MINISTRY OF SCIENCE AND HIGHER EDUCATION OF THE REPUBLIC OF KAZAKHSTAN

Non-commercial joint stock company "Kazakh national research

Technical University named after K.I. Satpayev

Institute of Geology and Oil and Gas named after K. Turysov

Department of Chemical and Biochemical Engineering

Burkit Ulan Beibutuli

Development of technology for obtaining click reagents for use on proteins.

DIPLOMA PROJECT

Major 6B05101 - Chemical and biochemical engineering

Almaty 2023

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Allowed to the Defence
Head of Department of Chemical and Biochemical Engineering
PhD professor
_____ Amitova A.A.
"__" _____2023

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Completed by — Burkit Ulan

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«25» May 2023.

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"06" 06 2023

ASSIGNMENT
On the completion of diploma project

Student Burkit Ulan
Topic: Development of technology for obtaining click reagents for use on proteins.
Approved by the order of University Rector №408 from 23.11.2022
Deadline for completion of work "1" May 2023
Summary of the thesis:

a) General aspect of click chemistry
b) How it can be used on proteins
c) Possible way of the production of click reagents
d) Conclusion. Results discussion

The list of graphical material is shown in 10 slides
Recommended mail bibliography: 38 references

SCHEDULE

on preparation of thesis

Name of sections, list of questions considered	Submission deadline	Note
Review of sources for diploma project	15.01.2023 – 14.02.2023	
Characteristics of click chemistry	15.02.2023 – 14.03.2023	
Production of such a click reagents that can be used on proteins	15.03.2023 – 04.04.2023	
Economic calculations	05.04.2023 – 04.05.2023	
Results discussion	05.05.2023 – 25.05.2023	

Signatures

Section Names	**Consultants (academic degree, rank)**	**Date of signing**	**Signature**
Review of Sources for Diploma Project	Khabiyev A.T. Doctor Ph.D., assoc. prof.	14.02.2023	
Characteristics of click chemistry	Khabiyev A.T. Doctor Ph.D., assoc. prof.	14.03.2023	
Production of such a click reagents that can be used on proteins	Khabiyev A.T. Doctor Ph.D., assoc. prof.	04.04.2023	
Economic calculations	Khabiyev A.T. Doctor Ph.D., assoc. prof.	04.05.2023	
Results discussion	Khabiyev A.T. Doctor Ph.D., assoc. prof.	25.05.2023	
Normcontroller	Khabiyev A.T. Doctor Ph.D., assoc. prof.	30.05.2023	

Research mentor_____Khabiyev A.T.

The student accomplished the task_____Burkit U.B.

Date «06» 06 2023

АННОТАЦИЯ

Диссертация на тему «Разработка технологии получения клик-реагентов для использования на белках». Цель проекта является проектирование технологии по производству клик-реагентов, задачей же является создать схему производства 500 кг желаемого клик-реагента. Проект содержит 38 страниц текста, в том числе 12 таблиц и 28 рисунков, и включает следующие компоненты: Введение; Информация о химии кликов; Какие виды клик-реагентов существуют; Их возможное использование на белках; Их использование при сшивании белков; Как производить клик-реагенты, которые можно использовать для сшивания белков; Техническая схема; Описание возможного производства клик-реагентов; Заключение; Библиография.

АҢДАТПА

«Ақуыздарда қолдану үшін клик реагенттерін алу технологиясын жасау» тақырыбына дипломдық жұмыс. Жобаның мақсаты – клик реагенттерін өндіру технологиясын жобалау болса, тапсырма – 500 кг қажетті клик реагентін алу схемасын құру. Жоба 38 бет мәтінді, оның ішінде 12 кесте мен 28 суретті қамтиды және келесі компоненттерді қамтиды: Кіріспе; Клик-химия туралы ақпарат; Клик реагенттерінің қандай түрлері бар; Олардың ақуыздарда қолданылуы мүмкіндігі; Олардың протеинді айқастыруда қолданылуы; Ақуыздарды айқастыру үшін қолдануға болатын клик реагенттерін қалай өндіруге болады; Техникалық схема; Клик реагенттерінің мүмкін өндірісінің сипаттамасы; Қорытынды; Библиография.

ABSTRACT

Thesis on the topic "Development of technology for obtaining click reagents for use on proteins." The goal of the project is to design a technology for the production of click reagents, while the task is to create a scheme for the production of 500 kg of the desired click reagent. The project contains 38 pages of text, including 12 tables and 28 figures, and includes the following components: Introduction; Click chemistry information; What types of click reagents exist; Their possible use on proteins; Their use in protein crosslinking; How to produce click reagents that can be used to crosslink proteins; Technical scheme; Description of the possible production of click reagents; Conclusion; Bibliography.

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INTRODUCTION

Click chemistry is a new chapter of contemporary chemistry which can enhance the industry by many times. Especially, recent studies have allowed humanity to fight modern industrial world in almost all the fields you can think of. Food industry, drug development, material science, biotechnology and many others. Being able how to use emerging fields of chemistry can allow our world to develop faster, and click chemistry is not an exception. Barry Sharpless won Nobel Prize award in 2022 for the development of click chemistry and biorthogonal chemistry. I believe that it is a starting point for the future of chemistry, as click reagents can make all the processes faster. For example, the reaction which needed plenty of heat to occur doesn't need it anymore because click chemistry can do it in a more rapid and efficient way. As a citizen of Kazakhstan, I strongly believe that the development of such reagents in our country will solve a lot of issues that are associated with industry. No more harmful wastes coming from factories which contaminate local soil and water, no more unavailable catalysts or extremely costly conditions and so on.

The field on which I decided to take a focus on is protein crosslinking with click chemistry combination. Such kind of enhancement can make the processes of crosslinking much easier and more efficient. The task which I have by the end of the project is to show the model of production of 500 kg of the product named BS3 crosslinker.

1 Click chemistry. General information

Click chemistry is a type of chemical synthesis used to combine two molecular entities in a simple and efficient way. It is not a single reaction, but rather a method of generating products that mimic the way nature creates substances by joining small, modular units. Click reactions are often used to join a biomolecule and a reporter molecule, but can also be used in chemoproteomics, pharmacology, and biomimetic applications. Click reactions have unique properties, including being one-pot reactions that generate minimal byproducts, are not affected by water, and have a high thermodynamic driving force leading to a high yield of a single product with high specificity. These properties make click reactions particularly useful for targeting and isolating molecules in complex biological environments, where products must be stable and byproducts non-toxic.

Scientists have developed specific and controllable bioorthogonal reactions to target particular molecules in complex cell lysates. Click chemistry has also been adapted for use in live cells, using small molecule probes that find and attach to their targets. Click reactions have been useful in pulldown experiments and fluorescence spectrometry, and novel methods have been developed to incorporate click reaction partners onto and into biomolecules. Click chemistry plays an important role in the field of chemical biology, intentionally and specifically coupling modular units to various ends. The term "click chemistry" was coined by K. Barry Sharpless in 1998, and was jointly awarded the Nobel Prize in Chemistry in 2022 with Carolyn R. Bertozzi and Morten P. Meldal for their development of click chemistry and bioorthogonal chemistry. [1][2]

1.1 Click reagents. How to obtain them

Within the time, new and new click reagents were found. Starting with azides, and now finishing with cyclooctenes. There are 5 main different types of click reagents at the moment:

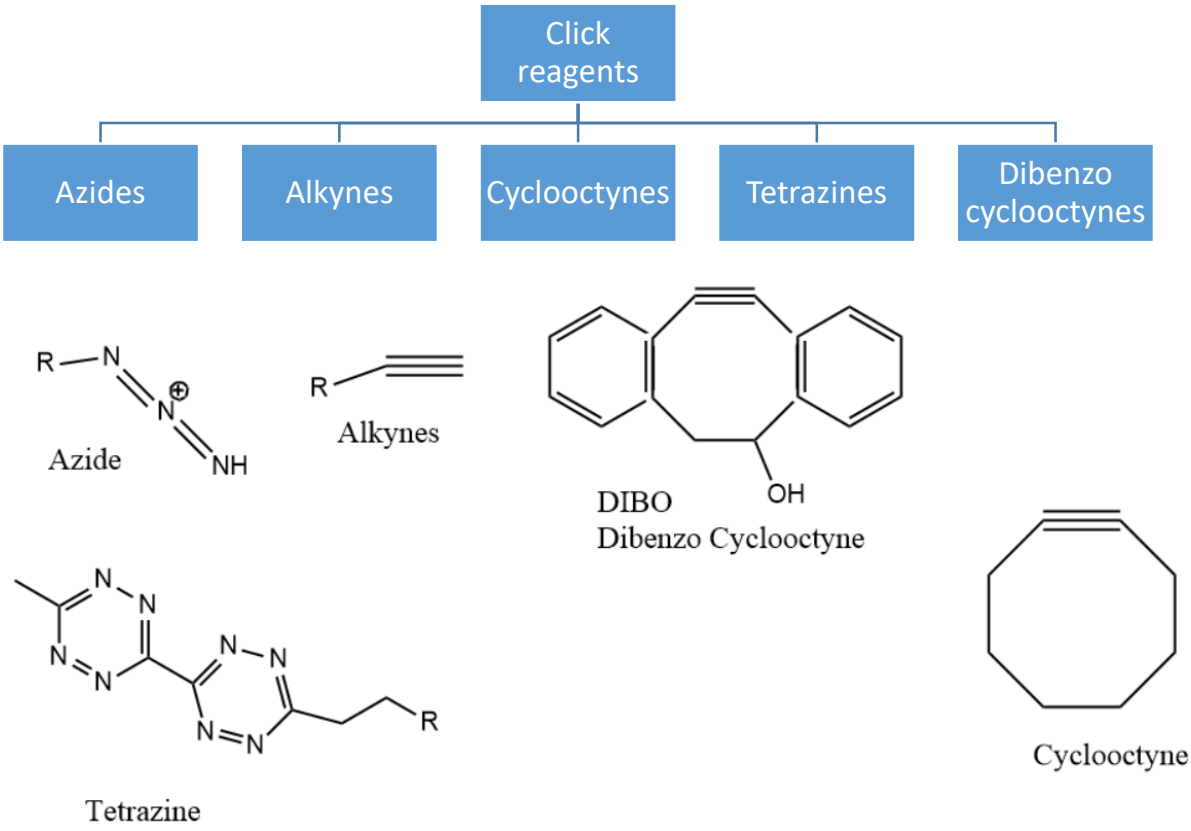


Figure 1 - types of different click reagents

Azides are molecules with abundant energy and are utilized in various fields with the structure $^{-}N=N^{+}=N^{-}$. Sodium azide, for instance, finds application as a preservative, mutagen, biocide, and assay reagent. Organic azides exhibit a wide range of organic reactions and play a crucial role in the azide-alkyne "click" reaction.

Alkynes: The azide-alkyne "click" reaction relies on incorporating alkynes as a crucial element for several reasons. Alkynes possess distinct reactivity and can engage in a remarkably efficient and selective reaction with azides, resulting in the formation of stable triazoles. This particular reaction, referred to as the Huisgen cycloaddition, represents one of the most widely employed click reactions. The presence of a terminal triple bond in alkynes offers a reactive site for the click reaction. The notable reactivity of alkynes towards azides enables rapid and specific bond formation under mild reaction conditions. Additionally, the click reaction involving alkynes and azides exhibits high efficiency, generating minimal unwanted byproducts while achieving a substantial yield of the desired product.

Cyclooctene is favored as a click reagent due to its distinctive reactivity and structural properties. It undergoes a strain-promoted click reaction with certain functional groups, such as cyclooctynes, without the need for a catalyst or high temperatures. This bioorthogonal reaction can be performed within biological systems, enabling selective labeling or modification of biomolecules without interfering with their native functions. Furthermore, click products formed with cyclooctene exhibit good stability, making them suitable for long-term applications. The reactivity of

cyclooctene is rapid and selective, leading to high yields of desired products. These qualities, including its compatibility in biological settings, contribute to the widespread use of cyclooctene as a valuable click reagent.

Tetrazines: Another good alternative to other click reagents are tetrazines, as they have good selectivity and reactivity. The general formula of them is C2H2N4 and they exist in different isomers. As well as other reagents, it has also various applications in particular fields.

Dibenzocyclooctynes: also an exciting substitute for previously mentioned reagents. It has got unique selectivity and reactivity, which makes it ideal for the future use in biorthogonal click reactions [3][4][5]

These are the main click reagents, and all of them are used in various click reactions.

