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Modification and characterization of chloro-sugar derivatives as anti-bacterial agents

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MODIFICATION AND CHARACTERIZATION OF CHLORO-SUGAR DERIVATIVES AS ANTI-BACTERIAL AGENTS

by

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Dedication

I would like to dedicate my work to my parents Kaushik Jani and Amita jani. They have provided me enormous support and encouragement throughout my college career and my life. Thank you for believing in myself. I am truly blessed for having you in my life. I also dedicate this thesis to my person Mayur Baraiya and my brother Devarshi Jani who never allowed me to give up. I will always appreciate all they have done for me.

Acknowledgment

I would like to express my appreciation to my professor, Dr. Ramanujachary Kandalam for his guidance and help throughout this research. He has the interest of his students at heart, he takes the time to get to know his students as individuals and for that, I am sincerely grateful. This would have not been possible without you. I have gained skills, knowledge and learned a lot about myself in the process that I will take with me into my next professional endeavor.

I also want to thank Prof. Dr Subash Jonnalagadda for being there whenever I needed him during my master program.

Abstract

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Sucralose is an artificial sugar substitute which is most commonly used sweetener among other artificial sweeteners. It is derived from sucrose through a complex chemical process that selectively substitutes three atoms of chlorine for three hydroxyl groups on sucrose molecule, which have shown some inhibition of bacterial growth in gut. The goal of the project was to substitute halide in sucralose in a way that it sustains potential anti-bacterial activity along with sweetening effect, which can be then incorporated into mouthwash formulation. Sucralose is very stable molecule and it also has other physico-chemical advantages which are suitable for our anticipated reactions. We initiated the modification of this chloro sugar starting from replacement by other halide molecule via S_N2 nucleophilic reaction. Additionally, we also reacted different functional group for chlorine replacement which involved click chemistry. Use of azide ion increased the complexity of the molecule since it has three nitrogen which undergoes rearrangement. To produce the library of target molecules hydroxyl group was protected by acetate group and used as starting material for further reactions. The compounds synthesized were characterized by different analytical techniques which might be potential anti-bacterial agents.

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Chapter 1

Sucralose

Introduction

Sucralose was accidently discovered in 1976 by Tate & Lyle, a British sugar company. Tate and Lyle were doing research on ways to use sucrose and its synthetic derivatives for industrial use, which is highly stable and non-toxic. Such sweetener would be easy to produce because sucrose is inexpensive and readily available.[1-2] Tate and Lyle have tested halogenated sugar in collaboration with Professor Leslie Hough and Shashikant Phadnis at Queens College in London. The interesting story behind the Sucralose (**figure 1**) discovery is, Phadnis was told to "test" a chlorinated sugar compound. Phadnis thought Hough asked him to "taste" the compound. He found the compound to be exceptionally sweet and has potency hundred times more than normal sugar.[1]

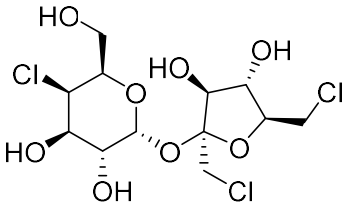


Figure 1. Chemical structure of sucralose

The Tate & Lyle research was based on investigating the sweetness of halogenated sugar spins.[3] Halogens were chosen because these are the elements that help dissolve one substance into another. The researchers determined five closely related

halogens which are fluorine, chlorine, bromine, iodine and astatine that change the sweetness of sugar molecule and found chlorine and bromine most effective. None were as sweet as the chlorinated version of sucralose.[3] The chlorine atom is present as highly electronegative region and the two chlorine atoms present in the fructose portion of the molecule lead to more hydrophobic properties which causes the drastically increased sweetness of sucralose.[4] Generation of sweet taste also comes from hydrophobic bonding of taste receptor with electronic bonding of sucralose. Chlorine was preferred because being lighter halogen it can more easily dissolves in other substances and combine readily with sucrose for sugar substitution.[3-4] The chlorine is chemically altered in sucralose to be very tightly bound so that it doesn't breakdown inside the human body. It provides stability to the molecule. That means during the complex process the chemical used were very toxic that prevent the dangerous chlorine molecule from detaching from sucralose inside the digestive system which could be a carcinogenic hazard.[4]

Artificial Sweeteners vs. Sucralose

Fahlberg discovered the sweet properties of saccharin, since then several artificial sweeteners have been discovered and produced.[5] Sucralose is 600 times sweeter than sucrose, three times as sweet as acesulfame potassium and aspartame, and twice as sweet as sodium saccharin. Neotame is 8000 times and advantame is 20000 times sweeter than sugar.[5] Among all these artificial sweeteners sucralose is the most used sweetener in the world.

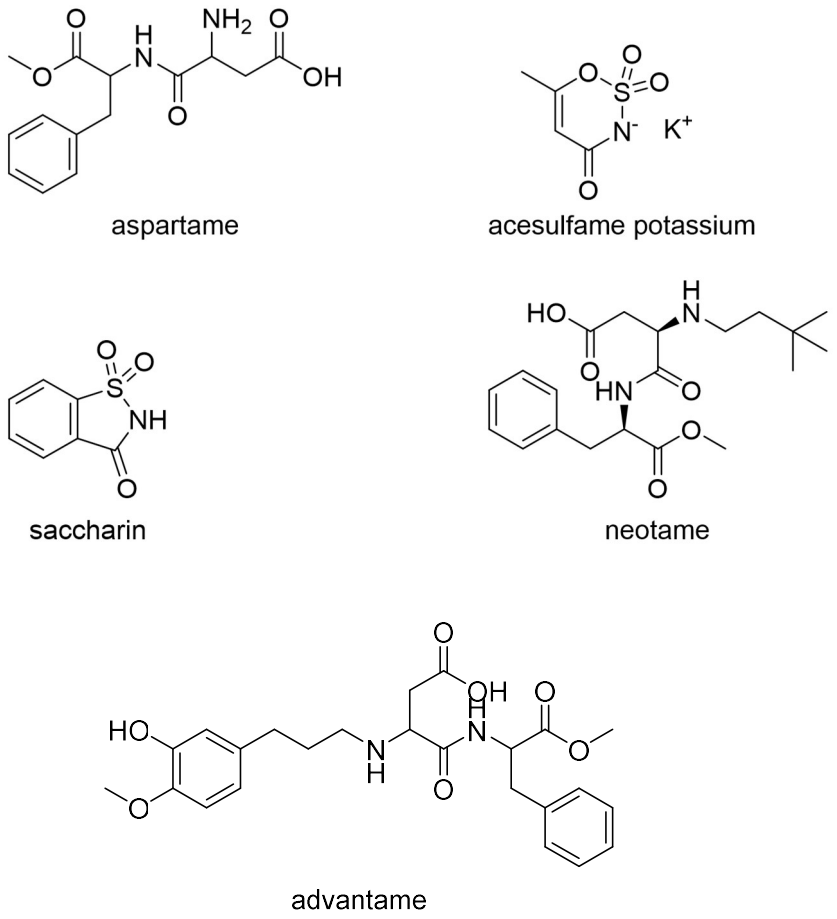


Figure 2. Artificial sweeteners

Artificial sweeteners may be non-nutritive, but they can still affect metabolism.[6] Artificial sweeteners are alternatives to sugar because they add virtually no calories to your diet and only a fraction is needed compared with the amount of sugar you would normally use for sweetness.[7] Although these claims appear promising, they have never been confirmed in any vigorously conducted trial or large epidemiological study. However

natural sugar substitutes may seem healthier than processed table sugar, their vitamin and mineral content isn't significantly different from that of sugar and both end up in body as glucose and fructose.[8]

