ORIGINAL ARTICLE

New Vitamin E Analogues

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Abstract

Lipid peroxidation is the mediator of several pathophysiological events such atherosclerosis, neurodegenerative disease and others. It is induced by reactive oxygen species that react with biological substrates, leading to cell damage. It is thought that Nicotinamide Adenine Dinucleotide Phosphate Hydrogen oxidases, as well as mitochondria dysfunction and other sources, are at the centre of these events, so it becomes an important therapeutic target. In order to retard this damage and the progression of the disease, the natural and synthetic antioxidant vitamin E (Tocopherol) has been studied extensively. In this study, we briefly address current knowledge on the function of vitamin E and try to emphasize its antioxidant properties versus its other properties. The purpose of this study is to design and synthesize a new vitamin E analogue that is placed outside cells. The precursor to a new vitamin E analogue bearing two charges is prepared from the reaction of the corresponding (6acetoxy-2,5,7,8-tetramethylchroman-2-yl) acyl chloride compound that was directly treated with an aniline-2,5-disulfonic acid tetrabutylammonium salt. The latter, a newly prepared compound, is considered a target. The new tocopherol analogue of the product was expected to exhibit protection of lipid membrane from the oxidative damage behavior of reactive oxygen species.

Keywords

Vitamin E; Tocopherol; Antioxidant

Introduction

When the body breathes in air, the unfunctionalized or damaged cells inside the body react with the oxygen from the air and produce highly reactive molecules known as free radicals. A free radical is defined as a molecule containing unpaired electrons that is generally more reactive than a molecule with paired electrons. These free radicals, or reactive oxygen species (ROS), including superoxides ($O_2^{\bullet-}$) and hydroxyl radicals (OH), are unstable species that can react with a wide range of biomolecules as does non-radical H_2O_2[1,2]. Reactive oxygen species is known as a metabolic product of the respiratory chain, and under normal conditions, these metabolic products are eliminated by enzymatic antioxidant defenses as well as nonenzymatic ones. Firstly, enzymatic mechanisms take place to prevent the modification of lipids, proteins and nucleic acids by oxidation, which is induced by free radicals. The enzymes used are superoxide dismutase (SOD) enzyme, which can convert the superoxide radical ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2), and catalase and glutathione peroxidase enzymes, which metabolize hydrogen peroxide into water and oxygen. When these enzymes fail to eliminate ROS, oxidative cell injury will be observed. For non-enzymatic mechanisms, small molecules such as vitamin E can

neutralize ROS and prevent oxidative damage[3]. Oxidative damage is the result of a redox imbalance between the production of ROS and the defense effects of biological antioxidant molecules (reviewed by Gutteridge and Mitchell)[4]. Reactive oxygen species may damage cellular macromolecules such as DNA and RNA and induce apoptosis, which is programmed cell death. These deleterious effects are more likely to be observed in the context of many life-threatening human diseases such as cancer, neurodegenerative disease, atherosclerosis, aging and sepsis. When low or normal levels of damage are observed, ROS acts as a signaling molecule to the repair system, which replaces the damaged biomolecules, including protein, lipids, and DNA, thus contributing to many physiological processes[2]. The oxidative stress involved in numerous diseases has attracted the attention of scientists and the general public to the role of antioxidants in maintaining human health. Our expectation is that antioxidants help in lowering the incidence of these diseases and maintaining a healthy quality of life[5]. There are two main sources of ROS formation: mitochondria and endothelial cells. Mitochondria are the powerhouses of the cell, which generate the ATP of the cells and control calcium ion concentration. Smith *et al.*[6] developed an effective strategy to deliver bioactive molecules in the development of mitochondria-specific therapies[6,7].

The second and major source of ROS is Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase. Researchers have tried to generate a small molecule that inhibits NADPH oxidase and protects against oxidative stress. Several drugs currently used in clinical practice have been shown to decrease NADPH oxidase function.

There are two ways of eliminating free radicals: through the administration of an antioxidant that enhances the activity of endogenous antioxidants, or through the inhibition of enzymes that generate ROS[8]. In order to counteract oxidative stress, the body produces antioxidants to destroy free radicals[2]. Antioxidants are defined as a substance present at a low concentration that significantly delays or inhibits oxidation of substrate, including proteins, enzymes or other small molecules[5]. Therefore, diet-derived antioxidants such as vitamin E, ascorbate, flavonoids and carotenoids are essential. These antioxidants naturally occur in diets rich in fruits, vegetables, and grains, which are loaded with antioxidants that protect the plant against oxidative stress[2]. Evans and Bishop discovered vitamin E in 1922[9]. In the 1950s, vitamin E was found to inhibit lipid peroxidation and other radical oxidative events[10].

Vitamin E consists of a chromanol ring, which acts as lipophilic antioxidant, and a polyprenyl side chain, which acts as a membrane structure-stabilizing agent. The heterocyclic chromanol ring is responsible for the compound's antioxidant activity, allowing the hydroxyl group at the chromanol ring to bind to lipophilic free radicals. In fact, the chroman ring is considered the core of the substructure of potential drug candidates[11].