1.2 Types of click reactions.

The classic click reaction, also known as CuAAC, involves the copper-catalyzed reaction of an azide with an alkyne to form a 5-membered heteroatom ring. This reaction was first reported in 1893 by Arthur Michael, and later took on Rolf Huisgen's name after his studies of their reaction kinetics and conditions. The copper-catalyzed version of this reaction gives only the 1,4-isomer, whereas Huisgen's non-catalyzed 1,3-dipolar cycloaddition gives both the 1,4- and 1,5-isomers. This reaction mechanism may involve a dicopper mechanism rather than a single catalytic copper atom.[6] However, the copper used in this reaction is cytotoxic, so solutions have been proposed to reduce the dosage needed. The RuAAC reaction catalyzed by ruthenium allows for the selective production of 1,5-isomers. Although Meldal and co-workers first reported the Cu(I)-catalyzed variant, they were overtaken by the publicly more recognized Sharpless, who described it as a reliable catalytic process offering "an unprecedented level of selectivity, reliability, and scope for those organic synthesis endeavors which depend on the creation of covalent links between diverse building blocks.[7]

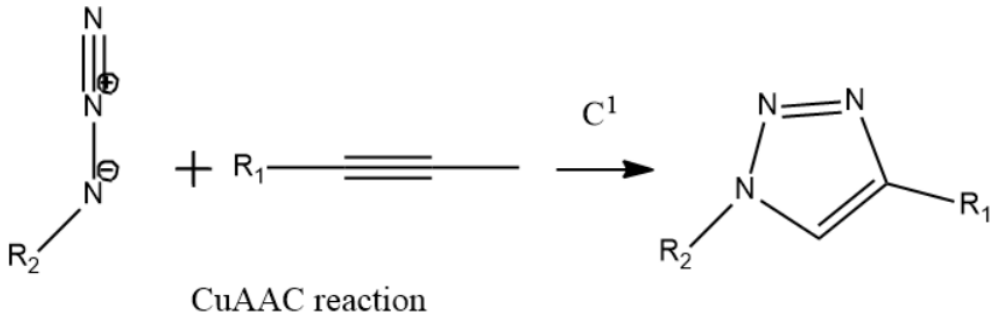


Figure 2 - CuAAC reaction

The Bertozzi group has improved one of Huisgen's copper-free click reactions known as strain-promoted azide-alkyne cycloaddition (SPAAC) to avoid the cytotoxicity of the CuAAC reaction. In SPAAC, the alkyne is introduced in a strained difluorooctyne (DIFO) rather than using Cu(I) to activate the alkyne. The electron-withdrawing propargylic gem-fluorines act together with the ring strain to destabilize the alkyne, which increases the reaction driving force and promotes the cycloalkyne to relieve its ring strain. This reaction proceeds as a concerted [3+2] cycloaddition similar to the Huisgen 1,3-dipolar cycloaddition, and other substituents such as benzene rings are also allowed on the cyclooctyne. Although the SPAAC reaction rate is slower than that of the CuAAC, it has been successfully used to detect azides in living systems. However, the low yield in the synthesis of cyclooctynes has limited the probe development for this reaction. Nonetheless, SPAAC has been used successfully with cyclooctyne derivatives such as DIFO, dibenzylcyclooctyne (DIBO), and biarylazacyclooctynone (BARAC) to detect azides in living systems.[8]

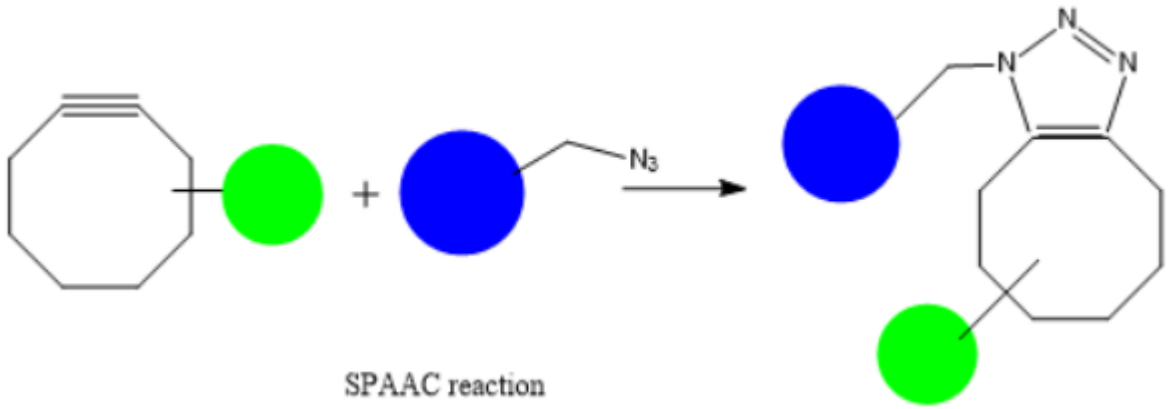


Figure 3 - SPAAC reaction

The Strain-promoted alkyne-nitrone cycloaddition (SPANC) is a type of click reaction that involves the reaction of a strained cyclooctyne with a nitrone dipole to form a N-alkylated isoxazoline product. The reaction is metal-free and has fast reaction kinetics, making it suitable for live cell labeling. The isoxazoline product is not as stable as the triazole product of the CuAAC and the SpAAC, but the reaction is still useful for labeling proteins containing serine as the first residue and for multiplex labeling.[9]

The alkene and azide [3+2] cycloaddition is a chemical reaction where an activated alkene, such as oxanorbornadiene, reacts with an azide, resulting in the formation of triazoles as a product. However, the triazoles formed in this reaction are not aromatic, unlike those formed in CuAAC or SPAAC reactions, and are therefore less stable. The activated double bond in oxanobornadiene produces a triazoline intermediate, which then undergoes a retro Diels-alder reaction spontaneously, releasing furan and forming 1,2,3- or 1,4,5-triazoles. Although this reaction is slow, it

is useful because oxabornodiene is relatively easy to synthesize. However, the reaction is not entirely selective for a particular chemical group or functional group (chemoselective).

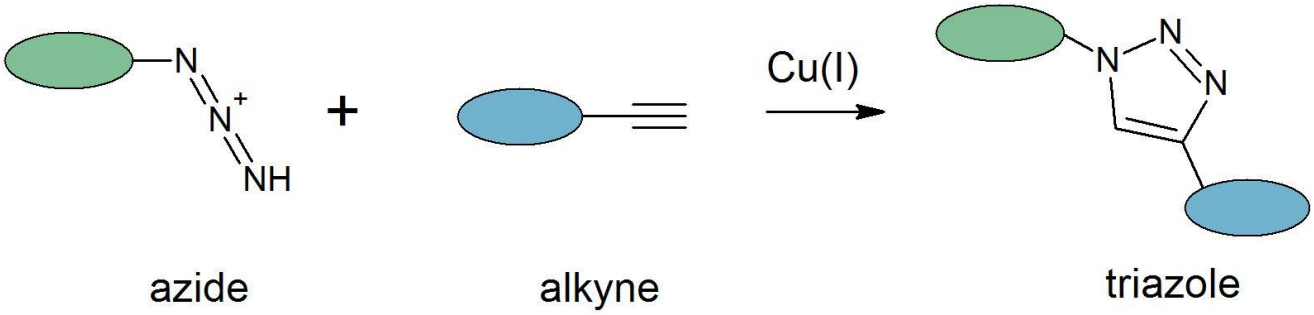


Figure 4 - Azide-alkene cycloaddition reaction

These are the most popular reactions that are used in industries. Some of them are applicable on proteins modification, labelling, and so on.

1.3 Different ways of using click reagents on proteins

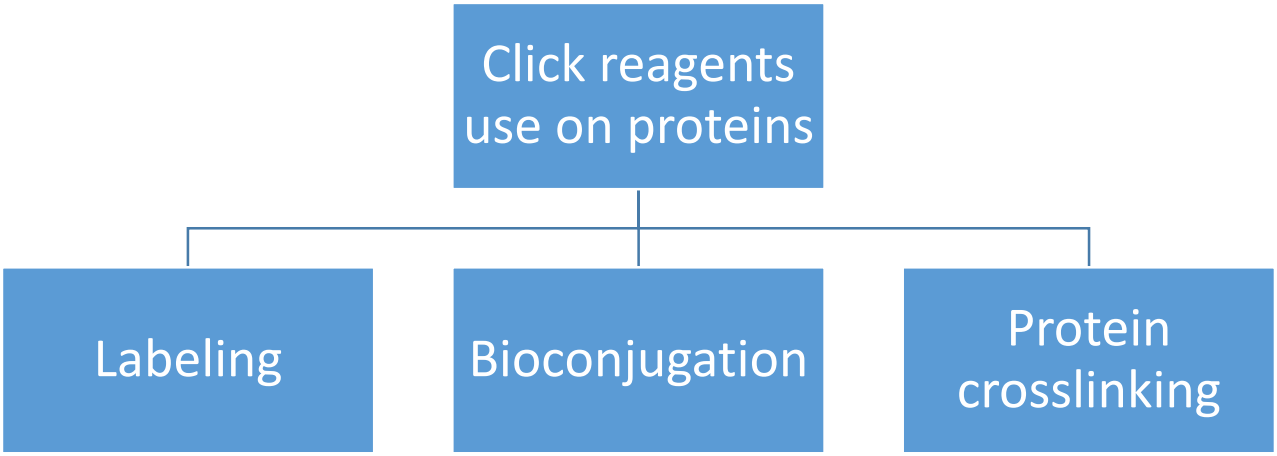


Figure 5 – Varieties of click reagents use on proteins

One of the application of click reactions in proteins can be labeling. The labeling method is based on two different types of reactions, strain-promoted alkyne-azide cycloaddition (SPAAC) and strain-promoted inverse-electron-demand Diels-Alder cycloaddition (SPIEDAC), which use noncanonical amino acids (ncAAs) with specific reactive groups to label proteins. To introduce the ncAAs into the protein of interest (POI) in a specific location, genetic code expansion technology is used. The protocol consists of two main steps: first, introducing an Amber stop codon at the desired site on the POI gene using site-directed mutagenesis, followed by transfection of mammalian cells with the mutant gene and another plasmid that encodes an orthogonal RS/tRNA pair. In the presence of the ncAA, the orthogonal RS/tRNA pair suppresses

the Amber codon and incorporates the ncAA into the POI. In the second step, the expressed POI is labeled with a reactive dye derivative supplied to the growth medium. The protocol includes cloning, transfection, and labeling steps, which take a total of 7-11 days.[10]

Another possible application is bioconjugation. Click reactions have two distinct characteristics that make them useful for modifying biomolecules. Firstly, they are bio-orthogonal, meaning that neither the reactants nor their products interact with functionalized biomolecules. Secondly, they can proceed easily under mild, non-toxic conditions, such as at room temperature and in water. Copper-catalyzed Huisgen cycloaddition, azide-alkyne [3 + 2] dipolar cycloaddition, Staudinger ligation, and azide-phosphine ligation are examples of click reactions that possess these qualities. Click chemistry allows for the selective modification of one cellular component while leaving others unharmed, making it a valuable tool in many applications. Although it is not a perfect technology for all applications, it has proven to be superior in many respects, including biocompatibility, selectivity, yield, and stereospecificity. As a result, it is expected to become a more common strategy in various fields in the future.[11]

However, there is one more specific field I would love to highlight. It is called protein crosslinking.

1.4 How click chemistry can be used in protein crosslinking

Various click reactions, carefully selected reactants, and catalytic conditions are widely employed in bioconjugation reactions, such as protein modification. These click approaches leverage the presence of highly reactive functional groups naturally occurring in biopolymers. For instance, amines and thiols found in lysine and cysteine, respectively, are utilized in modifying peptides and proteins. Similarly, reactions involving alcohols and thiols have been used to functionalize oligo/polysaccharide compounds. In addition to these functional groups, azides and alkynes can be introduced via chemical modification or through genetic encoding, where non-natural amino acids with clickable properties are incorporated. These non-natural functional groups enable precise modification of peptides and proteins. An example of such an approach is the use of ring strain-promoted azide-alkyne cycloaddition by Bertozzi and colleagues in 2007, where they modified the lipoyl domain of the full-length E2p protein with different tags.[12]

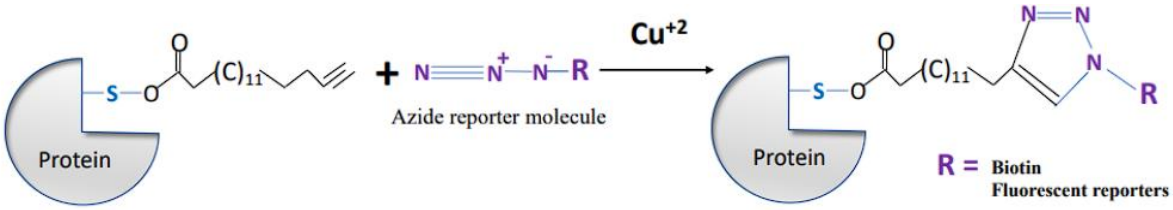


Figure 6 - CuAAC applied for Chemical proteomics

But, what is protein crosslinking?

1.4.1 Brief data about protein crosslinking

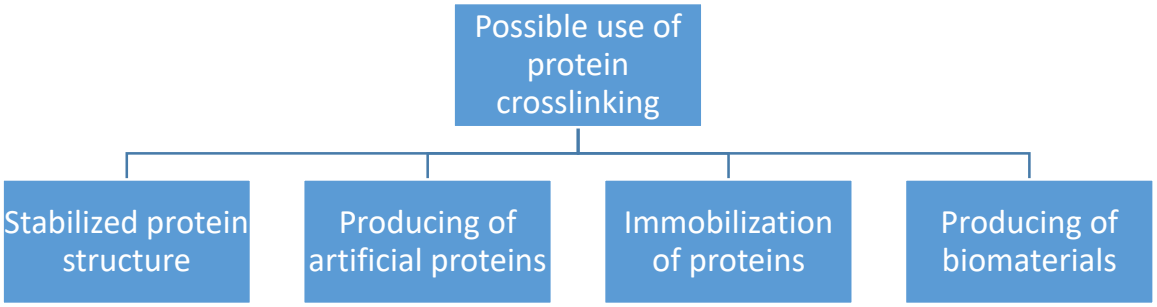


Figure 7 – application of protein crosslinking

The process of linking two or more proteins together by a covalent bond is known as crosslinking proteins. Crosslinkers, also known as crosslinking reagents, are molecules that have two or more reactive ends that are able to chemically attach to specific functional groups, such as primary amines or sulfhydryls, on proteins or other molecules.