Synthesis of Sucralose

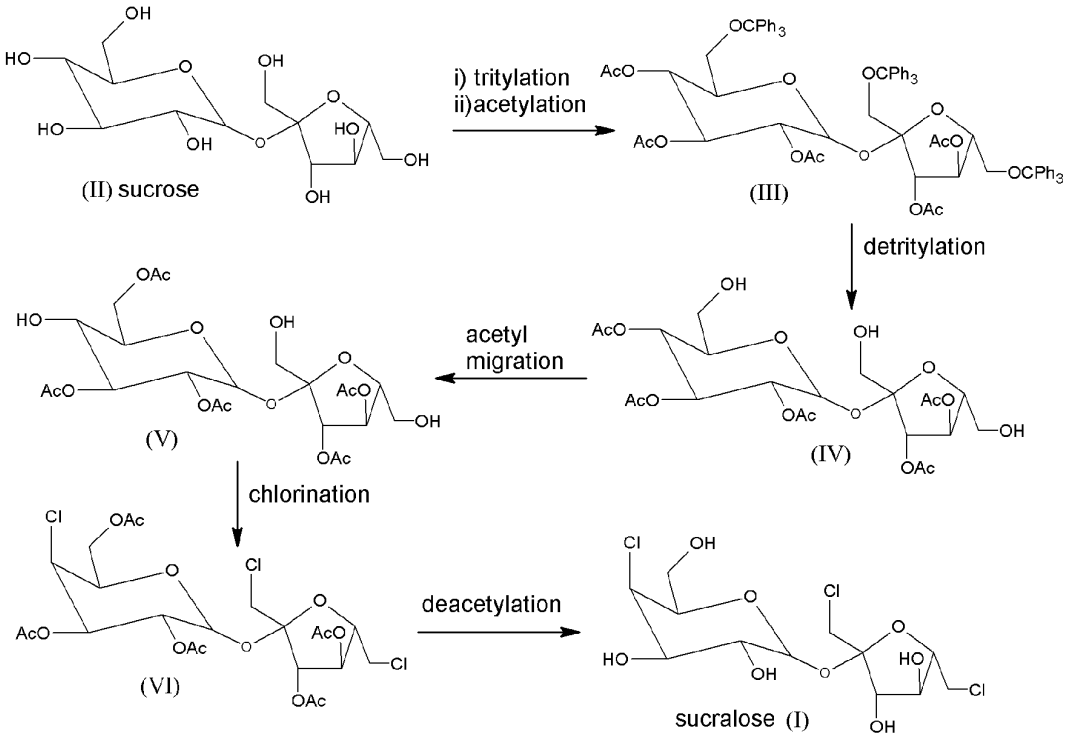


Figure 3. Synthetic process for selective chlorination of sucrose into sucralose

In the field of sucrochemistry, the discovery of an intensely sweet chloro sugar has initiated studies on chemical modification of sucrose by halogenation.[9] Sucralose is a synthetic trichlorinated disaccharide with the chemical name 1,6-dichloro-1,6-dideoxy-*β*-*D*-fructofuranosyl-4-chloro-4-deoxy-*α*-*D*-galactopyranoside. Sucralose is made from sucrose through a complex chemical process that involves chlorine and phosgene gas and

selectively substitutes three atoms of chlorine for three hydroxyl groups on sucrose molecule **figure 3**. This chlorination is achieved by selective protection of a primary alcohol group, followed by chlorination of the partially acetylated sugar with excess chlorinating agent, and then by removal of the acetyl groups to give the desired sucralose product.[11]

Splenda

Splenda is a commercial trademark of sucralose based artificial sweetener. FDA approved Splenda as sweetener in 1998 based on more than 110 animal and human safety studies.[10-11] The amount of sucralose can be consumed daily over a person's lifetime without any adverse effects is 9 mg/kg BW/day.[12] It has taste profile just like sugar without any unpleasant aftertaste.[13] It is also extremely heat stable 450 ˚F (232 ˚C) which makes it ideal to use in baking, aseptic processes and many other manufacturing processes that require elevated temperatures.[14] It is stable over broad range of acidic and alkaline conditions. It passes through the body unchanged and does not metabolize, so it has no calories. According to U.S FDA regulation food that contains less than 5 calories per amount serving is considered as zero calories.[15] Sucralose has long shelf life and is very stable through many processes and all physical states like solid or in liquid products. It possesses ingredient and process compatibility.[16] Additionally, sucralose has excellent solubility characteristic to use in food and beverages and compatible with food ingredients, preservative and flavors.[17] It works in favor of diabetic people because in body it does not recognize as sugar or carbohydrate. Thus, it does not affect carbohydrate metabolism,

glucose utilization and secretion of insulin. It does not support the growth of bacteria and does not promote tooth decay.[17]

Sucralose makes medication less effective thus limits the absorption of therapeutic drugs used for cancer and heart diseases ultimately making them less effective.[18] Some studies show that sucralose decomposes when baked and releases toxins called as chloroproanols.[19] It can alter insulin levels and blood sugar associated with inflammatory bowel disease and may alter the genes.[20] During the manufacturing it goes under process which involves complex chemicals. It means sucralose consumption can lead to ingestion of chemicals. Sucralose can cause GI problems, blurred vision, seizures, dizziness and migraine.[21] Data from humans and animals reflects sucralose as stable and lipophilic in nature. Sucralose being lighter molecule, distributed to essentially all tissues. About 15% of sucralose gets absorbed by GI tract.[22] It does not metabolize in the body therefore; it is non-caloric. 85% of consumed sucralose is excreted in the feces unchanged.[23]

Mechanism of Sweetness

Sucralose follows same mechanism as of sugar and is recognized by positive allosteric modulators of the human sweet taste receptor T1R. All compounds that produce a sweet taste bind to and activate the T1R2+T1R3 receptor.[24] Each T1R subunit is composed of 3 principal domains: an extracellular Venus-flytrap (VFT) domain at the N terminus, a seven transmembrane-spanning domain at the C terminus, and a cysteine-rich linker joining them.[25]