The human body prefers the α-tocopherol form when the chiral center is placed at position 2, 4′ and 8′ in the *R* configuration (2*R*, 4′*R*, 8′*R*-α-tocopherol or d-α-tocopherol)[9,10]. The structure activity relationship of this is that phenolic hydrogen present on α-tocopherol at the surface of the membrane or lipoprotein is considered an active site, whereas the long phytyl side chain restricts the mobility of α-tocopherol within the membrane and in the plasma lipoproteins as shown in Figure 1[5,12].

Many forms of vitamin E are oxidized by cytochrome P450 and then conjugated with either sulfate or glucuronide to be excreted in the urine or bile[9]. The most effective form of vitamin E is α-tocopherol. The α-tocopherol transfer protein (α-TTP) is responsible for maintaining plasma α-tocopherol levels. Hence, impaired activity in the gene responsible for α-TTP leads to severe vitamin E deficiency in a human being. Several symptoms are associated with vitamin E deficiency, such as ataxia with vitamin E deficiency, a rare condition resulting from the mutation of α-TTP, which impairs the body's ability to use vitamin E. This is associated with peripheral neuropathy, a progressive deterioration of the nerves, and hemolytic anemia: hemolysis of red blood cells before their lifespan is over. These conditions lead cells to oxidative stress or apoptosis.

Vitamin E has nonantioxidant roles beyond its antioxidant function. It modulates kinase activity by inhibiting protein kinase C, the key signaling molecule in the process of cell proliferation and differentiation, and regulates cytosolic phospholipase A_2, which reduces peroxylipid levels. It also enhances vasodilator release and inhibits platelet aggregation[9,10]. Therefore, it represents an alternative to the warfarin drug[13]. Additionally, it should be noted that the effect of vitamin E in heart disease is not related to its antioxidant properties but rather to its interference with vitamin

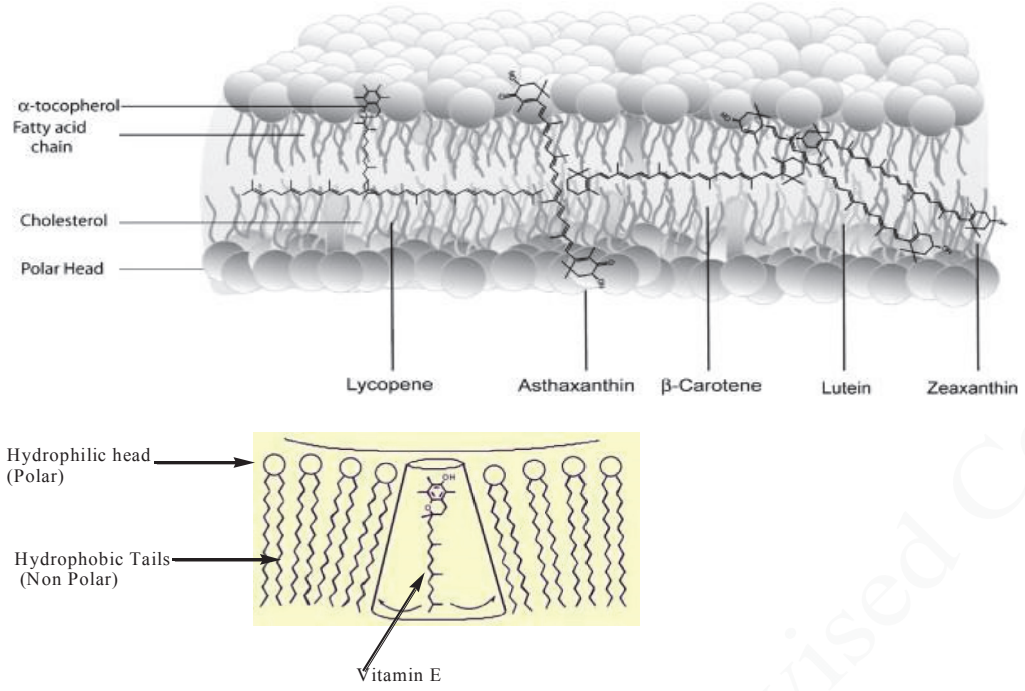


Figure 1. Schematic of α-tocopherol or vitamin E inside the phospholipid bilayer membrane

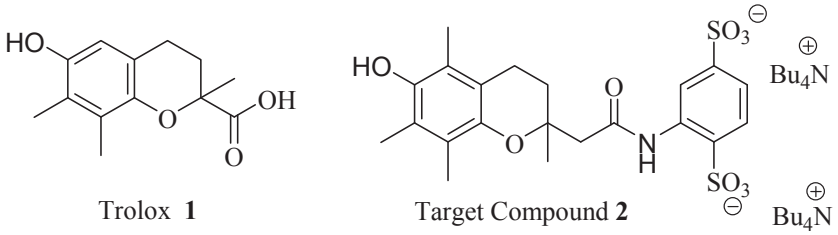


Figure. The structure of the target carboxamide 2, which is expected to be synthesized, is related to the structure of Trolox 1.