Several methods are available for examining the composition and interaction of proteins and for manipulating them to be used in detection procedures, which depend on chemically crosslinking, modifying or labeling proteins. Crosslinking refers to the formation of covalent bonds between two or more molecules. Modification refers to adding or removing chemical groups to change the original molecule's solubility or other properties. Labeling involves attaching a chemical group, such as a fluorescent molecule, to aid in molecule detection. This collection of crosslinking and modification methods used for proteins and other biomolecules in biological research is known as "bioconjugation" or "bioconjugate" technology. The term "conjugation" is a synonym for crosslinking.

When two groups on a single protein are attached, it creates intramolecular crosslinks that reinforce the tertiary or quaternary structure of the protein. Conversely, when two different proteins have their groups attached, it produces intermolecular crosslinks that reinforce the interaction between the proteins. In the case of a mixture of two purified proteins, intermolecular crosslinking generates a specific conjugate that can be used in detection procedures. When a protein is linked to a chemical group on a solid surface like a glass slide or resin bead, it becomes immobilized, which is the foundation for many assay and affinity-purification systems.

Crosslinking is a versatile technique used for various purposes. It can be used to stabilize protein tertiary and quaternary structure for analysis, identify unknown protein interactors or interaction domains, conjugate an enzyme or tag to an antibody or other purified protein, immobilize antibodies or other proteins for assays or affinity-

purification, and attach peptides to larger "carrier" proteins to facilitate handling and storage.

Crosslinkers are chosen based on their chemical properties, including their specificity for particular functional groups and other features that aid their use in specific applications. These properties include chemical specificity, such as whether the reagent has the same or different reactive groups at each end (homobifunctional or heterobifunctional), spacer arm length, including whether the linkage can be reversed or broken when desired, water-solubility and cell membrane permeability, and whether the reagent reacts spontaneously or requires activation at a specific time. [13]

Protein crosslinking is the process of merging proteins (more than 2) in order to achieve next goals:

• For having stabilized protein structure. With the help of crosslinking, it is possible that newly formed structure of proteins can be a great help to avoid unfolding or denaturation.

•To create artificial proteins. New protein-protein interaction is a great opportunity to get the newest forms of proteins.

•To immobilize proteins: Crosslinking can be used to immobilize proteins on surfaces or matrices. This can be useful for creating biosensors, drug delivery systems, or protein purification columns.

•For creation of biomaterials. Such kind of newly formed proteins can be used as biomaterial for creating hydrogels or scaffolds. Later they could be used in material engineering. [14]

For all the application listed above, there are different techniques that are used there.

1.4.2 What are the techniques for protein crosslinking

Protein crosslinking consists of many different techniques. Some of the most common are the next:

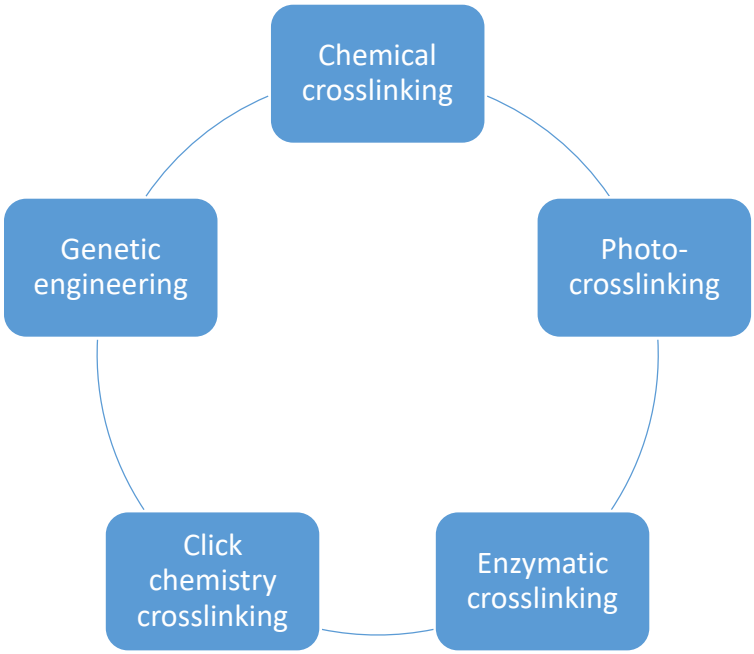


Figure 8 – methods of protein crosslinking

Chemical crosslinking is a process where two or more molecules are joined together by a covalent bond, and usually those reagents are called “crosslinkers”. According to purpose of the research, various reagents can be used, depending on factors like spacer length and reactivity. In recent decades, the usage of such reagents increased dramatically, as people have been using them in different fields connected with proteins modification, protein-protein interactions and so on. [15]

Over the past few decades, photo-crosslinking has garnered a lot of attention due to its specificity, controllability, and modularity.This powerful chemical tool is used to capture transient interactions and has become a preferred method for studying protein-protein interactions (PPIs) in live cells. Studying PPIs is crucial for understanding cellular functions and mechanisms. Photo-crosslinking methods are preferred for evaluating PPIs under conditions that are as close to native as possible because they can generate reactive species on-demand through UV light irradiation. A range of techniques, including fusion tags, metabolic incorporation, and amber codon suppression, have been used in live cells with various crosslinkers such as aryl azides, benzophenones, and diazirines. Mass spectrometry and immunological techniques are employed to identify crosslinked proteins and capture context-dependent and transient interactions. [16]

Next type of technique is called enzymatic crosslinking. It is divided into two parts (in vivo and in vitro). For in vivo processes protein covalent modification through posttranslational processes is crucial for both prokaryotic and eukaryotic cells as it enhances the structural and functional diversity of the proteome (Walsh 2006; Walsh et al. 2005). Specific enzymes have evolved for carrying out most of these modifications. These modifications are made to the functional groups of amino acid side chains, which may be present within sequence-specific recognition motifs. They involve various processes, such as the addition of organic molecules like cofactors,

oligosaccharides, nucleotides, lipids, and small groups such as methyl, acetyl, and phosphoryl groups, intramolecular transformations such as disulfide bond formation and proteolytic processing, and intermolecular crosslinking by covalent bond formation between individual protein molecules For in vitro enzymes that can crosslink proteins in living organisms have potential applications for a variety of uses in the laboratory. While several enzymes are capable of this, transglutaminases have been most commonly used for this purpose, along with sortase A and lysyl oxidase. The enzymes responsible for protein ubiquitination have not been explored for crosslinking applications, due to the complexity of the reaction and the need for ATP. Other oxidoreductases that typically work with nonproteinogenic substrates have been studied for their ability to synthesize new covalent bonds between proteins in vitro. Two types of crosslinking reactions can occur enzymatically, including direct covalent bonding through intermediates, and enzyme-mediated covalent bonding via reactive species generated by oxidoreductases. [17]

Genetic engineering is used in protein crosslinking by introducing genetically encoded noncanonical amino acids (ncAAs) into proteins. These ncAAs contain unique chemical groups that can be used to crosslink proteins either intra- or intermolecularly. To achieve this, a specific tRNA synthetase enzyme is evolved to recognize and incorporate the ncAA into a specific site of the protein during protein synthesis. Once the modified protein is expressed, it can be crosslinked using various methods, including photo-crosslinking or chemical crosslinking with specific reagents. By using genetic engineering, researchers can precisely introduce crosslinking sites into proteins and investigate protein-protein interactions, enhance protein stability, and improve the pharmacological properties of proteins. [18]

Finally, one more technique of protein crosslinking is processed with the usage of click reactions. The click chemistry reaction between azides and alkynes was used to synthesize the crosslinker, which is then used to chemically crosslink proteins. The resulting crosslinked proteins can be enriched and analyzed by mass spectrometry to identify the specific amino acid residues involved in the interaction, providing valuable information for understanding protein structures and interactions. Click chemistry offers several advantages in this context, including high efficiency, specificity, and versatility, making it a useful tool for protein crosslinking studies. [19][20][21]

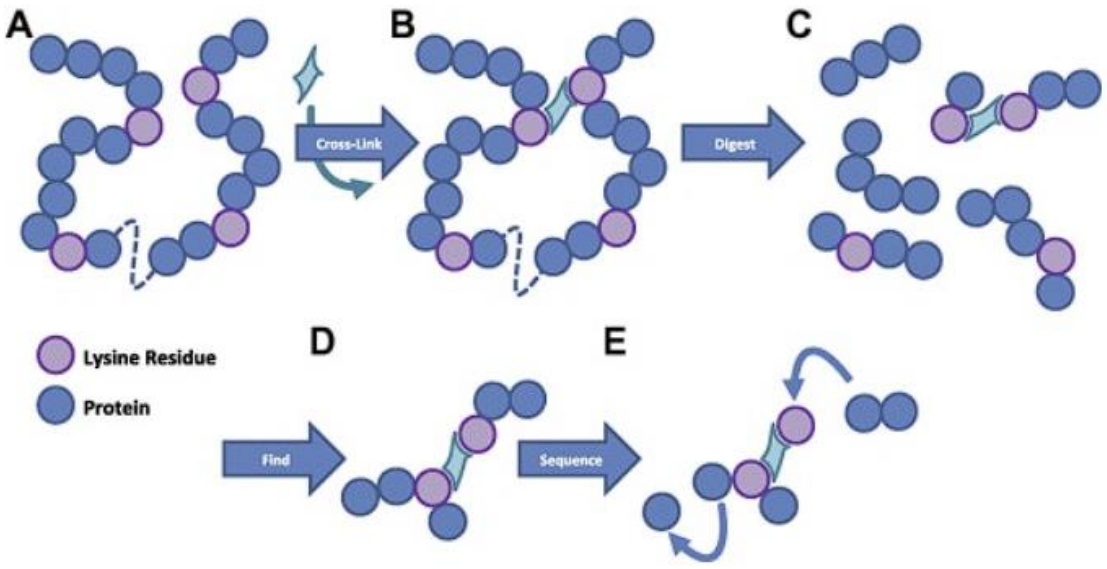


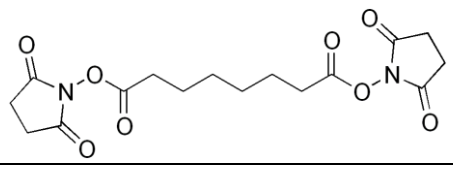
Figure 9 - The typical analysis of a cross-linked sample by shotgun proteomics

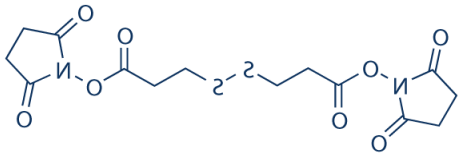
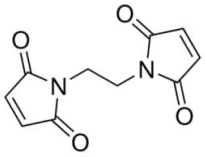
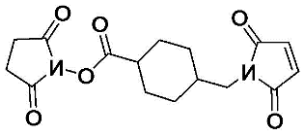
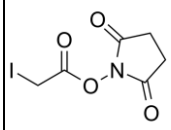
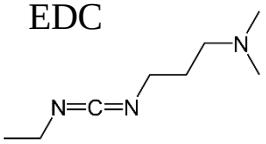
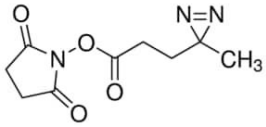
In order to realize the techniques, specific reagents are needed.