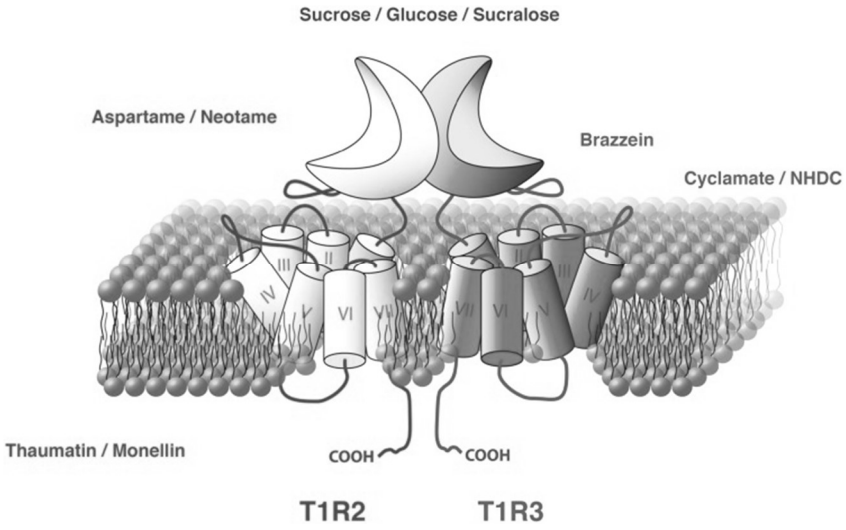


Figure 4. T1R2 and T1R3 with sucralose

Natural and artificial sugars (e.g., sucrose, glucose, and sucralose) bind to the VFT domains of both T1R2 and T1R3, whereas dipeptide sweeteners (e.g., aspartame and neotame) bind only to the T1R2 VFT domain. Each of these distinct sweetener-binding events leads to receptor activation; if they did not, there would be no accompanying perception of sweetness.[26]

Sweetness Determination

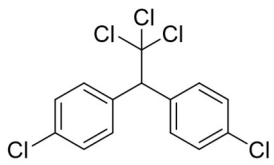
Scientists have taste panels to determine the sweetness. Highly trained researchers are provided with samples of water that have been artificially sweetened to varying degrees. Initially they are given plain water and then they taste samples with higher and higher concentrations until they start to taste different. When half of them can detect the change in taste it is called to be a threshold value of a compound. Scientists can measure

relative sweetness by comparing the threshold values for several types of sugar and sugar substitutes. Everyone has different sensitivities, but scientists estimate that the general population can detect a solution of about 0.5 percent sucrose—that's one teaspoon of table sugar dissolved into several cups of water. By comparison, one-six-hundredth of a teaspoon of sucralose would make the same impression on your taste buds.[27] Using trained panelists with proven sensitivity to artificial sweetener aftertastes, they generate sensory and temporal profiles that described and differentiated the aftertaste associated with each of the natural and artificial sweeteners.[27]

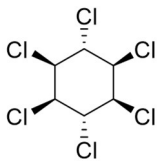
Sucralose - A Poisonous Chlorocarbon?

Sucralose is simply a chlorinated sugar.[28] Some studies show that sucralose was found when the researchers were looking for new types of insecticide. It contains chlorine, which is highly excitable, ferocious atomic element employed as biocide, disinfectants, insecticides in compound like DDT, Lindane, Chlordane. [29] So, when used with carbon, the chlorine atom in sucralose forms a covalent bond that results into organochlorine or simply: A Really Nasty Form of Chlorine (RNFOC). The RNFOC is lethal because it is fat soluble while rendering the natural defense mechanisms of the body helpless. Chlorocarbons are incompatible with normal human metabolic function.[28-29] Our mitochondrial and cellular enzyme systems are designed to completely utilize organic molecules containing carbon, hydrogen, oxygen, nitrogen and other compatible nutritional elements, but when chlorine is chemically reacted to carbon containing organic structure to make chlorocarbons, the chlorine and carbon atom bind to each other by mutually

sharing electrons in their outer shells. This arrangement adversely affects human metabolism.[29]



DDT



Lindane

Figure 5. Pesticides

By this process sucralose deliver chlorine directly into cells through normal metabolization and makes them effective as insecticides and preservatives which kills anything alive in the body to prevent bacterial decomposition.[29]

Effect on Gut Microbiome

The gut microbiome plays a key role in processes related to host health, such as food digestion and fermentation, immune cell development, and enteric nervous system regulation.[30] Sucralose consumption can alter the gut microbiota.[31] It reduces good gut bacteria by altering the quality and number of bacterial microbes in gut which may cause weight gain and obesity.[32] There are several studies which shows that sucralose containing products altered the rat gut microbiota and induce inflammatory lymphocyte infiltration.[33] Sucralose caused oral bacteria to proliferate less frequently being bacteriostatic which

prevents cavity formation. In gut it acts as bactericidal by inhibiting 50% of beneficial bacteria like streptococcus sorbinus, s. sanguis, s. challis, s. salivarius and actinomyces viscosus.[34] Scientists found that it can lead to obesity and diabetes because the sweeteners appear to change the population of intestinal bacteria that direct metabolism, the conversion of food to energy or stored fuel. They also analyzed a database of 381 men and women and found that those who used artificial sweeteners were more likely than others to be overweight.[35] Sucralose found to be particularly damaging to intestines since it increases pH level. It is considered as hepatotoxic and nephrotoxic. About 7% remains in your body after 5 days of consumption so continued consumption accumulates and finally leads to kidney damage.[36]

Hypothesis

Several studies indicate that the artificial sweeteners have biological activities and it is responsible to kill good gut bacteria. In our case Splenda®, a chloro -sugar sucralose, is the foundation of this project with the aim of modifying this commercially available chloro-sugar in such a way that the modified compound will kill oral microbes. Mouthwash are liquids required for oral health and hygiene. Most mouthwash preparation has fluorides to prevent cavities and periodontal disease.[37] Many mouthwashes brand these days have sucralose as an inactive ingredient. Here is the example of leading kid's mouthwash.

Active ingredients contain sodium fluoride, 0.02% and Inactive ingredients contains water, sorbitol solution, flavors, phosphoric acid, sucralose, cetylpyridinium chloride, disodium phosphate, FD&C yellow no. 6, FD&C blue no. 1, methanol

Mouthwash for children contains fluorides as active ingredient which is harmful if not delivered in right amount. Fluorides affects many tissues in body besides teeth and can cause osteosarcoma, bone fracture, genetic damage. For infants' fluoride provides more risk better than advantages and develops dental fluorosis and reduces IQ.[38] The functional groups in sucralose are hydroxyl group and chloride. Since substitution of an –OH group is relatively tough and is neither controlled nor selective due to the number of –OH groups in the molecule, substitution of halide is attempted in a way that it sustains anti-bacterial activity along with sweetening effect and flavoring agent, which can be then incorporate into mouthwash formulation and can replace the fluoride.