K (phyloquinine) metabolism, which can prevent thrombosis or clot formation, which in turn is linked to stroke and heart attack[14]. Evidence today proves that antioxidants act not only as radical scavengers but also as cellular signaling messengers that regulate the level of antioxidants[5].

Measuring Intra- and Extracellular Levels of ROS

In order to measure the extracellular levels of ROS, the cytochrome C assay has been used while the hydroethidine HE/ethidium ET fluorescent and dichlorofluorescein (DCF) probes have been used to detect and assess oxidative stress in mitochondria or intracellular ROS[15]. Although these methods for imaging intracellular and extracellular ROS lack sufficient resolution, they are still in use[16].

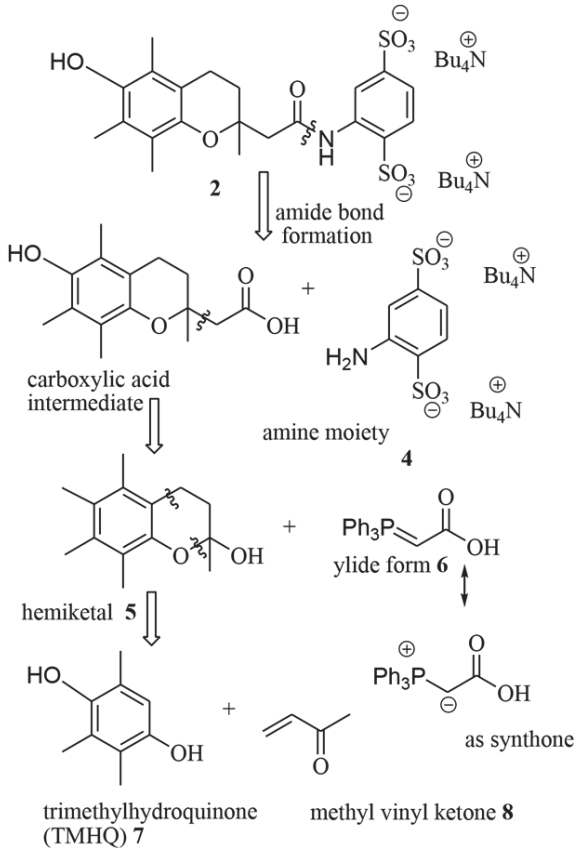
Materials and Methods

Understanding the detailed mechanisms of oxidative stress, particularly NADPH oxidase as a source of ROS could lead to success in designing an antioxidant molecule. In connection with the current project, the objective of this study is to develop a new vitamin E analogue with a chroman nucleus based on a structure similar to Trolox 1 but bearing an extra carbon (Figure 2). Trolox is commonly used as a standard in antioxidant assays and an adjuvant in certain cancer therapies.

The active compound is expected to show more potent inhibition of lipid peroxidation than Trolox, assuming that bis (sulfonate) 2 could be used as a control or reference in antioxidant assays, similar to Trolox[1]. The target compound acts in the extracellular matrix as a result of the charged head group, which provides highly water-soluble compounds so that extracellular ROS in both healthy and diseased people can easily be studied. In this research, the goal is to develop optimum synthetic methods for synthesizing the new vitamin E analogue from a commercially available source Trimethylhydroquinone (TMHQ) using a coupling method different from the conventional method.