1.4.3 What reagents are used for protein crosslinking

To select a cross-linker for a particular application, it is essential to consider the chemical reactivities, and the reaction's compatibility with the intended use. The optimal cross-linker must be determined experimentally based on specific characteristics such as chemical specificity, water solubility, spacer arm length, and cell membrane permeability. Cross-linkers typically contain two reactive groups, and the functional groups that can be targeted include primary amines, carbonyls, sulfhydryls, carbohydrates, and carboxylic acids. Photoreactive phenyl azide cross-linker may also be used for nonselective coupling. The selection of cross-linkers with specific features may be generated using a cross-linker selection guide. Additionally, it is also essential to consider features such as spontaneous or photoreactive groups, cleavability, and radiolabeling or tagging with another label when selecting a cross-linker. At the moment, there are a variety of available crosslinkers. [22]

Table 1 – types of different commercially available cross-linkers

Natural cross-links -S-S; Lys-to-Lys; Transglutaminase induced; Oxidation-derived; Glycation-derived;	Commercially available cross-linker DSS 

Chemo-selectivity: -NH2 (DSS, DTSP) -SH (bis(maleimido)ethane, BMOE) **Broad-specificity** Diazirenes (SDA) Formaldehyde **Hetero-bifunctional** (SMCC, EDC, SIA, SDA) **Affinity Handles,** (BDRG) **Click Chemistry handles** (CLIP) **Cleavable** (DTSP) **MS-Labile** (BDRG)	DTSP  BMOE  SMCC  SIA  EDC  SDA 

1.4.4 Click reagents that are used for protein crosslinking

There are variety of click reagents that can be used when proteins are crosslinked, but the most popular and efficient ones are the next:

•Diazirines: Diazirines are small, photoactivatable molecules that can crosslink to proteins upon UV irradiation. Examples of diazirine-based crosslinkers include Sulfo-DSS and Sulfo-SDA.[23]

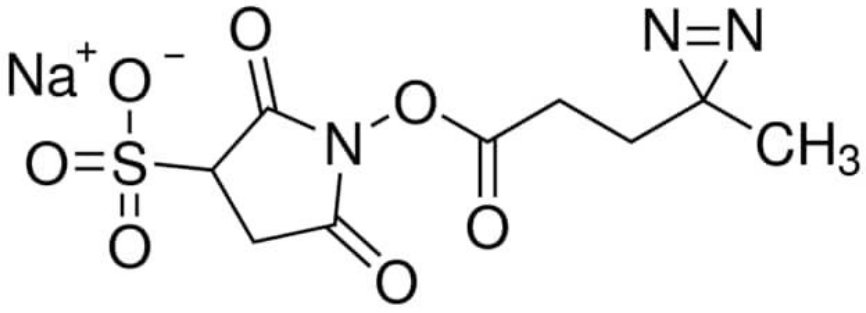


Figure 10 - Sulfo-SDA crosslinker

Alkynes: Alkynes are small molecules that can react with azides via copper-catalyzed azide-alkyne cycloaddition (CuAAC) to form a covalent bond. Examples of alkyne-based crosslinkers include BS3 and DSSO.[24] There are also one of the most commonly used type of crosslinker.[31]

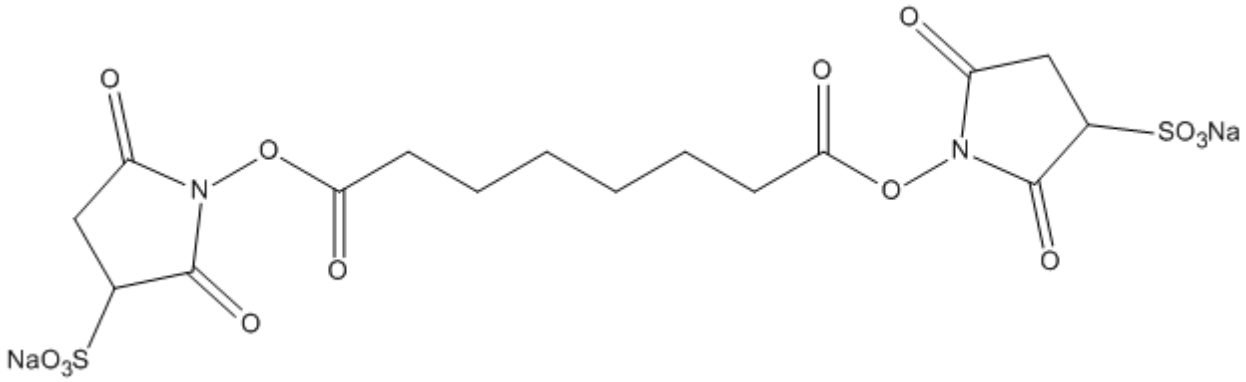


Figure 11 - BS3 crosslinker

Azides: Azides are small molecules that can react with alkynes via CuAAC to form a covalent bond. Examples of azide-based crosslinkers include DSG and NHS-SS-azide. [25]

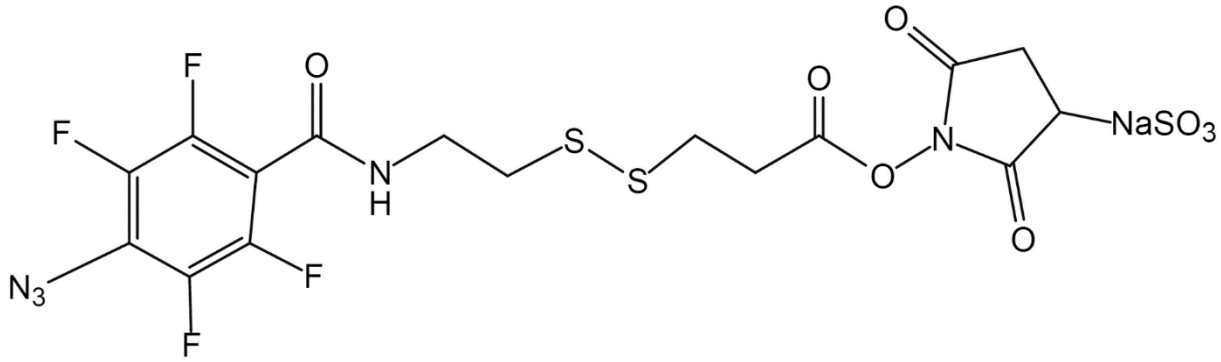


Figure 12 - NHS-SS-azide

2 Technological scheme and its description.

The click reagents I want to focus on are alkynes (BS3), as it is possible to use them in the industry where I plan to make researches (Food industry). It is commercially available reagent/crosslinker.

The list of needed reagents: Furan-2,5-dione, NaHSO3, NH4OH, NaOCl, maleic acid, ethanol, cyclooctane, H2O2, perrhenate, 1-ethyl-(3-(3-dimethylainopropyl-carbodiimide hydrochloride, dimethyl sulfoxide.

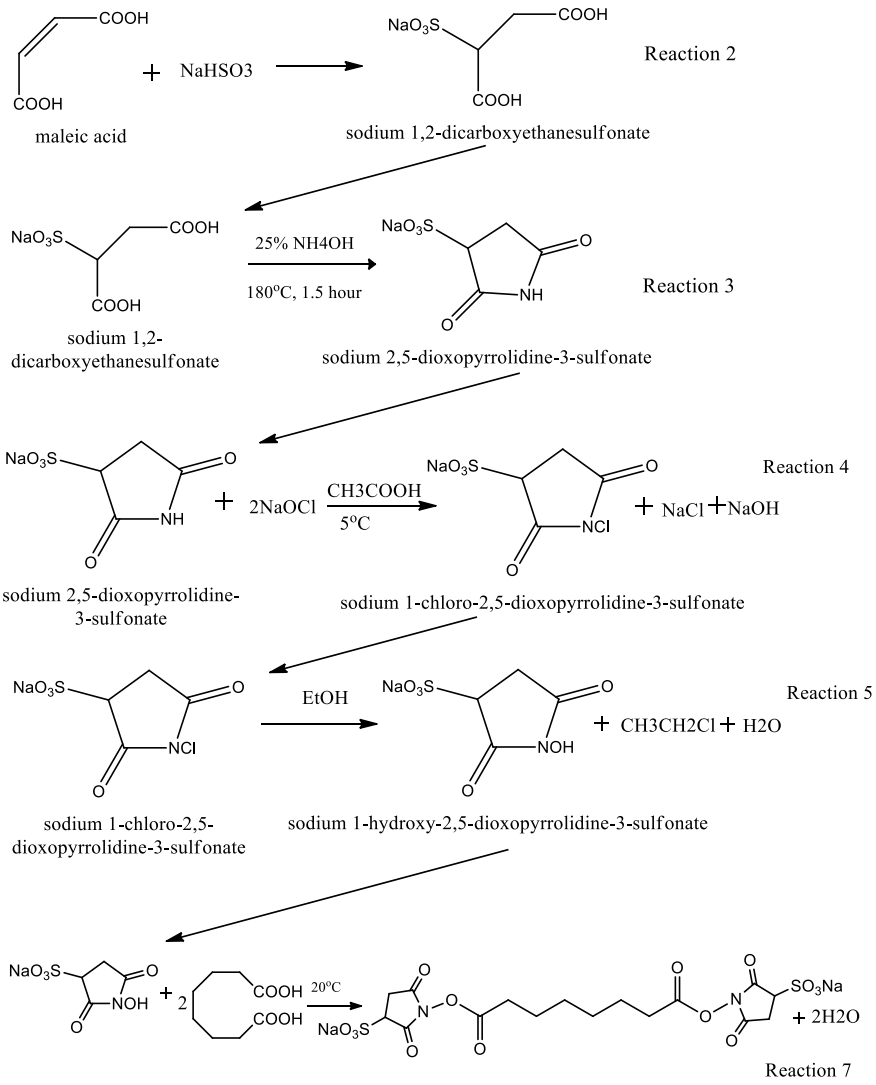


Figure 13 – Overall process of BS3 synthesis

In reaction №1, the first method of obtaining sodium 1,2-dicarboxyethanesulfonate (SDCES) from furan-2,5-dione is shown:

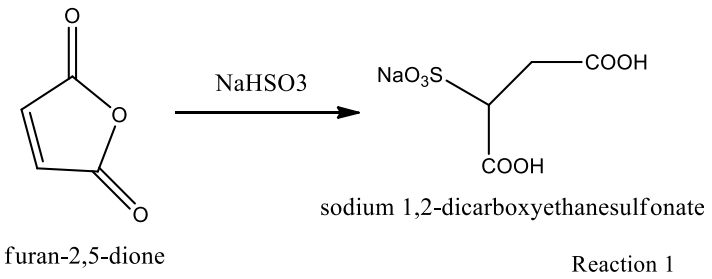


Figure 15 – The first reaction

In reaction №2, the second method of obtaining sodium 1,2-dicarboxyethanesulfonate (SDCES) from maleic acid is shown:

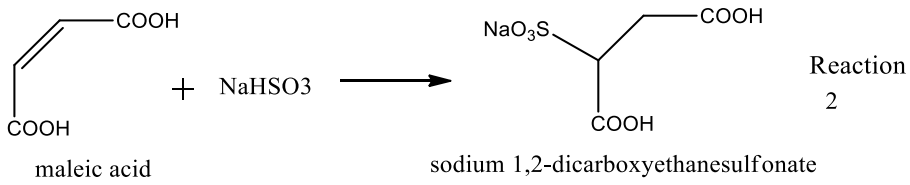


Figure 16 – The second reaction

In reaction №3, with the usage of NH4OH and SDCES, sodium 2,5-dioxopyrrolidine-3-sulfonate (SDOPS) is obtained.

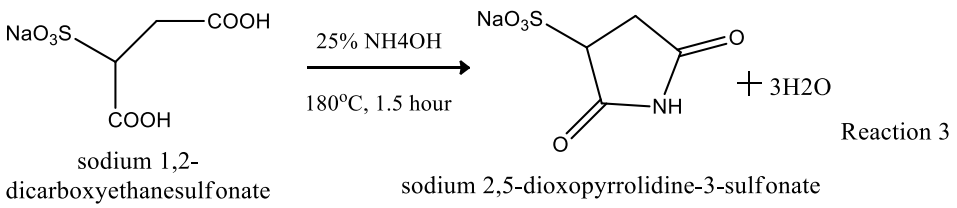


Figure 17- The third reaction

In reaction №4, from SDOPS and NaOCl, sodium 2,5-dioxopyrrolidine-3-sulfonate (SCDOPS) is produced. By products include NaCl and NaOH.

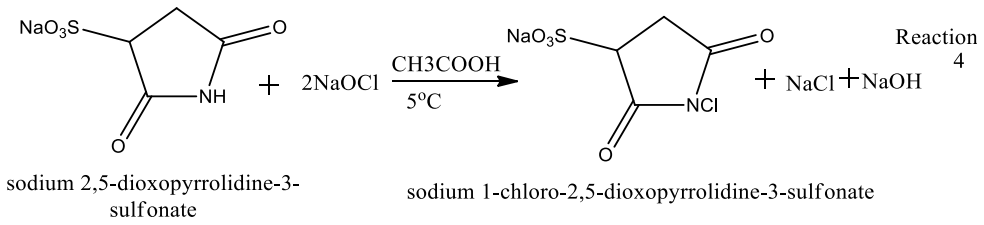


Figure 18 – The 4th reaction

In reaction №5, in order to get sodium 1-hydroxy-2,5-dioxopyrrolidine-3-sulfonate (HDOPS) ethanol and SCDOPS were reacted together. By product is CH3CH2Cl

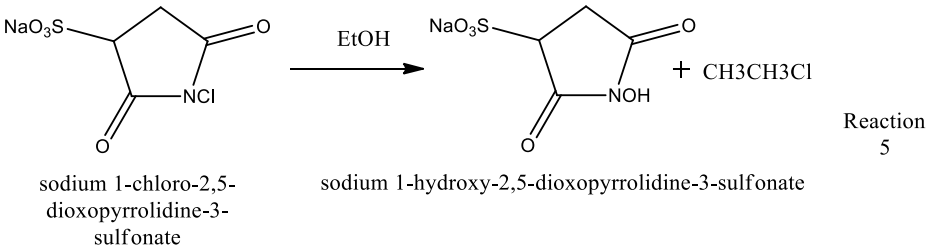


Figure 19 – The 5th reaction

In reaction №6, from cyclooctene and organic perrhenates, octanedioic acid was obtained.