Chapter 2

SN2 Reaction for Sucralose Derivatives

We identified the three chlorine atoms as a potential reaction site. Chlorine−carbon is a relatively weak bond and can be either broken by dechlorinated by other substitution. In our project we intended the preparation of sucralose derivatives by replacing 3 hydroxyl group of sucralose with another nucleophilic group to improve the desired activity. Here we are providing the synthetic reactions and the results.

SN2 is a bimolecular kind of nucleophilic reaction in which one bond is broken and one bond is formed simultaneously without forming an intermediate.[39] SN2 reactions give inversion of stereochemistry at the reaction center which is at position 6 in case of sucralose.[39] Steric effects like steric bulk, steric hinderance affects the reaction.[40] SN2 is only possible when back of molecule is not completely cluttered by alkyl groups so that approaching nucleophile can attack the carbon atom. If the molecule is large and has many substituents it might not make the reaction happen.[40]

Nature of the SN2 reaction is that the nucleophile must attack from the side of the molecule opposite to the leaving group. This geometry of reaction is called back side attack.[41-42] In a back-side attack, as the nucleophile approaches the molecule from the side opposite to the leaving group, the other three bonds move away from the nucleophile and its attacking electrons. Eventually, these three bonds are all in the same plane as the carbon atom. As the bond to the leaving group breaks, these bonds retreat farther away from the nucleophile and its newly formed bond to carbon atom.[41] As a result of these geometric

changes, the stereochemical configuration of the molecule is inverted during an SN2 reaction to the opposite enantiomer. This stereochemical change is called inversion of configuration.[43]

Preparation of Sucralose Derivatives

We started the synthesis of sucralose derivatives by using sucralose as a precursor. This reaction depends on nucleophile, substrate, solvent and leaving group.[44] All reactions were performed in both protic and aprotic solvent solvents like methanol, ethanol, DMF, DMSO etc. for the comparison. For halo derivatives higher rates were observed in DMSO. Sometimes lipophilic quaternary salt or crown ethers were used to increase the rate of reaction. The various functional groups used were halogens, azide, dithiocarbonate, amines, nitro, phosphine, thiocyanate, etc.

The study began by using different conditions to determine which one is best suitable to produce stable molecule with high percentage yield. Sucralose upon reaction with nucleophile can give three possible derivatives. Where Nu^- = azide, dithiocarbamate, nitro, amines, halogens, phosphines, thiocyanates.

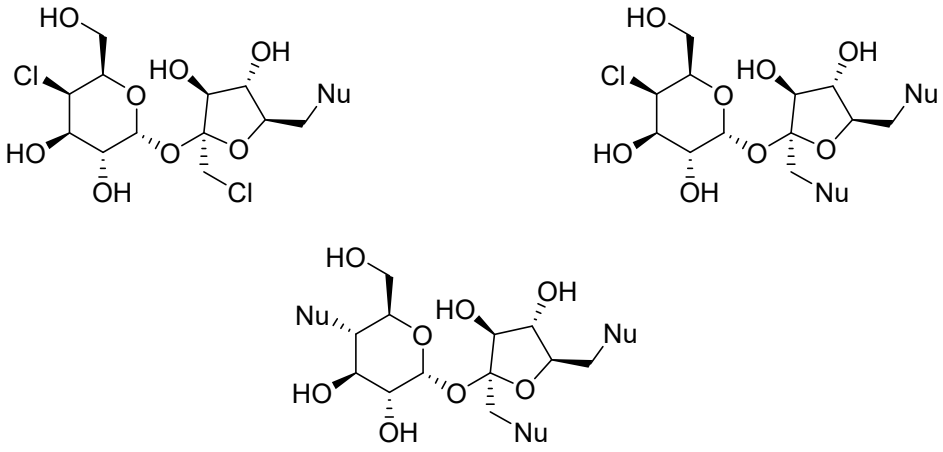


Figure 6. Possible structural derivatives from SN2 reaction

Preparation of Halogen Derivatives

In order to begin our experiments, the first step we started with was to react sucralose in a small-scale quantity with halogen elements to produce halogen derivatives(figure 6). The reaction began with 497 mg of sucralose dissolved in mixture of acetonitrile: water(10 ml), this reaction was run overnight at 80˚C. It was being monitored by thin layer chromatography (TLC, 80% ACN: DCM in saturated NaCl solution) for completion of the reaction. The experiments were set to run in different reaction conditions such as overnight and for one day by using various solvents, acetonitrile: water, acetonitrile and water at temperatures like 80˚C, 40˚C and RT. TLC showed formation of new compound. The resulting solution was vacuum evaporated to

remove ACN. This procedure was repeated one more time to remove any organic impurities. Sucralose being water soluble we assumed that the product would be water soluble as well. The aqueous phase was then freeze dried to obtain the product.

The end product was evident by TLC but for nuclear magnetic resonance (H^1NMR) no characteristic signal was found at specific δ corresponding to the proton. Also, for IR (Infrared spectroscopy) stretch of halogen functional group was not observed.

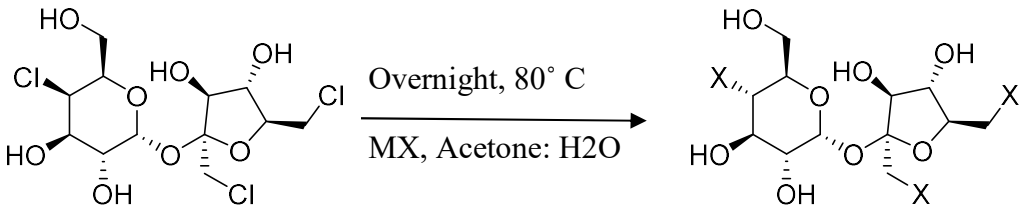
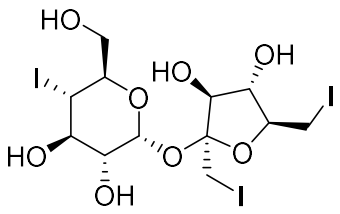
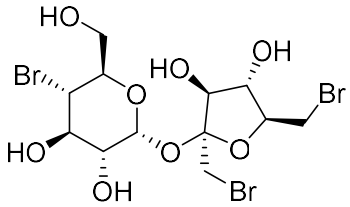
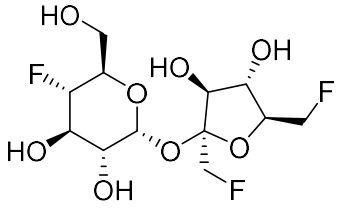


Figure 7. Preparation of halo derivatives

Table 1

Halogen derivative products with different reaction conditions.