Results

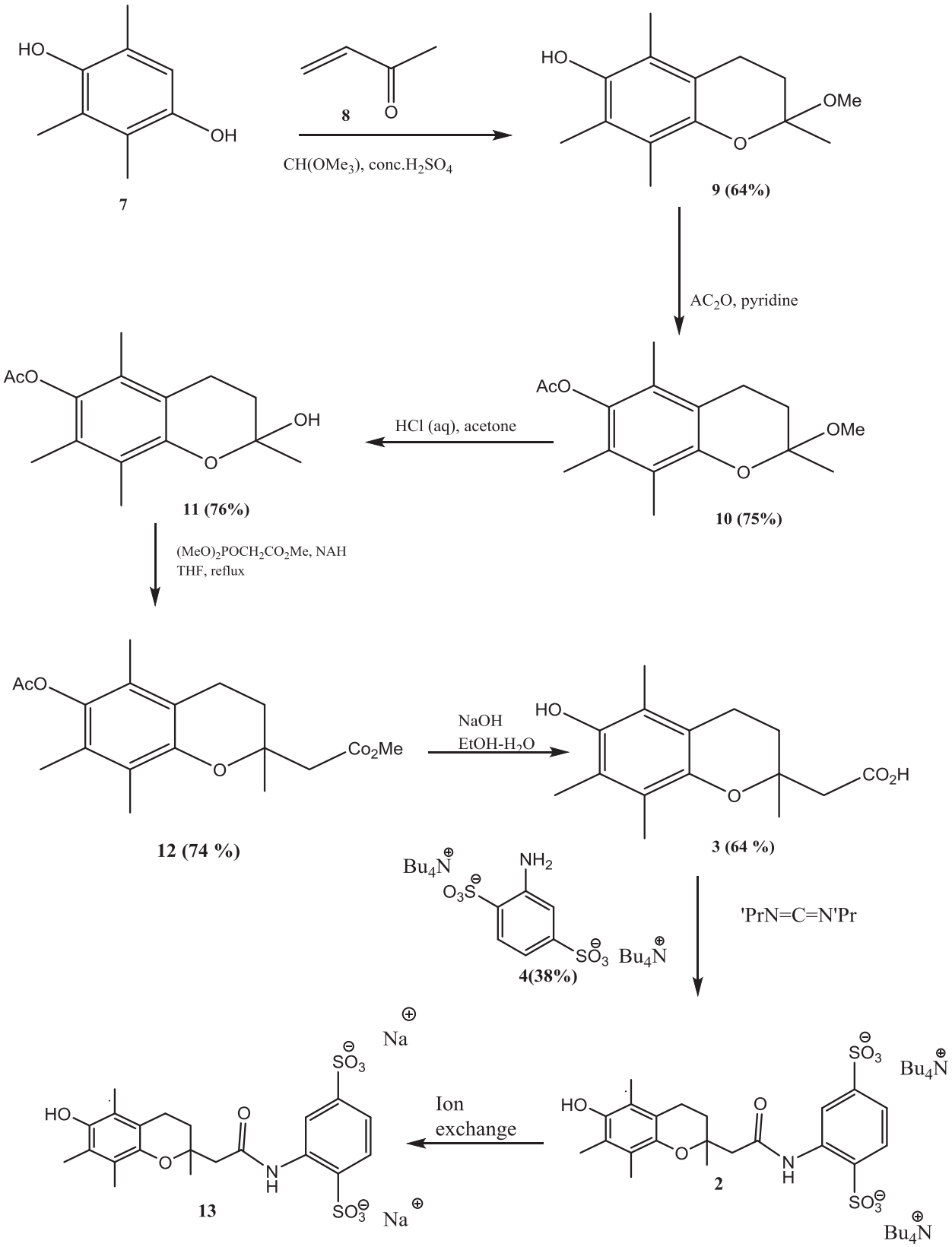
In retrosynthetic analysis (Scheme 1) of the target, the cleavage of the amide bond in the target compound (2) leads to carboxylic acid (3) and amine moiety (4). Further disassembly of carboxylic acid (3) intermediate gives hemiketal (5) and ylide (6). The hemiketal (5), in turn, can be prepared from TMHQ (7) and methyl vinyl ketone (8).



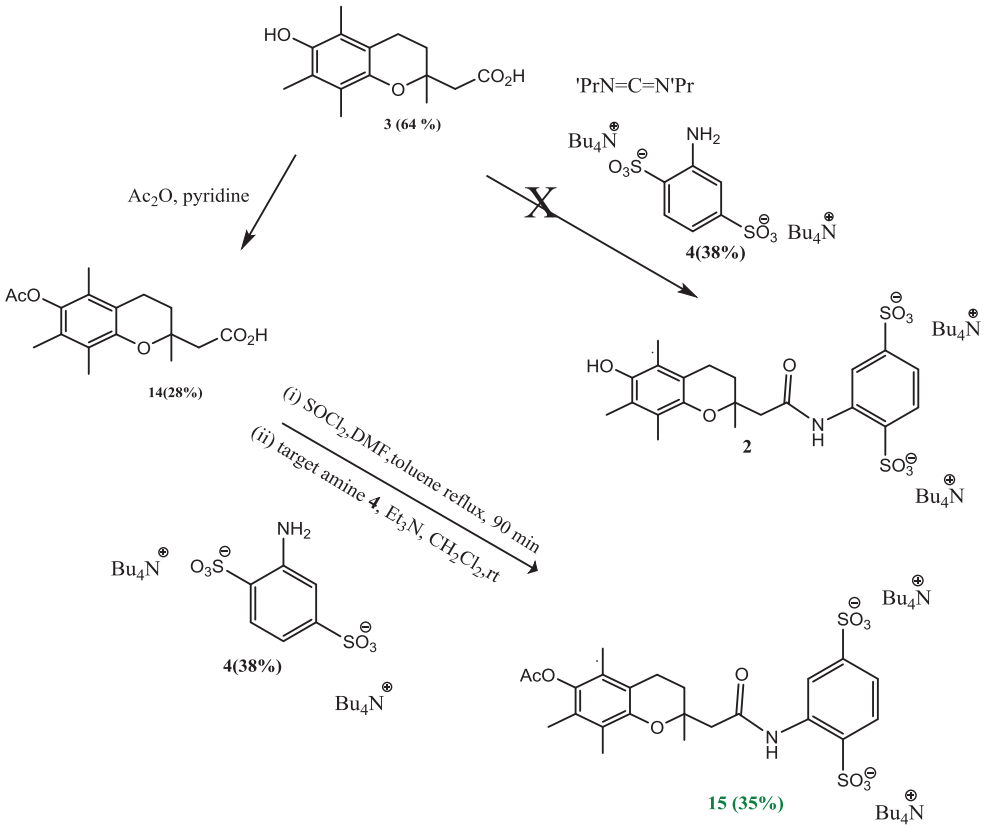
Scheme 1. Retrosynthetic analysis of the target compound 2.