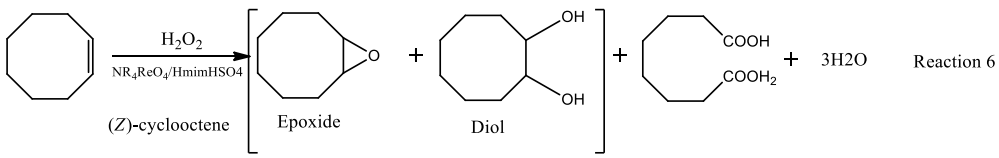


Figure 20 – The 6th reaction

In reaction №7, final BS3 crosslinker is produced by adding two final(HDOPS and OCDA) components in specific solution.

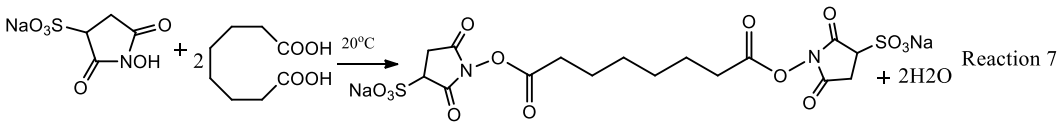


Figure 21 – The 7th reaction

Method of reaction 1: The method involves adding maleic anhydride (98.0 g) to a 1000 ml three-neck flask. The temperature is maintained at 80°C and a solution of sodium bisulfite (167.0 g of sodium bisulfite dissolved in 600 g of water) is slowly introduced with continuous stirring. Once the addition is complete, the reaction mixture is stirred for 5 hours at 80°C. After the reaction is complete, 1350 ml of water is added to the reaction mixture to produce a 10% solution of sodium sulfosuccinate. This solution is then passed through a 50 mm diameter ion exchange column packed with 1 L of strong acid cation exchange resin. The product is collected when the pH of the liquid from the bottom of the ion exchange column drops below 1, and it is obtained as an aqueous solution of sulfosuccinic acid. The product yield is determined to be 91% by detecting sodium ion concentration below 0.05%. However, the reaction's reagents cost is very high, so it is better to use alternative method which is described in "Method of reaction 2" [34] [33]

Method of reaction 2: The reaction usually takes place in the presence of an aqueous solution of the bisulfite or metabisulfite reagent at room temperature or slightly elevated temperature, with the reaction mixture being stirred or agitated to ensure proper mixing of the reagents. [32]

Method of reaction 3: Following that, it should be added to 25% NH4OH at 180℃ and stirred for 1.5 hours. [39]

Method of reaction 4: In the process of converting succinimide to N-chlorosuccinimide, a reactor with stirrer and heater is used. Acetic acid, water, and 'waste water' containing succinimide (approximately 80 mmol) are added sequentially to the reactor. The mixture is cooled in an ice-water bath to ensure that the internal temperature remains below 5 °C. A 10% bleach solution is added slowly through the addition funnel while maintaining an internal temperature of less than 8 °C. After the addition is complete, the mixture is stirred for an additional hour. The resulting mixture is then filtered by liquid-liquid extractor and dried up. The product, N-chlorosuccinimide, is obtained as a white powder with a yield of approximately 90%. [35]

Method of reaction 5: The conditions for the reaction between N-bromosuccinimide (NBS) and ethanol (EtOH) are typically carried out in an inert atmosphere, such as nitrogen or argon gas, to prevent the formation of unwanted byproducts. The reaction is typically carried out at room temperature or slightly elevated temperatures, with stirring to promote mixing of the reagents. The stoichiometry of the reaction may vary depending on the desired product, and the reaction may be monitored by various analytical techniques, such as gas chromatography or infrared spectroscopy. [36]

Method of reaction 6: The oxidation of cyclooctene to suberic acid using the perrhenate-containing composite ionic liquids as green catalysts requires a composite ionic liquid of perrhenate and an imidazolium-based ionic liquid, hydrogen peroxide as the oxidant, a temperature of 80°C, a reaction time of 4 hours, and a molar ratio of cyclooctene to the composite ionic liquid catalyst of 1:6. These conditions are optimized for a green and efficient method for the synthesis of suberic acid, which has potential applications in the production of various polymers and materials. [37]

Method of reaction 7: With 1-ethyl-(3-(3-dimethylamino)propyl)-carbodiimide hydrochloride In dimethyl sulfoxide at 25°C; for 12h; [38]

2.1 Model based on principal scheme

The overall model is shown on the Figure 22:

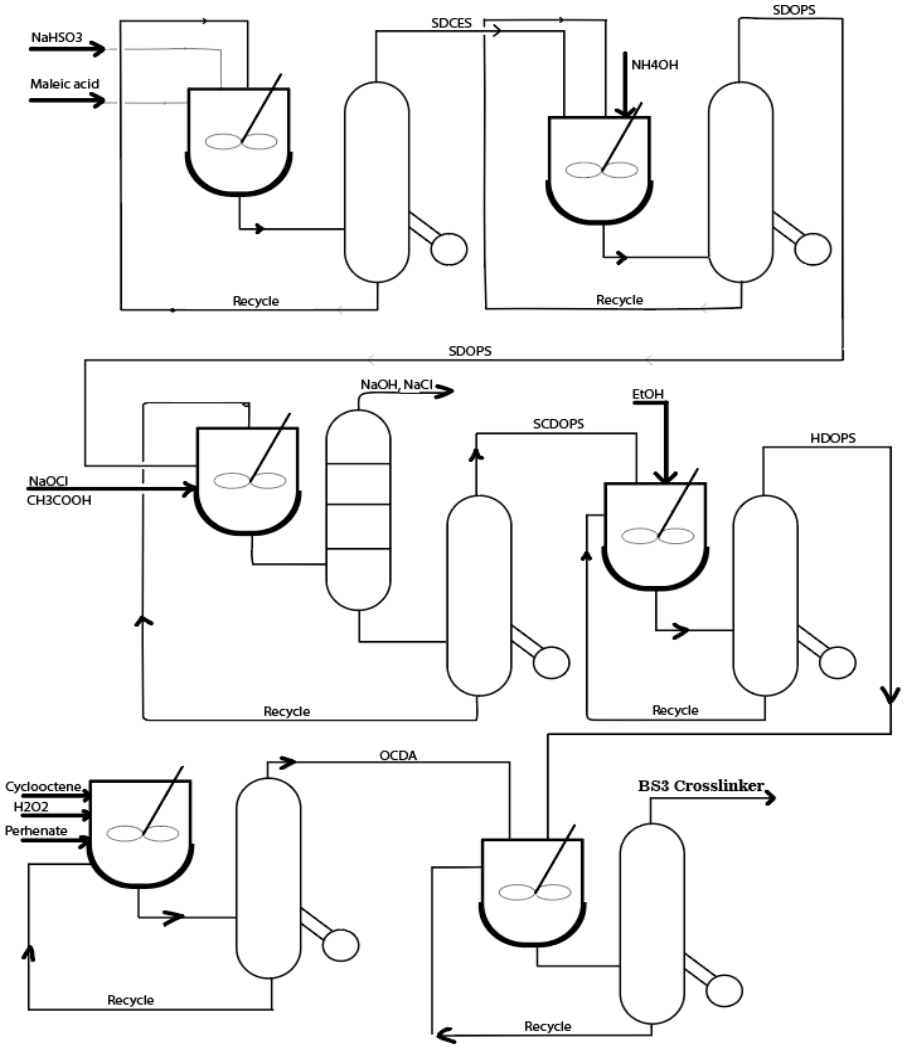


Figure 22 – The model of production

Principal technological scheme: The very first reaction is not beneficial, as maleic anhydrid cost too much money in comparison with maleic acid. So, it was decided that the second reaction is much better in terms of cost of reagents and purity

of product. The type of a reactor which is used here is called CSTR reactor with the embedded function of temperature control and stirrer. This type of reactor is planned to be used in other reactions as well. In order to have a higher purity, rotary vacuum evaporator is used here as well in order to separate unreacted reagents from the desired product (SDCES). The details are shown in the Figure 23.

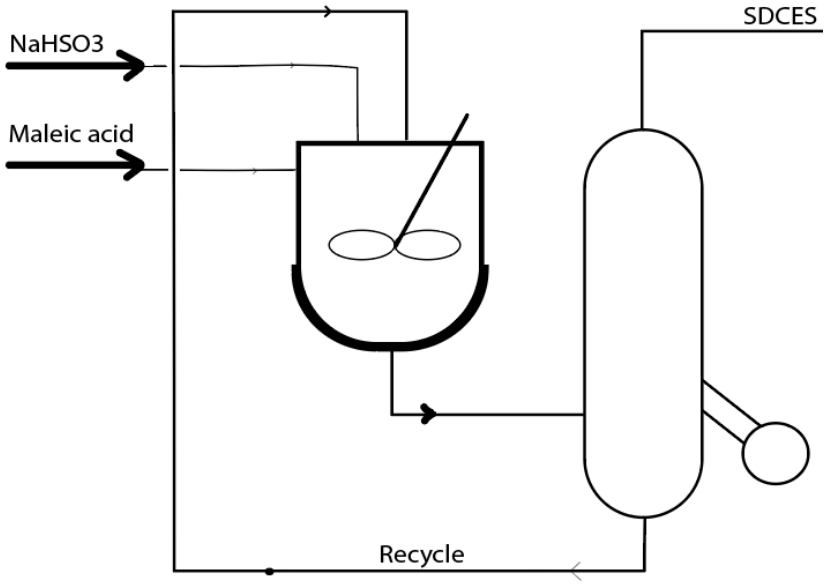


Figure 23 – The first step (The production of SDCES)

The following step is about getting SDOPS. The same reactor type is used as previously. Another product which is produced here is water, so it is possible to use rotary vacuum evaporator again. In addition, with the help of it, it is possible to separate SDOPS from unreacted reagents and send them over to the recycling.

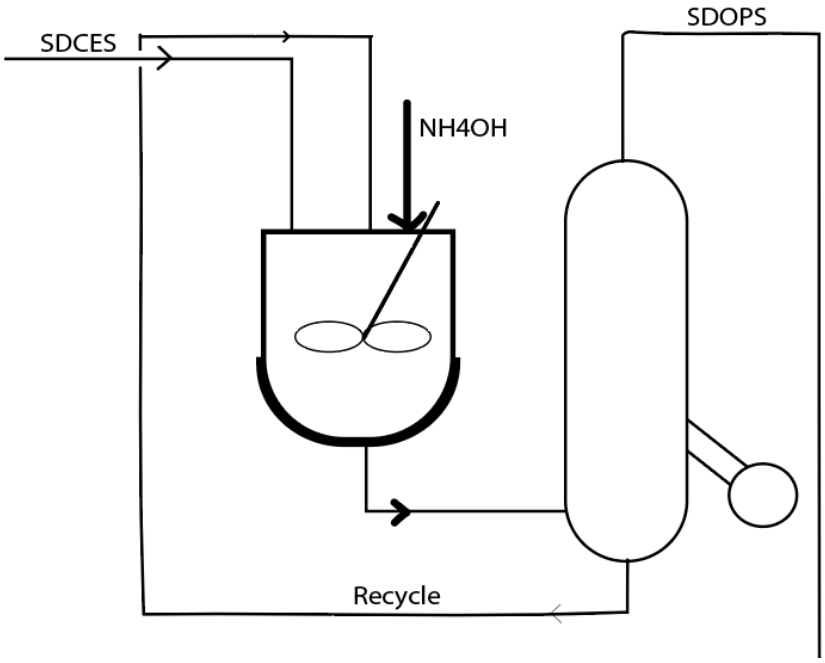


Figure 24 – The second step (The production of SDOPS)

After that, SDOPS is reacted with NaOCl and CH3COOH, which forms SCDOPS(desired product), NaCl, and CH3COOH. To separate the desired product, liquid-liquid extraction method is used. Afterwards, with the help of a rotary vacuum evaporator, reagents are distinguished from SCDOPS, and sent to recycle.

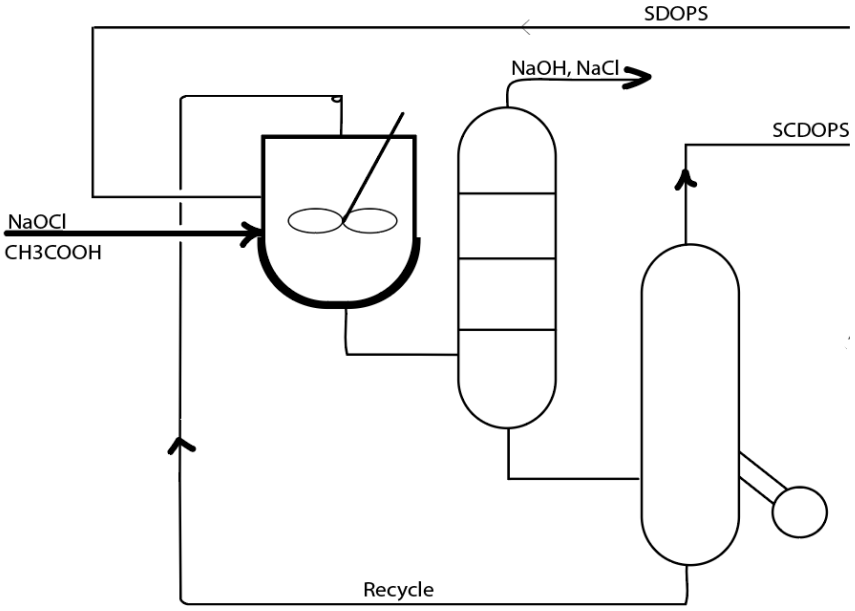


Figure 25 – The third step (The production of SCDOPS)

The 5th reaction includes the formation of HDOPS out of SCDOPS and Ethanol. Another product is CH3CH2Cl which is separated with the help rotary vacuum evaporator.