Condition	Products
i. KI, Acetone: H2O, 80˚C, Overnight ii. NaI, Acetone: H2O, Reflux, 24h	
i. Acetonitrile, Overnight, RT	
ii. KF, Acetonitrile: H2O, 40°C, 18-20h iii. AgF, H_2O, RT, overnight	

Note. For all reactions: The starting material was Sucralose; 497 mg scale reactions; 1:6 eq of starting material to halogen; 10 mL of solvent at different temperatures

Replacement of Chlorine by Azide

Sodium azide has traditionally been used as the source of azide anion for the synthesis of alkyl azides.[45] Azido group react with variety of compounds. The synthesis using sodium azide is hazardous because of its explosive and toxic nature.[46] Azide ion displaces halide ion from a primary or secondary alkyl halide to give an alkyl azide. The reaction has been routinely used for the preparation of alkyl azides from the corresponding alkyl chlorides and NaN_3 or HN_3/pyridine by treatment with sodium azide at 60˚C.[47] With alkali metal azides it is usually helpful to use a polar solvent typically DMF or DMSO, although acetone or even alcohols have found some use to provide some homogeneity. While good results can generally be obtained when DMF or DMSO is used, there are difficulties associated with azide isolation from such solvents.[48]

The study began trialing different conditions to see which was able to work best for our components in order to produce the highest percent yield. Beginning with 1:3 ratio of starting material to sodium azide in water-acetone mixture. The reaction was running at room temperature for overnight. After making the observation for completion of reaction we decided to move with methanol as a solvent in place of water-acetone mixture under same condition. The reactions were not proceeding the way we expected so we increased the ratio of starting material to azide to 1:6. The conditions used were DMSO at 80˚C for 24 hours. Additionally, the reaction did not proceed in tetrahydrofuran or diethyl ether.

NaN_3 (1.98 mmol,0.12 gm) was stirred in 10 ml of DMF until it dissolved. To this solution sucralose (0.33mmol,0.2 gm) was added and the mixture was stirred until sucralose was dissolved and gave turbid solution. The reaction was stirred for 24 hrs. at

60˚C until all the starting material had been consumed which was analyzed by TLC for completion of reaction. The reaction was quenched with H2O (25mL). The final solution was freeze dried to obtain product. Treatment of sucralose with a slight excess of sodium azide(6 equivalent) in DMF at 60˚C gives sucralose azide in high yield. Azide derivative of sucralose was obtained from sucralose with NaN_3/DMF at 60˚C, did not reacted under different conditions.

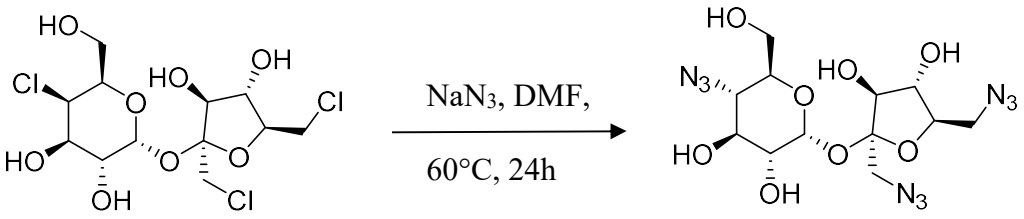


Figure 8. Preparation of azide derivative

Click Chemistry

Click chemistry refers to a group of reactions that are fast, simple to use, easy to purify, versatile, Regio specific, and give high product yields.[49] It is thermodynamically favored reaction which can lead to one specific product via cycloaddition reaction.[50] Click chemistry encompasses a group of powerful linking reactions that are simple to perform, have high yields, require no or minimal purification, and are versatile in joining diverse structures without the prerequisite of protection steps.[51] In Huisgen 1,3 dipolar cycloaddition the regioselectivity of reaction depends on electric and steric effects.[52] It typically does not require temperature elevation but can be performed over a wide range of

temperatures (0-160°C), in a variety of solvents (including water), and over a wide range of pH values (5 through 12).[51-52] In this azide reacts with terminal alkyne to give 1,2,3-triazole. Cu in water can be used to improve the reaction.[53]

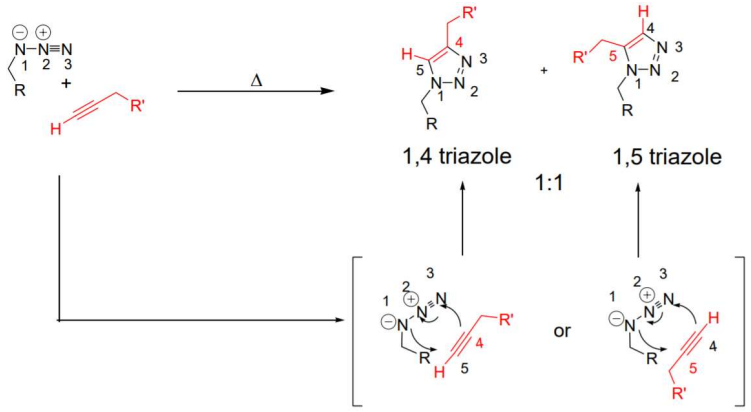


Figure 9. Mechanism of click chemistry

Since we have potentially prepared organic azide by reacting sodium azide and halogenated alkyl compound we thought of performing click chemistry. In this reaction the azide (1.98 mmol,0.12 gm) reacts with Phenylacetylene(5.94 mmol,0.6 g) in presence of diisopropylethylamine as a base and acetonitrile was used as solvents. TLC was used for confirmation of formation of new compound. The resulting solution was extracted with ethyl acetate(3 × 10 ml) and water. It was then dried over Na_2SO_4 and concentrated under vacuum to obtain final product.

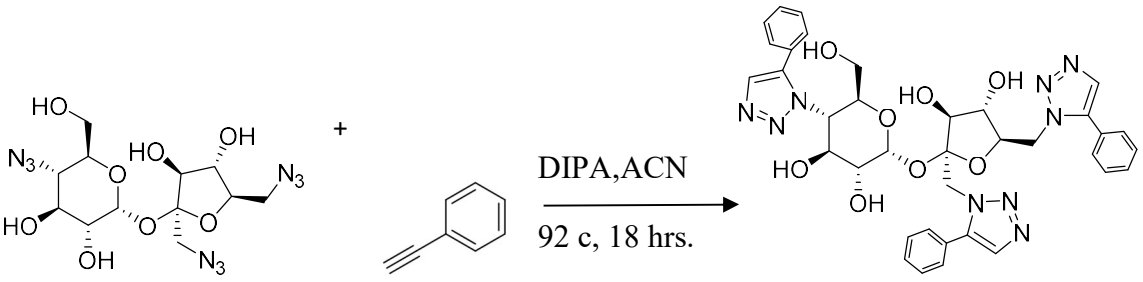


Figure 10. Click reaction of azide

Replace -Cl by Amine(N-Alkylation)