In the forward direction, a classical, simple and economical approach for synthesizing alpha-tocopherol is illustrated in Scheme 2. The synthesis of chromans nucleus involved the acid-catalyzed reaction starting with TMHQ (7), where the phenol compound is dissolved in methanol in the presence of dehydrating agents such as Trimethyl orthoformate. Then, let it react with protonated methyl vinyl ketone (8) in the presence of acidic conditions such as sulfuric acid yields acetal racemic-2-methoxy2,5,7,8-tetramethyl-chroman-6-ol (9). Then, any conventional method of esterification or etherification can be utilized to protect the free hydroxyl group in compound 9. The preferred methods of acetylation is to allow compound 9 to react with a reactive derivative of the acetyl functional group of an organic acid, such as acid anhydrides. This often occurs in the presence of a base such as pyridine as catalyst, so the acetylating of alcohol involves the replacement of the hydroxyl group with the acetyl group and yields a specific ester compound (10). Then, the method of acid hydrolysis was obtained through treatment of acetal 10 with hydrochloric acid to hydrolyze the ether group to form the corresponding hydroxyl group hemiketal (11). The latter is subjected to the Horner-Wadsworth-Emmons (HWE) reaction. Hemiketal (11) was added to a solution of sodium hydride as an alkali metal strong base in dry tetrahydrofuran organic solvent, and 2.3 equivalent of the trimethyl phosphonoacetate were prepared in order to form anions of trimethyl phosphonoacetate. Hemiketal (11) was added to the anion form of trimethyl phosphonoacetate, and the reaction was carried out at 0°C to the reflux temperature of the solvent and at atmospheric pressure to give ester (12). This is followed by hydrolysis of the ester-protecting group to yield antioxidant (6-hydroxy-chroman-2-yl) acetic acid (3), which is an intermediate in the preparation of alpha-tocopherol[17,18]. The next step is an attempt to synthesize an amide target (2). Then, 2 is subjected to ion exchange chromatography to afford 13.

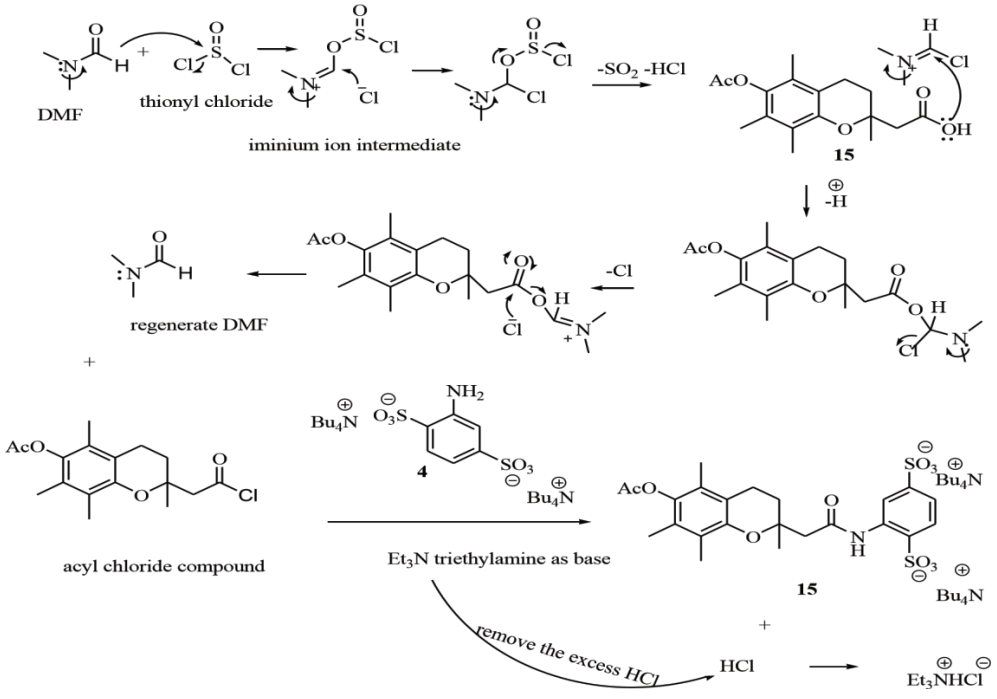
The process depends on charge interactions between substrate 2 and the immobilized charge in the resin of choice. Choosing cation exchange chromatography where a positive charge ion on substrate 2 binds to the negative charge ion on the resin. Although the ion exchange process was not performed in the lab due to the short duration of the summer project, the process is expected to be run in



Scheme 2. Simple and economical approach for synthesizing alpha-tocopherol.



Scheme 3. Making final product 15 in a lab.