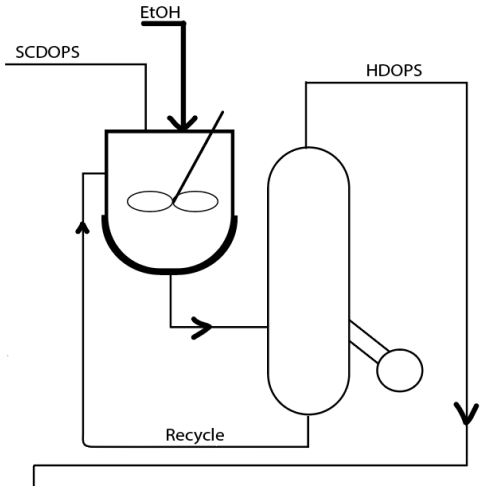


Figure 26 – the 4th step (The production of HDOPS)

The next part includes the synthesis of OCDA. It is formed from cycloctene(boiling point 148℃), H2O2 (150℃), and Perhenate (190℃). In order to divide OCDA from H2O and unreacted reagents, a rotary vacuum evaporator is used. Boiling point of OCDA is about 230℃

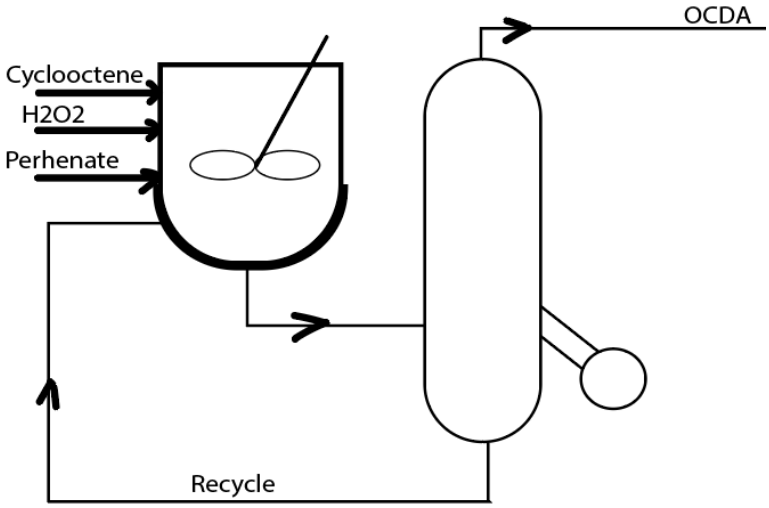


Figure 27 – The 5th step (The production of OCDA)

The final step is about combination of OCDA (230℃) and HDOPS. The same batch reactor with stirrer and heat controller is used. To separate water from BS3,

vacuum evaporator is used. At the end, final product is manufactured.

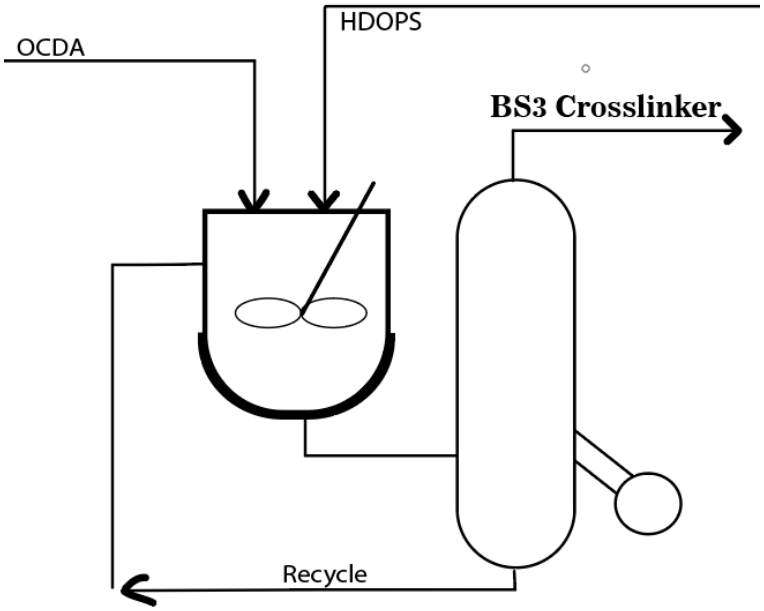


Figure 28 – The 6th step (The production of BS3)

2.2 Material balance

The following model based on the principal scheme. Overall, it consists of 6 main steps.

The 6th stage: At the beginning, the planned production of BS3 crosslinker will be 500 kilograms a year. Knowing that molar mass of BS3 is 572 g/mol, OCDA's molar mass is 174, HDOPS's molar mass is 434.

5000000/572=874.126 mol.

So, needed mass of OCDA is 874.126*174=152097.9 g =152.1 kg

Mass of HDOPS is 874.126*434=379370.684 g=379.4 kg

The 5th stage: The first reagent is OCDA and needed mass of it is 152097.9 g

Molar mass is 174, so 152097.9/174=874.126 mol

So, needed mass of Cyclooctene is 110*874.126=96153.845 g=96.2 kg

Needed mass of H2O2 is 4*34*874.126=118881.136 g=119 kg

The 4th stage: The second reagent is HDOPS. Needed mass of it is 379370.68g

379370.68/217=1748.3

Mass of SCDOPS is 235*1748.3=410850.5g=410.9 kg

Mass of ethanol is 46*1748.3=80421.8 g= 80.4 kg

The 3rd stage: For SCDOPS:

Mass of SDOPS is 201*1748.3=351408.3 g=351.4 kg

Mass of 2NaOCl is 74.5*2*1748.3=260452 g=260.4 kg

The 2nd stage: For SDOPS:

Mass of SDCES is 220*1748.3=384626 g=384.6 kg

Mass of NH4OH is 35*1748.3=61190.5 g=62 kg

The 1st stage: For SDCES:

Mass of C4H4O4 is 116*1748.3=202803 g=202.8 kg

Mass of NaHSO3 is 104*1748.3=181823.2 g=181.8 kg

The calculation of raw materials begins with the final stage of the process (6th stage). To stage No. 6 taking into account losses (10%) the mass of BS3 must be 450 kg. (m_t*0.9). After the recycling, the purity becomes 1%.

Table 2 – Material balance for batch reactor No.6 and rotary vacuum evaporator No.6

For batch reactor No.6			
Incoming reagents		Outcoming product	
	kg		Kg
OCDA	152.1	BS3	450
HDOPS	379.4	H2O	31.5
Total:	531.5		531.5
For rotary vacuum evaporator No.6			
Incoming reagents		Outcoming product	
	kg		kg
BS3	450	BS3	45
OCDA+HDOPS	50		
		Lost product:5 kg	

At stage No. 5, taking into account losses (9%), mass of OCDA must be 138.5 kg: The lost part is 13.7 kg (m_t*0.91). With the recycling process the losses should be diminished to 2%. (3 kg)

Table 3 – Material balance for batch reactor No.5 and rotary vacuum evaporator No.5

For batch reactor No.5			
Incoming reagents		Outcoming product	
	kg		Kg
Cyclooctene	96.2	OCDA	138.5
H2O2	119 kg	H2O	47.2
		Unreacted reagents	29.5
Total:	215.2		215.2
For rotary vacuum evaporator No.5			
Incoming reagents		Outcoming product	
	kg		kg
OCDA	138.5	OCDA	149.2

Unreacted reagents	29.5		
		Lost product: 3 kg	

At stage No. 4, taking into account losses (8%), mass of HDOPS must be 349.5kg: The lost part is 30.5 kg (m_t*0.92). With recycling it decreases to 1%. (3.75 kg)

Table 4 – Material balance for batch reactor No.4 and rotary vacuum evaporator No.4

For batch reactor No.4			
Incoming reagents		Outcoming product	
	kg		Kg
EtOH	80.4	HDOPS	349.5
SCDOPS	410.9	CH3CH2Cl	112.76
		Unreacted reagents	29
Total:	491.3		491.3
For rotary vacuum evaporator No.4			
Incoming reagents		Outcoming product	
	kg		kg
HDOPS	349.5	HDOPS	370.65
Unreacted reagents	29		
		Lost product: 3.5 kg	

At stage No. 3, taking into account losses (11%), mass of SCDOPS must be 365.7 kg: The lost part is 45.2 kg (m_t*0.89). Liquid-liquid extractor is used in order to separate NaOH and NaCl from it. Then with the help of recycling, the purity reaches 98%. (Lost part of SCDOPS is 8.2 kg)

Table 5 – Material balance for batch reactor No.3, rotary vacuum evaporator No.3, and liquid-liquid extractor No.1

For batch reactor No.3			
Incoming reagents		Outcoming product	
	kg		Kg
NaOCl	260.4	SCDOPS	365.7
SDOPS	351.4	NaOH, NaCl	200.9 kg
		Unreacted reagents	45.2
Total:	611.8		611.8

For liquid-liquid extractor No.1			
Incoming reagents		Outcoming product	
	kg		kg
SCDOPS +unreacted reagents	410.9	SCDOPS +unreacted reagents	410.9
NaOH, NaCl	200.9		
For rotary vacuum evaporator No.3			
Incoming reagents		Outcoming product	
	kg		kg
SCDOPS	365.7	SCDOPS	402.4
Unreacted reagents	45.2		
		Lost product: 8.5 kg	

At stage No. 2, taking into account losses (8%), mass of SDOPS must be 323.29 kg: The lost part is 28.112 kg. (m_t*0.92). Using the rotary vacuum evaporator, the purity becomes 1%. (3.5kg)

Table 6 – Material balance for batch reactor No.2 and rotary vacuum evaporator No.2

For batch reactor No.2			
Incoming reagents		Outcoming product	
	kg		Kg
NH4OH	62	SDOPS	323.29
SDCES	384.6	Unreacted reagents	28
		H2O	94.4
Total:	446.6		446.6
For rotary vacuum evaporator No.2			
Incoming reagents		Outcoming product	
	kg		Kg
SDOPS	323.29	SDOPS	347.9
Unreacted reagents	28		
		Lost product: 3.5 kg	

At stage No. 1, taking into account losses (9%), mass of SDCES must be 350 kg:
The lost part is 34.6 kg. (m_t*0.91). With recycling the loss decreases to 1% (3.8 kg of loss)

Table 7 – Material balance for batch reactor No.1 and rotary vacuum evaporator No.1

For batch reactor No.1			
Incoming reagents		Outcoming product	
	kg		Kg
C4H4O4	202.8	SDCES	350
NaHSO3	181.8	Unreacted reagents	34.6
Total:	384.6		384.6
For rotary vacuum evaporator No.1			
Incoming reagents		Outcoming product	
	kg		kg
SDCES	350	SDCES	380.75
Unreacted reagents	34.6		
		Lost product: 3.8 kg	

Overall material balance for the production is shown on the table No.8

Table 8 – Material balance for overall the process

Incoming product	kg	Lost product	kg
SDCES	384.6	SDCES	3.8
SDOPS	351.6	SDOPS	3.5
SCDOPS	410.9	SCDOPS	8.5
HDOPS	379.4	HDOPS	3.5
OCDA	152.1	OCDA	3
BS3	500	BS3	5
Total:	2178.4	Total	27.3

2.3 Economic calculations

Economic calculations include almost all the aspects of the successful manufacture of the given product. It includes: reagents, equipment, workers' salary, building for rent, communal payments, logistics, production costs and others.

Table 9 – The cost of reagents

Reagents' name:	Overall cost:
H2O2 (119kg)	350$
Cyclooctene (96.2kg)	116402$
Ethanol (80.4kg)	100$
NaOCl (260.4kg)	130$
C4H4O4(203kg)	2030$
NaHSO3(182kg)	95$
	Total: 119107$

Table 10 – The cost of equipment

Type of equipment and its quantity:	Overall cost:
6 stirred tank reactors	48000$
6 rotary vacuum evaporators	30000$
1 liquid-liquid extractor	3000$
1 packaging machine	3500$
Pipeline	3500$
	Total: 88000$

Table 11 – Workers' salary

Name of position:	Salary (A year)
Plant manager	20000$
Process Engineer	17000$
Reactor Operators (2)	15000$
Quality Control (QC) Analyst	15000$
Maintenance Technicians	14000$
Safety Officers (2)	9000$
Logistics/Supply Chain Manager	13000$
Laboratory Technician	16000$
Cleaning staff	15000$
	Total: 134000$

Table 12 – Other costs

Type of expenditure:	Price (Per year):
Buying an empty building (1000 m^2)	50000$ (One-time payment)
Communal payments	15000$
Production costs (10% out of the whole equipment cost)	8800$

	Total: 73800$

The expected manufacture of BS3 crosslinker is 495 kg. 1 kg of it cost 1080$. In total, our income should be around 1080*495=534600$. Overall expenditure is equal to 414907$. So the net profit is 119693$.