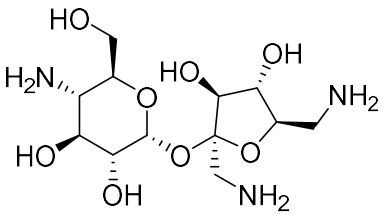
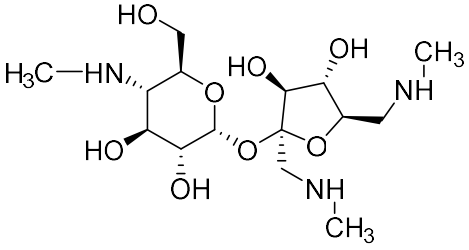
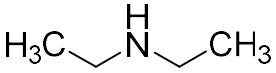
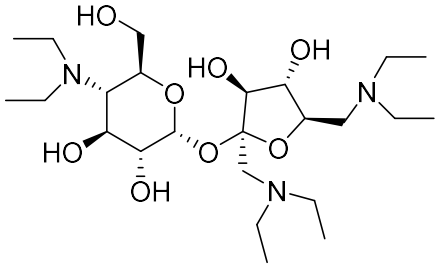
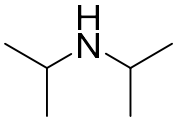
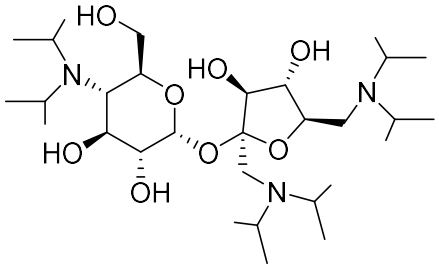
Amines have a unique biological property, because of that it is widely used in drug discoveries as Pharmacophores. Hence it holds significant role to a variety of diseases. Amine alkylation (amino-de-halogenation) is a type of organic reaction between an alkyl halide and ammonia or an amine.[54] The *N*-alkylation reaction(Hoffman reaction) is often unselective.[55] The reaction usually requires activated aryl halides, such as those with strong electron-withdrawing groups ortho or para to the halogen atom. Formation of tertiary amines *via N*-alkylation of amines by alkyl halides can also occur in aqueous media under mild conditions. This is useful method to form C–N bond without using any transition metal catalysts.[56] The exact amount of each product obtained depends on the precise reaction conditions and on the relative amounts of starting amine and alkyl halide.[57] It is impossible to get this reaction to just stop after one alkylation. So even if you add only one equivalent, you'll still get a mixture of product.[58]

We treated aryl halide which is sucralose (1.1 mol eq.) with excess of amine (1.5 mol eq.) at elevated temperature using polar aprotic solvents to get amine derivative of

sucralose. After completion of the reaction (monitored by TLC) the reaction mixture was obtained as yellow oil, which was evaporated to dryness under reduced pressure. The residue was dissolved in 10 mL of CH_2Cl_2 and washed with 10 mL of distilled water. The aqueous layer was washed with 3 x 10 mL fractions of CH_2Cl_2. The collected organic fractions were dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield the crude product.

Table 2

Amine derivatives.

Reactant	Solvent	Temperature	Product
NH_3	H_2O	RT, overnight	
H_2N-CH_3	Acetone	80˚C, reflux overnight	
	Ethanol	RT , overnight	
	ACN	60˚C,12 hrs.	

Note: For all reactions the starting material was Sucralose; 1.1:1:5 eq of starting material to amine; 10 mL of solvent at different temperatures

Menshutkin Reaction

The Menshutkin reaction converts a tertiary amine into a quaternary ammonium salt by reaction with an alkyl halide.[59] Though alkyl chlorides are poor alkylating agents, amines should be handled in chlorinated solvents such as dichloromethane and dichloroethane, especially at high temperatures, due to the possibility of a Menshutkin reaction.[59] Sucralose was reacted to triethylamine in 1:3 equivalent, in presence of dichloromethane as solvent at room temperature for overnight and after that refluxed at 40˚C for 48 hours. Similar reaction was tried with tertiary phosphines using sucralose as starting material. For this reactions Ion exchange column was used for separation of final product.

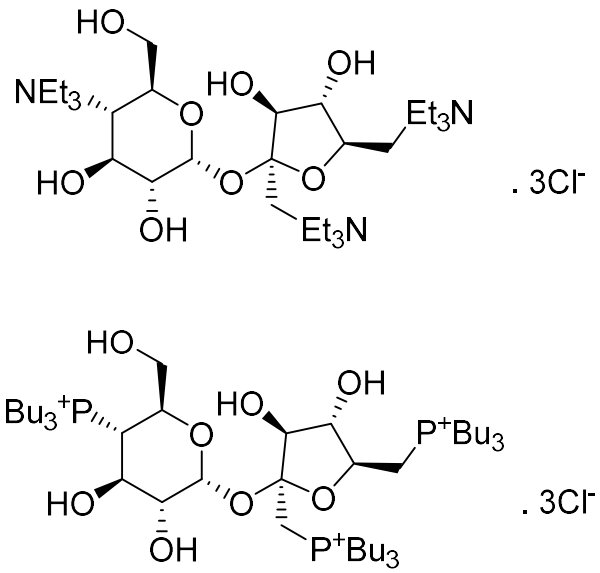


Figure 11. Quaternary derivatives of sucralose

Chapter 3

Reactions with Protected OH's

A protecting group is introduced into a molecule by chemical modification of a functional group to block its reactivity under the reaction condition required to make modification in molecule.[60] It increases the overall yield and efficiency of the synthesis. The presence of a large number of the same functional group at the carbohydrate skeletons, requires specific protecting group manipulations, which includes introduction of ester, ether, and acetal group. The protecting groups inhibit the participation of the substituted hydroxyl, or other functional groups in certain chemical transformations.[60]

During the designing of a synthesis strategy, consideration of economic factors is very important along with other characteristics. The required properties of an ideal protecting group includes, Readily available reagents are necessary for its introduction and removal; it should be readily characterized, its introduction is not accompanied by the formation of a new asymmetric center, but if this cannot be avoided, only one stereoisomer must be present; it should be stable in most of the chemical transformations; and it should be compatible with the work-up conditions, Efficient and selective removal. A further advantage is that the resulting protected compound is highly hydrophobic, thus making extractive work-ups easier, and if the product is crystalline, to offer convenient purification. Another advantage is that monitoring of the compound protected with the given group becomes easier.[61]

We started synthesis of sucralose Penta acetate as a precursor from sucralose as starting material. To a dry round-bottomed flask fitted with a reflux condenser and a drying tube is added 2.0 g (5.8 mmol) of sucralose, 1.0 g (12 mmol) of anhydrous sodium acetate and a boiling chip. Carefully add 10 mL (10.8 g, 106 mmol) of acetic anhydride and heated until the mixture just refluxes. Keep the reaction refluxing until all the reactants have dissolved (5-10 min). Then heat for an additional 5 min. Cooled and then pour the contents of the reaction flask into an Erlenmeyer flask that contains 50 g of ice and 50 mL of water. Use a glass stirring rod to stir the mixture for 5-10 min until the product collects as a thick syrup on the sides and bottom of the flask and the glass rod. Decanted the water from the syrup. Add 100 mL of distilled water to the flask and stir for 5 min so all the product is washed by the water. Decanted this water wash and repeated twice using an additional 100 mL of distilled water each time. Carefully decant the final wash water from the product and crystallized to obtain final product. Added 10 mL of 95% ethanol to the flask. Heat this on a steam bath until the product dissolves. Stopper the flask and let it stand overnight at room temperature (20 C). The crystals are collected by suction filtration, washed with 5 mL of cold 95% ethanol and air dried.