Scheme 4. Proposed mechanism of the target compound 15 (step 7).

future work. In terms of making amide according to the literature, Zhu *et al.*[15] successfully synthesized amide linkage upon a carboxylic acid used as prodrug carrier and an amine compound used as a drug-like molecule in the presence of 1,3dicyclohexylcarbodiimide or (N,N'-dicyclohexylcarbodiimide) DCC as a coupling agent placed in dimethylformamide (DMF). DCC is widely used in organic synthesis, peptide synthesis and protein, and excess is used ensuring completion of the reaction. It can be replaced with 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDAC-HCl), although this compound is less effective compared with DCC/DMF. EDAC-HCl can be removed in the aqueous phase.

This study provides a macromolecular prodrug delivery system of amide susceptible to enzymatic hydrolysis. In another example of amide formation, Ali *et al.*[19] employ a convenient coupling method by reaction of carboxylic acid group of an acylated amino acid using a DCC coupling agent in the presence of 1-hydroxy-benzotriazol (HOBT) as an additive to reduce racemization. This method creates many amide compounds that are expected to have anti-HIV-1 activity. Unfortunately, none of them showed selectivity toward anti-HIV-1 activity. Extensive efforts were made in the laboratory to form an amide using coupling agent DCC and 4-dimethylaminopyridine (DMAP), as base catalysis failed. The high polarity of the amine compound used in this study may cause the method of coupling to not work.

So this was not an efficient method of synthesising amide functional group. Firstly, protect the free hydroxyl groups in the compound 3 at the position C-6 and leave the carboxylic acid group free. The protection is achieved through introducing an acyl functional group by treatment of 3 with acid anhydride in presence of pyridine, as previously mentioned. Then, make acyl chloride the reactive derivative of carboxylic acids by replacing the free hydroxyl group with chloride through exposure 14 to thionyl chloride and a few drops of DMF, as catalysts accelerate the reaction. Then, the mixture is directly reacted with aniline-2, 5-disulfonic acid tetrabutylammonium salt 4 target amine compound to obtain the final product 15. The available DCC coupling agent was used instead of DIC (step 6) in laboratory. The proposed mechanism of the final product is shown in Schemes 3 and 4.

Conclusion

Vitamin E is widely recognized as one of the most powerful antioxidants currently available. It inhibits the formation of ROS-induced lipid peroxyl radicals, thereby protecting cells from lipid peroxidation. Increasing levels of lipid oxidation in membranes and organelles combined with decreasing levels of antioxidant defense mechanisms or overproduction of free radicals require the antioxidant properties of vitamin E to reverse[5]. Potential therapeutic agents of antioxidant substances and the pathogenic factors of ROS have recently received increased research attention. Oxidative stress results from an imbalance between the metabolic products of the respiratory chain (ROS) and antioxidant molecules. Oxidative stress plays a significant role in aging, cancer, cardiovascular and neurodegenerative disease. Thus, antioxidants must be carefully designed with a strong understanding of how ROS are involved in physiological and pathological processes[20]. In this study, the precursor to a new vitamin E analogue bis (sulfonate) bearing two negative charges has been prepared in 7 synthetic steps and will be tested in the future.

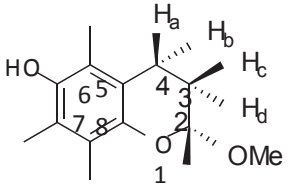
Experimental Section

General technical chemicals used in this study were of analytical grade and were obtained from Sigma Aldrich and Lancaster Synthesis, England Company of Chemical Synthesis. These commercial reagents and materials were used as received. All solvents were of reagent grade, and dry solvents were purified using a Pure Solv 500 MD solvent purification system (multiple dispensing system). To determine the purity of a substance, thin-layer chromatography (TLC) was used to separate the mixtures. TLC was generally used to monitor the progress of reactions and was carried out using silica gel 60 F_{254} pre-coated aluminum sheets from EM Science. TLC was run for these plates, with 2:1 petroleum ether-ethyl acetate as a mixture of polar and nonpolar solvents, unless otherwise noted. Spots were detected with UV light (254 nm). This procedure allows a suitable solvent to be chosen for purification *via* column chromatography. Column chromatography is one of the most commonly used purification methods. In this technique, a solvent is run through a stationary phase (SiO_2 column), and crude reaction mixtures are loaded onto the column, which is created

by tightly packing a slurry of Fisher matrix silica 60 into a glass chromatographic column, ensuring that no air bubbles are present. The compound is then eluted with an appropriate solvent system or mobile phase as determined *via* TLC. Fractions were then collected in several test tubes and identified based on the previous TLC conditions. Nuclear magnetic resonance (NMR) spectroscopy was used to elucidate the structures of any organic compounds involved in the experiment. Chemical proton shifts and ^{13}C NMR chemical shifts are reported in ppm relative to residual $CHCl_3$ (δ=7.26). The spectra were recorded on a Bruker DPX 400 spectrometer (400 MHz and 125 MHz for (^1H and ^{13}C NMR, respectively). The samples were dissolved in chloroform D (CDCl3), and chemical shifts were obtained in ppm with respect to (CDCl3), except for one sample, which was dissolved in DMSO-d_6 instead. This procedure allowed purity to be assessed following column chromatography or recrystallization. In addition, infrared spectra were recorded on Shimadzu IR, Fourier Transform FtTIR-8400S to determine functional groups in molecules by measuring the amount of radiation absorbed by compounds within the infrared region of the electromagnetic spectrum from 4000 to 600 cm^{-1} frequency. Mass spectrometer was also used. Samples were first subjected to a beam of electrons with sufficient energize to ionize them. Thus the sample ionized into cations and anions were separated according to their mass and charges, with the separation then measured. Each bar in mass spectrum represents specific mass to charge ratio of an ion and the length of the bar represents the abundance of the ion[21].