CONCLUSION

The production of such a novel compound, which has an ability to improve the various sectors of industry in Kazakhstan, can create a hub according to which our country can become a center of click chemistry. By now, there isn't a specific country which specializes on them, however, Kazakhstan has got all the needed resources to become the first.

One primary example here is the modification of BS3 crosslinker with the help of click reactions, such as copper-free or copper catalyzed. This can help us to modify any biomolecules and introduce any kind of desired additional enhancement.

According to the results of the diploma project, the BS3 crosslinker has been successfully "theoretically" synthesized. Its production is trade secret, so it is not available on the internet. The only source of infomation which I could use here is the "reaxys" (A database of all the known reaction all over the world), where I found a lot of useful information concerning the synthesis, starting with the first step and finishing with the last one.

During the calculations of 500 kg of desired product, it was revealed that sometimes there are 2 or even more methods of synthesis, so I had to choose the best options in order to have a higher interest in the end by choosing the cheapest and efficient reagents. The perfect example here is the 1st reaction which was described above. It is one way of synthesizing SDCES, but, there is also a 2nd reaction with the same product. In addition, the 2nd reaction had cheaper prices and higher purity which made it as an ideal option to choose.

Unfortunately, the rate of unreacted reagents was high in each step (About 9-10%), so it was decided to use recycle technique by using recycling. The desired 500 kg of product was theoretically produced with some minor wastes (1-4%).

List of symbols and abbreviations:

CuAAC	copper-catalyzed azide-alkyne cycloaddition
SPAAC	copper-free strain-promoted azide-alkyne cycloaddition
DIBO	Dibenzo Cyclooctyne
RuAAC	ruthenium-catalyzed azide-alkyne cycloaddition
DIFO	difluorooctyne
BARAC	biarylazacyclooctynone
SPANC	Strain-promoted alkyne-nitrone cycloaddition
SPIEDAC	strain-promoted inverse-electron-demand Diels-Alder cycloaddition
POI	Protein of interest
nsAA	noncanonical amino acid
PPI	protein-protein interactions
DSS	Disuccinimidyl suberate
DTSP	3,3'-dithiodipropionic acid di(N-hydroxysuccinimide ester)
BMOE	bismaleimidoethane
SMCC	sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate
SIA	succinimidyl iodoacetate
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
SDA	NHS-Diazirine
BDRG	1,2-bis(maleimido)ethane or N,N'-methylenebis(acrylamide)
DSSO	disuccinimidyl sulfoxide
BS3	Bissulfosuccinimidyl suberate
SDCES	Sodium 1,2- dicarboxyethanesulfonate
SDOPS	Sodium 2,5- dioxopyrrolidine-3-sulfonate
SCDOPS	Sodium 2,5-dioxopyrrolidine-3- sulfonate
HDOPS	Sodium 1-hydroxy-2,5-dioxopyrrolidine-3- sulfonate
OCDA	Octanedioic acid

Bibliography:

1. "The Nobel Prize in Chemistry 2022". NobelPrize.org. Retrieved 2022-10-05.
2. R. A. Evans (2007). "The Rise of Azide–Alkyne 1,3-Dipolar 'Click' Cycloaddition and its Application to Polymer Science and Surface Modification". Australian Journal of Chemistry. 60 (6): 384–395. doi:10.1071/CH06457.
3. Kolb, H. C., Finn, M. G., & Sharpless, K. B. (2001). Click Chemistry: Diverse Chemical Function from a Few Good Reactions. Angewandte Chemie International Edition, 40(11), 2004-2021.
4. BroadPharm. (2021). Click Chemistry Reagents. https://broadpharm.com/product-categories/click-chemistry-reagents
5. Liang, Y., Mackey, J. L., Lopez, S. A., Liu, F., Houk, K. N., & Wu, P. (2014). Mechanistic insights into the copper(I)-catalyzed azide–alkyne cycloaddition. Journal of the American Chemical Society, 136(35), 12736-12744.
6. L. Liang and D. Astruc: "The copper(I)-catalysed alkyne-azide cycloaddition (CuAAC) "click" reaction and its applications. An overview", 2011; 255, 23–24, 2933–2045, p. 2934
7. Zhang, Li; Chen, Xinguo; Xue, Peng; Sun, Herman H. Y.; Williams, Ian D.; Sharpless, K. Barry; Fokin, Valery V.; Jia, Guochen (November 2005). "Ruthenium-Catalyzed Cycloaddition of Alkynes and Organic Azides". Journal of the American Chemical Society.
8. MacKenzie, DA; Sherratt, AR; Chigrinova, M; Cheung, LL; Pezacki, JP (Aug 2014). "Strain-promoted cycloadditions involving nitrones and alkynes—rapid tunable reactions for bioorthogonal labeling". Curr Opin Chem Biol. 21: 81–8. doi:10.1016/j.cbpa.2014.05.023.
9. Lang, K.; Chin, J. (2014). "Bioorthogonal Reactions for Labeling Proteins". ACS Chem. Biol. 9 (1): 16–20. doi:10.1021/cb4009292. PMID 24432752
10. Nikić I, Kang JH, Girona GE, Aramburu IV, Lemke EA. Labeling proteins on live mammalian cells using click chemistry. Nat Protoc. 2015 May;10(5):780-91. doi: 10.1038/nprot.2015.045. Epub 2015 Apr 23. PMID: 25906116.
11. Nwe K, Brechbiel MW. Growing applications of "click chemistry" for bioconjugation in contemporary biomedical research. Cancer Biother Radiopharm. 2009 Jun;24(3):289-302. doi: 10.1089/cbr.2008.0626. PMID: 19538051; PMCID: PMC2811415.
12. Xi, W., Scott, T. F., Kloxin, C. J., & Bowman, C. N. (2014). Click Chemistry in Materials Science. Advanced Functional Materials, 24(18), 2572–2590. doi:10.1002/adfm.201302847A practical approach to crosslinking. Mattson, G., et al. Molecular Biology Reports (1993) 17:167-183
13. Wong SS, Jameson DM. Chemistry of protein and nucleic acid cross-linking and conjugation. 2. Boca Raton: Taylor & Francis; 2012.

14. Arora B, Tandon R, Attri P, Bhatia R. Chemical Crosslinking: Role in Protein and Peptide Science. Curr Protein Pept Sci. 2017;18(9):946-955. doi: 10.2174/1389203717666160724202806. PMID: 27455969.
15. Arora B, Tandon R, Attri P, Bhatia R. Chemical Crosslinking: Role in Protein and Peptide Science. Curr Protein Pept Sci. 2017;18(9):946-955. doi: 10.2174/1389203717666160724202806. PMID: 27455969.
16. Heck T, Faccio G, Richter M, Thöny-Meyer L. Enzyme-catalyzed protein crosslinking. Appl Microbiol Biotechnol. 2013 Jan;97(2):461-75. doi: 10.1007/s00253-012-4569-z. Epub 2012 Nov 25. PMID: 23179622; PMCID: PMC3546294.
17. Xuan W, Shao S, Schultz PG. Protein Crosslinking by Genetically Encoded Noncanonical Amino Acids with Reactive Aryl Carbamate Side Chains. Angew Chem Int Ed Engl. 2017 Apr 24;56(18):5096-5100. doi: 10.1002/anie.201611841. Epub 2017 Apr 3. PMID: 28371162; PMCID: PMC5543777.
18. Stadlmeier M, Runtsch LS, Streshnev F, Wühr M, Carell T. A Click-Chemistry-Based Enrichable Crosslinker for Structural and Protein Interaction Analysis by Mass Spectrometry. Chembiochem. 2020 Jan 15;21(1-2):103-107. doi: 10.1002/cbic.201900611. Epub 2019 Nov 28. PMID: 31593346; PMCID: PMC6980279.
19. M. Ehrlich, M. J. Gattner, B. Viverge, J. Bretzler, D. Eisen, M. Stadlmeier, M. Vrabel, T. Carell, Chem. Eur. J. 2015, 21, 7701–7704.
20. M. Grimm, T. Zimniak, A. Kahraman, F. Herzog, Nucleic Acids Res. 2015, 43, W362–W369.
21. https://www.korambiotech.com/upload/bbs/2/Cross-LinkingTechHB.pdf
22. Mackinnon AL, Taunton J. Target Identification by Diazirine Photo-Cross-linking and Click Chemistry. Curr Protoc Chem Biol. 2009 Dec;1:55-73. doi: 10.1002/9780470559277.ch090167. PMID: 23667793; PMCID: PMC3647699.
23. Barysz HM, Malmström J. Development of Large-scale Cross-linking Mass Spectrometry. Mol Cell Proteomics. 2018 Jun;17(6):1055-1066. doi: 10.1074/mcp.R116.061663. Epub 2017 Apr 7. PMID: 28389583; PMCID: PMC5986251.
24. Schock M, Bräse S. Reactive & Efficient: Organic Azides as Cross-Linkers in Material Sciences. Molecules. 2020 Feb 24;25(4):1009. doi: 10.3390/molecules25041009. PMID: 32102403; PMCID: PMC7070646.
25. Genomatica, Inc. Methods for producing dicarboxylic acids. US Patent application US20160031790A1, filed Jul 31, 2015, and published Feb 4, 2016.
26. Woodward, R. B.; Gosteli, J.; Ernest, I.; Friary, R. J.; Nestler, G.; Raman, H.; Sitrin, R.; Suter, Ch.; Whitesell, J. K. J. Am. Chem. Soc. 1973, 95, 6853–6858.
27. Sato, K., Aoki, M., and Noyori, R., Science, 1998, vol. 281, p. 1646.
28. Noyori, R., Aoki, M., and Sato, K., Chem. Commun., 2003, p. 1977.
29. Wang, J.Y., Zhou, M.D., Yuan, Y.G. et al. Oxidation of cyclooctene to suberic acid using perrhenate-containing composite ionic liquids as green catalysts.

Russ J Gen Chem 85, 2378–2385 (2015). https://doi.org/10.1134/S1070363215100254

30. Belsom, A., & Rappsilber, J. (2021). Anatomy of a crosslinker. Current Opinion in Chemical Biology, 60, 39-46. https://doi.org/10.1016/j.cbpa.2020.07.008

31. Farkhani, Dariush, Khalili, Ali Asghar and Mehdizadeh, Ali. "The Role of Surface Active Agents in Sulfonation of Double Bonds" Tenside Surfactants Detergents, vol. 51, no. 4, 2014, pp. 352-355. https://doi.org/10.3139/113.110317

32. Patent: CN108003069A Preparation method for sulfosuccinic acid

33. CN108003069A - Method for preparing N-chlorosuccinimide." (n.d.). Espacenet. Retrieved April 28, 2023, from https://worldwide.espacenet.com/patent/search/family/062057198/publication/CN108003069A?q=pn%3DCN108003069A

34. Xu, J., & Yang, Z. (2013). Convenient and Environment-Friendly Synthesis of Sulfonyl Chlorides from S-Alkylisothiourea Salts via N-Chlorosuccinimide Chlorosulfonation. Synthesis, 45(12), 1675–1682. doi:10.1055/s-0033-1338743

35. Duan, X., Liu, X., Cuan, X., Wang, L., Liu, K., Zhou, H., … Wang, J. (2019). Solvent-Controlled Synthesis of Thiocyanated Enaminones and 2-Aminothiazoles from Enaminones, KSCN, and NBS. The Journal of Organic Chemistry, 84(19), 12366–12376. doi:10.1021/acs.joc.9b01722

