry it is important to create a connection between the tested object (drug or biomolecule) and a fluorescent probe that allows to track the metabolism of the tested molecule and the place of its accumulation. In recent years, there has been a clear increase in interest in bioorthogonal chemistry among chemists, as evidenced by the Nobel Prize awarded last year for achievements in this field.

The chemical reactions used in bioorthogonal chemistry are divided into two groups: polar reactions and cycloadditions, but the second group constitutes the vast majority. It includes 1,3-dipolar cycloadditions and Diels-Alder reactions. Only two papers involving application of the hetero-Diels-Alder reaction with inverse electron demand of 1-oxa-1,3-butadienes in bioorthogonal chemistry have been published so far.

The main goal of the research carried out as part of this doctoral thesis was to expand the base of bioorthogonal reactions with new examples of hetero-Diels-Alder reactions with 1-oxa-1,3-butadienes. In order to generate bioorthogonal ligation, the so far unused „click" hetero-Diels-Alder reaction with inverse electron demand of 5-arylidene derivatives of 1,3-dimethylbarbituric acid (as 1-oxa-1,3-butadiene systems) and alkenes was used. Substrates for the above-mentioned cycloaddition reaction: a dienophile with the tested object in the form of a taxol vinyl thioether and a diene with a fluorescent tag in the form of a 5-arylidene derivative of 1,3-dimethylbarbituric acid combined with fluorescein isothiocyanate were obtained through several-stage syntheses. The conducted hetero-Diels-Alder reaction with the use of model compounds, i.e. dienophile and diene not equipped with the tested object and fluorescent probe, led to the formation of the desired product in the form of one diastereomer. The kinetic studies made it possible to determine the second-order rate constant k_2 of the above cycloaddition reaction, which was $(1.6 \pm 0.1) \times 10^{-2}$ M^{-1} s^{-1} and it is definitely greater than the value of k_2 for the first-generation 1-oxa-1,3-butadienes hetero-Diels-Alder reaction and slightly lower than the k_2 value for the second-generation 1-oxa-1,3-butadienes cycloaddition. The stability studies of compounds under physiological conditions showed that the product of the hetero-Diels-Alder reaction has high stability in the pH range of 4.0 - 7.4 and that the synthesized heterodiene undergoes to a negligible degree

of unfavorable Michael addition reaction with reactive amino acids that occur in the cell environment. The performed biological research showed the usefulness of the proposed bioorthogonal cycloaddition reaction. The hetero-Diels-Alder reaction carried out inside tumor cells of U-2 OS osteosarcoma with use of previously obtained substrates enabled the imaging of the anti-cancer drug - taxol in living cells.

In addition, further research was done to find a faster hetero-Diels-Alder reaction characterized by a higher value of the rate constant k_2 compared to the previously obtained value ($(1.6 \pm 0.1) \times 10^{-2}$ M^{-1} s^{-1}). In the cycloaddition reaction with the previously synthesized heterodiene, dienophiles characterized by high reactivity in hetero-Diels-Alder reactions with tetrazine derivatives were tested. One of the dienophiles was a norbornene derivative and the other dienophile was a cyclopropene derivative. The attempts were unsuccessful - the reactions did not lead to the obtaining of cycloadducts. As part of this doctoral thesis, the synthesis of fluorescent dyes with the 1-oxa-1,3-butadiene system was also carried out. The molecules of these dyes would act as a heterodiene and a fluorescent marker at the same time. The dyes were obtained in high yields and tested in a cycloaddition reaction with a dienophile - vinyl thioether. However, the substrates used in the reaction did not react with each other. In the last stage of the research, the synthesis of 5-arylidene derivatives of 1,3-dimethylbarbituric acid with *para* or *para* and *meta* substituents in the aromatic ring in the form of electron-withdrawing groups was performed. These compounds were used in kinetic studies of hetero-Diels-Alder reaction with a vinyl thioether, which was to check the influence of the mentioned groups on the value of the rate constant k_2 and to indicate the derivative, the use of which in the cycloaddition reaction would lead to the highest possible value of the constant k_2. The conducted research showed that the hetero-Diels-Alder reaction using a derivative with substituents *para* and *meta* in the aromatic ring in the form of two acetyl groups gives the best prospects for the future. In the case of this compound, the value of the rate constant k_2 was the highest 1.4 ± 0.1 M^{-1} s^{-1} and is within the range of values characterizing very fast cycloaddition reactions of tetrazine derivatives with norbornene derivatives.