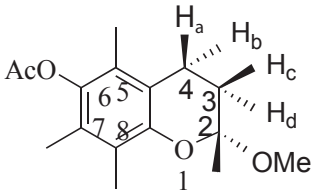
Procedure

Preparation of the compound 9: 2-methoxy-2, 5, 7, 8-tetramethylchroman-6-ol.



TMHQ 7 (20.0 g, 131 mmol, 1 eq.) was dissolved in methanol (100 mL), and trimethyl orthoformate (43.2 mL, 394 mmol, 3 eq.) was added to the solution, which was then stirred under an argon atmosphere. Methyl vinyl ketone 8 (21.2 mL, 262 mmol, 2 eq.) was added slowly; this was followed by the addition of concentrated sulphuric acid (3.24 mL) to the stirred solution. The reaction mixture was stirred under an argon atmosphere for 24 hours at room temperature. Then, the methanol solvent was evaporated under reduced pressure, the reaction mixture was quenched with water (100 mL) and the product extracted with ethyl acetate (EtOAc) (3 x 60 mL). The combined EtOAc extracts were washed with brine (3 x 60 mL) and dried over sodium sulfate (Na_2SO_4). Then filtered and concentrated under reduced pressure to yield a pale yellow, amorphous solid (20 g, 64%) that was sufficiently pure to continue. One g of crude product was recrystallized from methanol to yield acetal 9 as a yellow amorphous solid (300 mg, 19 %). ^1H NMR (400 MHz, $CDCl_3$) δ= 4.24 (1 H, s, OH), 3.20 (3H, s, OCH_3), 2.74 (1H, ddd, J= 16.4, 12.5 and 6.8 Hz, H_a of C-4), 2.55 (1H, ddd, J = 16.5 and 6.7 and 1.9 Hz, H_b of C-4H-4b), 2.16 (2H, m, CH_3Ar) 2.12-2.08 (7H, m, 2x CH_3Ar + H_c of C-3), 1.80 (1H, ddd, J= 13.4, 12.6 and 6.6 Hz, H_d of C-3), 1.53 (3H, s, CH_3C2); ^{13}C NMR (125 MHz, $CDCl_3$): δ= 145.40, 143.76, 122.27, 120.82, 118.57, 118.42, 97.21, 48.82, 31.95, 23.12, 20.00, 12.15, 11.60, 11.22 ppm. The ^1H and ^{13}C NMR data agree with the results published in the literature[22].

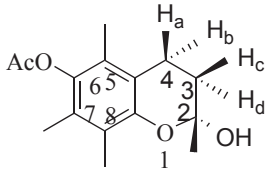
Preparation of compound 10: 6-acetoxy-2-methoxy-2, 5, 7, 8-tetramethylchroman.



Acetal 9 (20.0 g, 87.7 mmol, 1 eq.) was dissolved in pyridine (27 mL) and acetic anhydride (40 mL, 105 mmol, 5 eq.). The reaction mixture was stirred under an argon atmosphere for 18 h at room temperature. Then the reaction mixture was poured into 400 mL of ice water and transferred to a separating funnel. The product was extracted with DCM (300 mL). The combined organic layer was washed with brine (2 x 50 mL); the organic solution was dried over Na_2SO_3, filtered and concentrated under reduced pressure to yield (17.8 g, 75 %) an orange oily solid of product acetate 10, which was used crude in the next reaction. 1 g of crude product 10 was purified by column chromatography [SiO_2, petroleum ether: EtOAc (2; 1)]

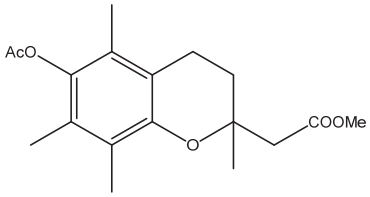
to yield 164 mg (12%) of material for characterization. R_f [SiO_2, petroleum ether: EtOAc (2; 1)]: 0.34. ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.22 (3H, s, OCH_3), 2.72 (1H, ddd, J= 16.4, 12.8 and 6.8 Hz, H_a of C-4), 2.53 (1H, ddd, J= 16.4, 11.6 and 2.4 Hz, , H_b of C-4), 2.33 (3H, s, H_3CCO_2), 2.14 (3H, s, CH_3Ar), 2.09 (1H, ddd, J=12.8, 6.8 and 2.4 Hz, Hc of C-3), 2.03 (3H, s, CH_3Ar), 1.98 (3H, s, CH_3Ar), 1.80 (1H, ddd, J= 13.2, 12.8 and 6.4 Hz, H_d of C-3), 1.54 (3H,s, CH_3C2); ^{13}C NMR (125 MHz, $CDCl_3$): δ=169.64, 147.69, 141.41, 126.67, 124.96, 122.70, 118.80, 97.50, 48.92, 31.64, 23.13, 20.56, 19.86, 12.93, 12.05, 11.66 ppm. The ^{1}H and ^{13}C NMR data agree with the findings reported in the literature.[23]

Preparation of compound 11:2-hydroxy-6-acetoxy-2, 5, 7, 8-tetramethylchroman.