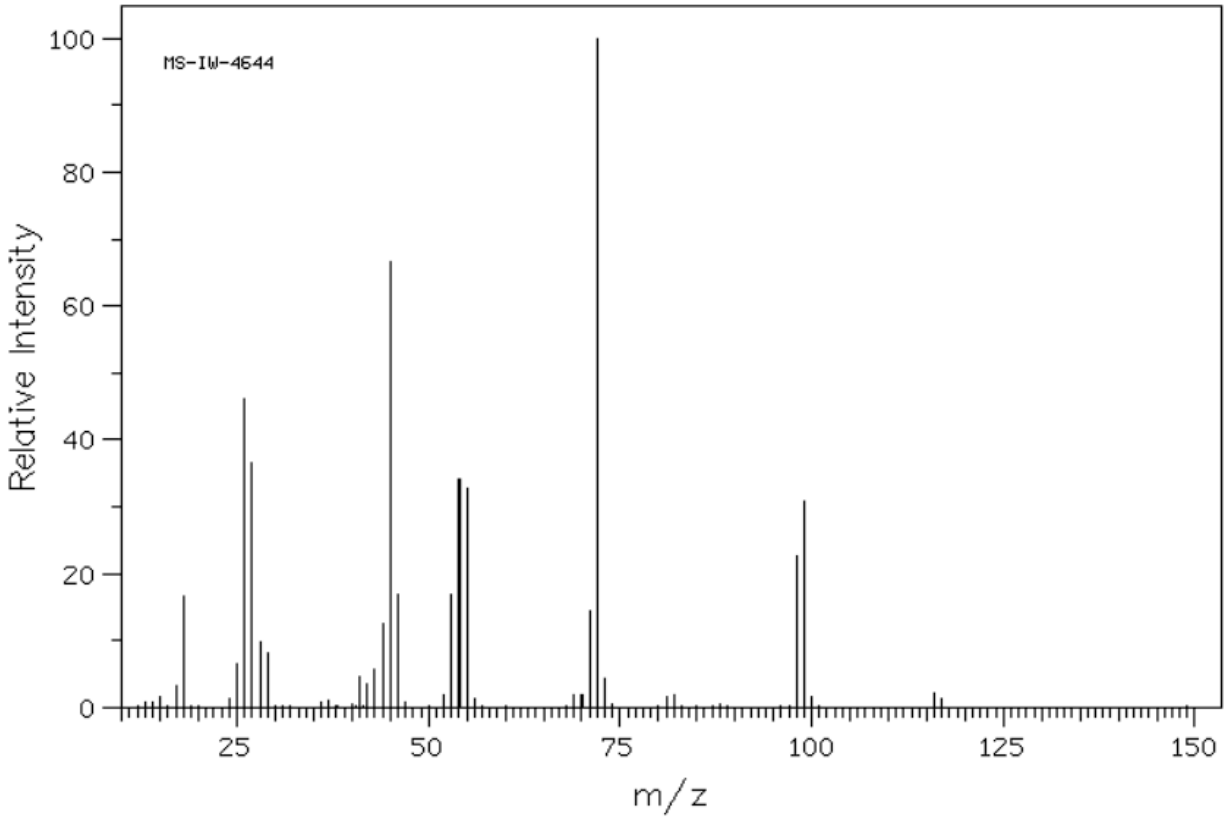
36. Wang, J. Y., Zhou, M. D., Yuan, Y. G., Fu, N. H., & Zang, S. L. (2015). Oxidation of cyclooctene to suberic acid using perrhenate-containing composite ionic liquids as green catalysts. Russian Journal of General Chemistry, 85(10), 2378–2385. doi:10.1134/s1070363215100254

37. CN 112979674 A. "A method for preparing a material for flexible integrated circuit boards," filed October 27, 2020.

38. US8017790B2 Method for producing pyrrolidones from succinates from fermentation broths

ANNEX A

MS-IW-4644 SDBS NO. 1065
maleic acid
C4H4O4 (Mass of molecular ion: 116)



Source Temperature: 150 °C
Sample Temperature: 130 °C
Direct, 75 eV

НЕКОММЕРЧЕСКОЕ АКЦИОНЕРНОЕ ОБЩЕСТВО «КАЗАХСКИЙ НАЦИОНАЛЬНЫЙ ИССЛЕДОВАТЕЛЬСКИЙ ТЕХНИЧЕСКИЙ УНИВЕРСИТЕТ имени К.И.САТПАЕВА»

РЕЦЕНЗИЯ

на дипломный проект
(наименование вида работы)

Бүркіт Ұлан Бейбітұлы
(Ф.И.О. обучающегося)

6В05101 – Химическая и биохимическая инженерия
(шифр и наименование специальности)

На тему: «Разработка технологии получения клик-реагентов для использования на белках.»

ЗАМЕЧАНИЯ К РАБОТЕ

"Дипломный проект на тему "Разработка технологии получения клик-реагентов для использования на белках" представляет собой исследование и разработку методов, связанных с клик химией, а также применение этих методов для модификации белков и их сшивания.

Проект содержит подробное описание клик химии и его общие принципы. Он освещает различные типы клик-реакций и способы их получения. Также представлены различные подходы к использованию клик-реагентов на белках, включая их применение в процессе сшивания белков.

Описываются методы и техники сшивания белков, а также используемые в этом процессе реагенты. Проект рассматривает клик-реагенты, которые наиболее эффективны при сшивании белков.

В работе приводится технологическая схема и ее описание, а также модель, основанная на основной схеме. Приводится материальный баланс и экономические расчеты.

Проект является актуальным и интересным исследованием, которое имеет потенциал для дальнейшего развития в области модификации белков и их сшивания. Автор проекта продемонстрировал глубокое понимание клик химии и его применения на примере белков

В целом, дипломный проект "Разработка технологии получения клик-реагентов для использования на белках" представляет значимый вклад в область клик-химии в Казахстане и его применения в белковой биохимии. Работа заслуживает положительной оценки за обширность исследования, четкое представление информации и потенциал для дальнейших исследований в этой области."

Оценка работы

Дипломный проект на тему «Разработка технологии получения клик-реагентов для использования на белках.» оцениваю на хорошо, и считаю, что Бүркіт Ұлан заслуживает квалификации бакалавра по образовательной программе 6В05101 – “Химическая и биохимическая инженерия”

Рецензент
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(должность, уч. степень, звание)
Шарипов Р.Х.
(подпись)
«6» июня 2023г.

МИНИСТЕРСТВО НАУКИ И ВЫСШЕГО ОБРАЗОВАНИЯ РЕСПУБЛИКИ КАЗАХСТАН

ОТЗЫВ

НАУЧНОГО РУКОВОДИТЕЛЯ

на дипломную проект
(наименование вида работы)

Бүркіт Ұлан Бейбітұлы
(Ф.И.О. обучающегося)

6В05101 – Химическая и биохимическая инженерия
(шифр и наименование специальности)

Тема:
Разработка технологии получения клик-реагентов для использования на белках.

Дипломный проект на тему "Разработка технологии получения клик-реагентов для использования на белках" представляет собой ценный вклад в область химии и биохимии, исследующей методы взаимодействия белков с помощью клик-химии. Работа обладает хорошо структурированным содержанием, позволяющим читателю получить представление о клик-химии, клик-реагентах и их применении в кросс-связывании белков.

Автор проекта достаточно полно исследовал основы клик-химии, предоставив общую информацию о ее принципах и применении. Особое внимание уделено получению клик-реагентов и описанию различных способов их использования на белках. Важно отметить, что проект также содержит подробные данные о кросс-связывании белков, методиках и техниках, используемых при проведении этого процесса, а также применяемых реагентах, включая клик-реагенты.

Особенно интересным аспектом данного проекта является технологическая схема и описание модели производства клик-реагентов. Автор предоставили материальный баланс и провели экономические расчеты, что говорит о серьезном подходе к практической реализации данной технологии. Результаты и обсуждение проекта наглядно демонстрируют его актуальность и потенциал для дальнейшего развития в области химии и биохимии.

Я хотел бы отметить, что дипломный проект демонстрирует глубокое понимание клик-химии и ее применения в кросс-связывании белков. Автор продемонстрировал способность анализировать сложные научные концепции и применять их на практике. Его тщательная работа по изучению литературы, проведению экспериментов и анализу данных является заметной особенностью этого проекта.

В целом, дипломный проект на тему "Разработка технологии получения клик-реагентов для использования на белках" является отличной работой, полной интересных исследовании

Дипломный проект на тему «Разработка технологии получения клик-реагентов для использования на белках.» оцениваю на 95 баллов, и считаю, что Бүркіт Ұлан Бейбітұлы заслуживает квалификации бакалавра по специальности 6В05101–“Химическая и биохимическая инженерия”

Научный руководитель
Ассистент проф кафедры ХиБИ
(должность, уч. степень, звание)
Хабиев А.Т.
(подпись)
«31» мая 2023г.

Метаданные

Название
Development of technology for obtaining click reagents for use on proteins.docx

Автор
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Научный руководитель / Эксперт
Алибек Хабиев
Подразделение
ИГиНГД

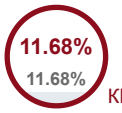
Список возможных попыток манипуляций с текстом

В этом разделе вы найдете информацию, касающуюся текстовых искажений. Эти искажения в тексте могут говорить о ВОЗМОЖНЫХ манипуляциях в тексте. Искажения в тексте могут носить преднамеренный характер, но чаще, характер технических ошибок при конвертации документа и его сохранении, поэтому мы рекомендуем вам подходить к анализу этого модуля со всей долей ответственности. В случае возникновения вопросов, просим обращаться в нашу службу поддержки.

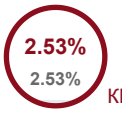
Замена букв	ß	0
Интервалы	A→	0
Микропробелы	·	0
Белые знаки	ß	0
Парафразы (SmartMarks)	a	73

Объем найденных подобий

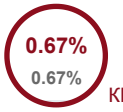
Обратите внимание!Высокие значения коэффициентов не означают плагиат. Отчет должен быть проанализирован экспертом.



КП1



КП2



КЦ

25
Длина фразы для коэффициента подобия 2

7467
Количество слов

48359
Количество символов

Подобия по списку источников

Просмотрите список и проанализируйте, в особенности, те фрагменты, которые превышают КП №2 (выделенные жирным шрифтом). Используйте ссылку «Обозначить фрагмент» и обратите внимание на то, являются ли выделенные фрагменты повторяющимися короткими фразами, разбросанными в документе (совпадающие сходства), многочисленными короткими фразами расположенные рядом друг с другом (парафразирование) или обширными фрагментами без указания источника ("криптоцитаты").

10 самых длинных фраз

Цвет текста

ПОРЯДКОВЫЙ НОМЕР	НАЗВАНИЕ И АДРЕС ИСТОЧНИКА URL (НАЗВАНИЕ БАЗЫ)	КОЛИЧЕСТВО ИДЕНТИЧНЫХ СЛОВ (ФРАГМЕНТОВ)	
1	https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-crosslinking-protein-modification.html	80	1.07 %
2	http://en.wikipedia.org/wiki/Click_chemistry	50	0.67 %
3	http://en.wikipedia.org/wiki/Click_chemistry	33	0.44 %
4	http://en.wikipedia.org/wiki/Click_chemistry	26	0.35 %

5	https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-crosslinking-protein-modification.html	24	0.32 %
6	http://en.wikipedia.org/wiki/Click_chemistry	23	0.31 %
7	Labeling proteins on live mammalian cells using click chemistry Edward A Lemke, Gemma Estrada Girona, Iker Valle Aramburu, Jun Hee Kang,Ivana Nikić;	20	0.27 %
8	Labeling proteins on live mammalian cells using click chemistry Edward A Lemke, Gemma Estrada Girona, Iker Valle Aramburu, Jun Hee Kang,Ivana Nikić;	19	0.25 %
9	http://en.wikipedia.org/wiki/Click_chemistry	19	0.25 %
10	http://en.wikipedia.org/wiki/Click_chemistry	17	0.23 %

из базы данных RefBooks (0.95 %)

ПОРЯДКОВЫЙ НОМЕР	НАЗВАНИЕ	КОЛИЧЕСТВО ИДЕНТИЧНЫХ СЛОВ (ФРАГМЕНТОВ)	
Источник: Paperity - abstrakty			
1	Labeling proteins on live mammalian cells using click chemistry Edward A Lemke, Gemma Estrada Girona, Iker Valle Aramburu, Jun Hee Kang,Ivana Nikić;	71 (6)	0.95 %

из домашней базы данных (0.32 %)

ПОРЯДКОВЫЙ НОМЕР	НАЗВАНИЕ	КОЛИЧЕСТВО ИДЕНТИЧНЫХ СЛОВ (ФРАГМЕНТОВ)	
1	Yezhenova Anelya.docx [redacted] 5/18/2020 Satbayev University (ИХиБТ)	24 (3)	0.32 %

из программы обмена базами данных (0.00 %)

ПОРЯДКОВЫЙ НОМЕР	НАЗВАНИЕ	КОЛИЧЕСТВО ИДЕНТИЧНЫХ СЛОВ (ФРАГМЕНТОВ)

из интернета (10.41 %)

ПОРЯДКОВЫЙ НОМЕР	ИСТОЧНИК URL	КОЛИЧЕСТВО ИДЕНТИЧНЫХ СЛОВ (ФРАГМЕНТОВ)	
1	**http://en.wikipedia.org/wiki/Click_chemistry**	**413 (32)**	**5.53 %**
2	**https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-crosslinking-protein-modification.html**	**208 (13)**	**2.79 %**
3	https://doczz.net/doc/8191158/large-scale-chemical-cross-linking-mass-spectrometry-pers...	28 (2)	0.37 %
4	https://www.quantabiodesign.com/wp-content/uploads/Click-Chemistry-with-dPEG-Products-2021.pdf	26 (5)	0.35 %
5	http://www.geolog-technical.kz/images/pdf/g20195/138-143.pdf	14 (1)	0.19 %
6	https://handwiki.org/wiki/Chemistry:Click_chemistry	14 (2)	0.19 %
7	https://official.satbayev.university/en/geology-oil-gas-business	14 (2)	0.19 %
8	https://safety.fsu.edu/safety_manual/Azide%20Compounds.pdf	13 (1)	0.17 %

9	https://www.ambeed.com/products/1173081-96-3.html	13 (1)	0.17 %
10	https://patents.google.com/patent/US5187299A/en	12 (2)	0.16 %
11	https://pubmed.ncbi.nlm.nih.gov/28371162/	12 (1)	0.16 %
12	http://rd.springer.com/protocol/10.1007/978-1-60327-360-2_2	10 (1)	0.13 %

Список принятых фрагментов (нет принятых фрагментов)

ПОРЯДКОВЫЙ НОМЕР	СОДЕРЖАНИЕ	КОЛИЧЕСТВО ИДЕНТИЧНЫХ СЛОВ (ФРАГМЕНТОВ)