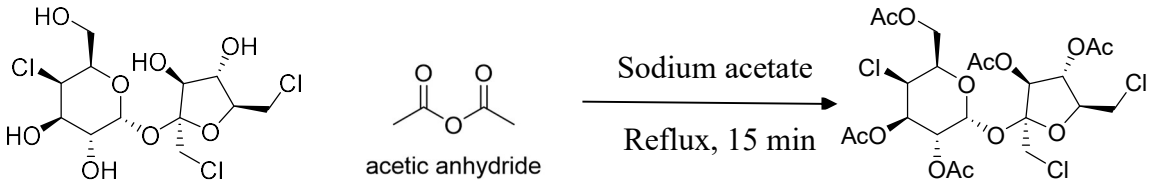


Figure 12. Preparation of sucralose penta acetate

The five Hydroxyl groups on the sucralose are protected by converting the –OH to $–OCOCH_3$, which might minimize the interference due to the reactivity of hydroxyl group. This change in structure made the purification process much simpler since this compound is less miscible in H_2O and better soluble in organic solvents. Further reactions were performed using sucralose Penta acetate as starting material.

Result: The product has the following properties: FT-IR (CHC13), 2946, 1744,1369, 1222,1074, and 1043 cm-' and MP = 89˚C[62]

Reactions with Protected OH's

Heterocyclic amines are innovative approach of developing antifungal drugs.[63] Some examples include nitroimidazole, midazolam and many other drugs. These are highly polar compounds, so we tried to replace chlorine with amines and generated library of these compound that can potentially act like antibacterial agent.

Sucralose Penta- acetate (0.2 g, 0.33 mmol) was reacted to secondary amines in 1:3 equivalent in DMF, in presence of K2CO3 as a base at 60˚C for different times depending on completion of reaction which was monitored by TLC. The resultant solution was extracted in dimethyl chloride and water mixture(1:1). The water layer was washed with 10 ml of DCM. The organic layer was vacuum evaporated to obtain final product.

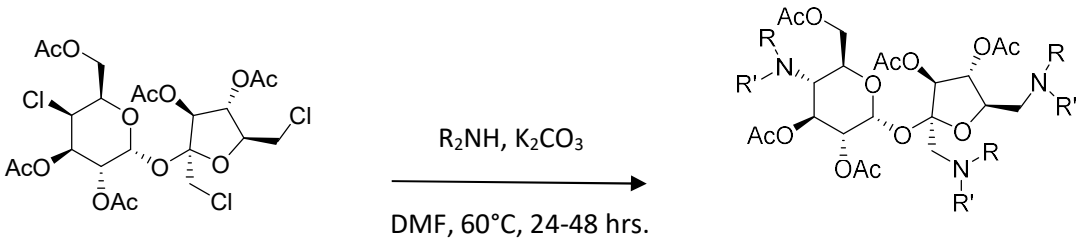


Figure 13. Reaction with protected OH's

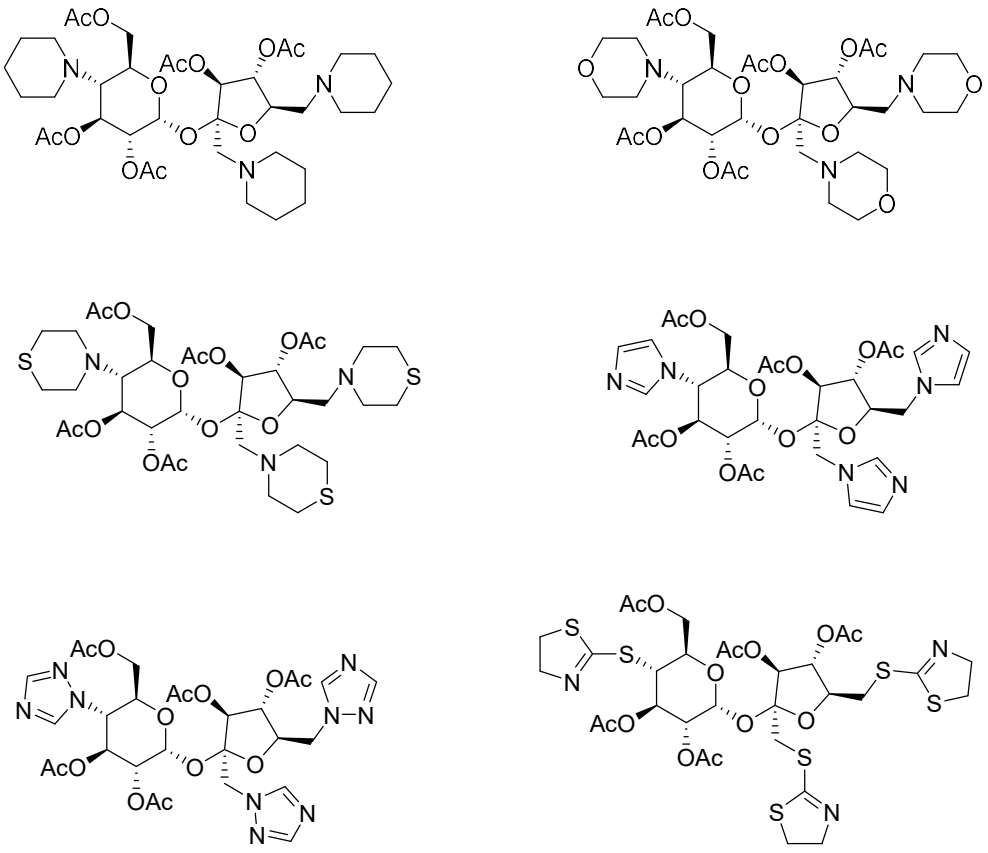


Figure 14. Sucralose penta acetate derivative

Experimental NMR

Sucralose- ^{1}H NMR: δ 3.32 (1H, dd, *J* = 10.2, 2.6 Hz), 3.39-3.49 (2H, 3.44 (d, *J* = 9.1 Hz), 3.44 (dd, *J* = 9.2, 9.1 Hz)), 3.70-3.84 (4H, 3.79 (td, *J* = 6.0, 2.7 Hz), 3.74 (dd, *J* = 10.2, 3.5 Hz), 3.83 (d, *J* = 6.0 Hz), 3.83 (d, *J* = 6.0 Hz)), 4.13-4.14 (2H, 4.14 (s), 4.14 (s)), 4.16-4.18 (2H, 4.17 (d, *J* = 3.3 Hz), 4.17 (d, *J* = 3.3 Hz)), 4.22-4.33 (2H, 4.26 (dt, *J* = 9.2, 3.3 Hz), 4.31 (dd, *J* = 3.5, 2.7 Hz)), 4.63 (1H, d, *J* = 2.6 Hz).

Sucralose Penta acetate-^{1}H NMR: δ 2.05-2.07 (12H, 2.06 (s), 2.06 (s), 2.06 (s), 2.06 (s)), 2.06 (3H, s), 4.14-4.21 (5H, 4.14 (s), 4.17 (td, *J* = 3.3, 2.7 Hz), 4.20 (d, *J* = 3.3 Hz), 4.20 (d, *J* = 3.3 Hz), 4.14 (s)), 4.39 (1H, dd, *J* = 3.5, 2.7 Hz), 4.41-4.44 (2H, 4.42 (d, *J* = 3.3 Hz), 4.42 (d, *J* = 3.3 Hz)), 4.58 (1H, dt, *J* = 9.3, 3.3 Hz), 4.79 (1H, d, *J* = 2.6 Hz), 5.02-5.15 (3H, 5.11 (dd, *J* = 9.3, 7.4 Hz), 5.05 (d, *J* = 7.4 Hz), 5.05 (dd, *J* = 10.2, 2.6 Hz)), 5.21 (1H, dd, *J* = 10.2, 3.5 Hz).

Chapter 4

Separation Techniques

Ion Exchange Column

This technique exchanges ions between two electrolytes, cation which exchange positively charged ions and anion which exchange negatively charged ions using an electrolyte solution as eluent. In most cases it is used in the processes of purification, separation, and decontamination of aqueous and other ion-containing solutions.[64] There are also amphoteric exchangers that can exchange both cations and anions simultaneously.

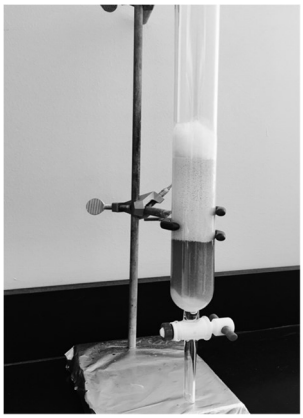


Figure 15. Ion exchange column

Ion exchanges can be unselective or have binding preferences for certain ions or classes of ions, depending on their chemical structure. Ion exchange is a reversible

process, and the ion exchanger can be regenerated or loaded with desirable ions by washing with an excess of these ions.[64]

$$Dowex^- + NH_4^+ OH^- \longrightarrow Dowex^- NH_4^+ + OH^-$$

Dowex® is negatively charged cation exchange resin which exchange positively charged ions. It was soaked in 0.1 M NH_4OH solution before adding it to column Solution containing product was added to resin which now contains NH_4^+ ion. NH_4^+ is replaced by product which is positively charged(figure 13). NH_4^+ and other impurities will not be able to adhere to resin and it will wash out. 10% acetone-water buffer solution was added as eluent to elute the Product.

Extraction

Extraction is a separation process consisting separation of a substance from a matrix. It includes Liquid-liquid extraction, and Solid phase extraction.[65] Here we have used liquid-liquid extraction to separate compound based on their solubility using aqueous and organic solvents. We have used ethyl acetate as organic phase and water phase to separate a solute from one phase from the other. A separatory funnel was used for this process. The organic solvent used for extraction must meet a few criteria. It should readily dissolve substance to be extracted and do not react with the substance to be extracted. It must be immiscible in water which is the usual second solvent for extraction. Low boiling point helps to easily remove from the product. Common extraction solvents are diethyl ether and methylene chloride.[66]

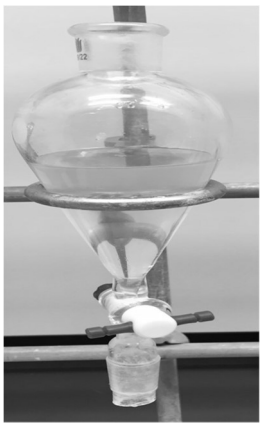


Figure 16. Liquid-liquid extraction

For reactions with sucralose Penta acetate this technique was used. As our starting material is soluble in organic solvent, we extracted it with dimethyl chloride and water mixture. Then the organic layer was vacuum evaporated to get desired product.

Lyophilization

Freeze-drying works by freezing the material and then reducing the surrounding pressure to allow the frozen water in the material to sublimate directly from the solid phase to the gas phase. Freeze-drying causes less damage to the substance than other dehydration methods using higher temperatures.[67] There are some advantages of this method. It processes a liquid with ease and thereby simplifies aseptic handling. Enhances the stability of a dry powder as well as the product stability in a dry state and removes water without having to heat the product excessively.[68]

Figure 17. Freeze dryer

Freezing takes place in stage one of the lyophilization process. It can take place in the freeze dryer. Freezing temperatures are around -40°C. There is no thawing in the second stage the product goes from frozen state to dry powder through the process of sublimation. Freeze-drying works by freezing the liquid material and then reducing the surrounding pressure to allow the frozen water in the material to sublimate directly from the solid phase to the gas phase and that gives a dry powder. Depending on the type of product and quantity, it can take 12-72 hours to go through all these stages.[68]

The reaction in which sucralose was used as starting material we chose this technique. As our starting material is water soluble so we assumed that the product will be water soluble as well. For our compound instead of going into organic layer it was dissolved in mixture of DMF and water, so the extract was lyophilized to get the product. Freezing was done by freeze drying a compound in centrifuge tube using liquid nitrogen. The freezing point for water is 0°C where as DMF freezes at -61°C. To produce larger

crystals, the product should be frozen slowly. Initial drying phase is slow because too much heat can alter the substance. During that 95% of water from mixture is sublimated. The product mixture must be maintained to prevent melt-back otherwise it collapses during primary freeze drying. Freeze-drying also causes less damage to the substance.

Conclusion

Sucralose modification was attempted by several functional group. Compounds were analyzed using different analytical techniques. No compound was confirmed as potential molecule to be considered as anti-bacterial. Based on these less than desirable results, a series of reaction conditions were attempted in order to facilitate the desirable reaction. A variety of functional groups were screened by using different solvents including methylene chloride, acetonitrile, DMF, Acetone and biphasic systems. Unfortunately, all the reaction conditions examined led to the formation of various byproducts, including the unwanted quaternary ammonium salt. Eventually, after additional repeated attempts our persistence finally led to a conclusion that sucralose is very difficult to modify due to its structural properties.

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