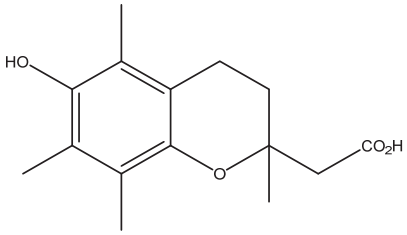
Acetate 10 (14.5 g, 61.3, 1 eq.) was dissolved in a mixture of acetone (73 mL) and (44 mL) of water followed by the addition of 12.3 mL of conc. HCl dropwise. The reaction mixture was stirred at room temperature for 30 min. Then the solvent was evaporated under reduced pressure until the solid was formed then filtered using a Buchner Funnel and suction flask connected with vacuum and washed with water to yield hemiketal 11 as a yellow amorphous solid (12.4 g, 76%) sufficiently pure for the next step. Then 148 mg of the crude product was recrystallized from acetone and dried in rotavapor vacuum to yield 26 mg (13%) of pure product, which was characterized.^{1}H NMR (400 MHz, $CDCl_3$)δ= 2.75-2.71 (1H, m, H_a of C-4), 2.62 (1H, ddd, J= 16.4, 6.4 and 3.2 Hz, H_b of C-4), 2.32 (3H, s, CH_3COO), 2.15 (3H, s, CH_3Ar), 2.09 (3H, s, CH_3Ar), 2.07-2.04 (1H, m, H_c of C-3), 2.01 (1H, s, OH), 1.98 (3H, s, CH_3Ar), 1.84-1.76 (1H, m, H_d of C-3), 1.60 (3H, s, CH_3C2). The ^{1}H NMR data agree with the literature. [23]

Preparation of compound 12: Methyl 6-acetoxy-2, 5, 7, 8-tetramethylchroman-2- acetate



To a stirred suspension of NaH (2.60 g, 108 mmol, 2.2 eq.) in dry THF (100 mL) under an argon atmosphere (11.2 mL, 69.5 mmol, 2.3 eq.) trimethyl phosphonoacetate was added slowly dropwise while placed in (0°C). After addition of trimethyl phosphonoacetate an ice bath was removed and the reaction mixture was stirred for 30 min at RT. Then hemiketal 11 (8.00 g, 30.3 mmol, 1 eq.) was added to the mixture after dissolved in 60 mL of dry THF. The reaction was stirred over 15 h at RT under an argon atmosphere. After that the reaction mixture was stirred, heated at reflux for 4 h. The reaction mixture was cooled to ambient temperature, after which 250 mL of water was added to it. Extraction with diethyl ether (3 x 40 mL) followed. The combined extracts were washed with brine (3 x 40 mL), and the organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give ester 12 (7.12 g, 74% crude yield) as brown oil that was used for the next step. Selected data taken from spectrum provided evidence of the compound; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.65 (3H, s, OCH_3), 2.29 (3H, s, H_3CCO_2), 2.06 (3H, s, CH_3Ar), 2.00 (3H, s, CH_3Ar) 1.96 (3H, s, CH_3Ar), 1.40 (3H, s, CH_3C2).

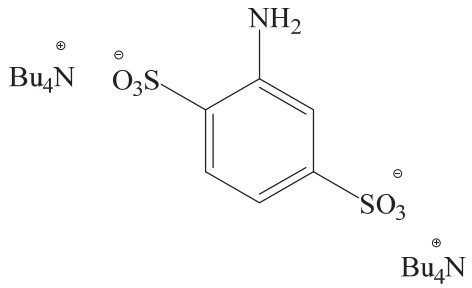
Preparation of compound 3: 2-(6- hydroxy-2, 5, 7, 8-tetramethylchroman-2- yl) acetic acid



The crude of ester 12 (6.00 g, 18.8 mmol, 1 eq) was dissolved in EtOH (93 mL) and stirred at RT. NaOH base (10.8 g, 267 mmol, 14 eq.) was dissolved in 93 mL of water and was added dropwise to the stirred mixture. The reaction was stirred for 4 h at RT. The reaction mixture was washed with (240 mL) hot hexane to 50 ^{0}C). Then the mixture was diluted with 684 mL ice-water and acidified by addition of (82.7 mL) of conc. HCl dropwise. Thus formed the solid was removed by a Buchner Funnel and suction flask connected with vacuum and washed with with H_2O and EtOH to yield carboxylic acid 3 intermediate as amorphous solid (3.20 g, 64%) sufficiently pure for the next step. ^{1}H NMR (400 MHz, DMSO-d_6) δ=12.14 (1H, s, COOH), 7.42 (1H, s, OH), 2.47-2.42 (6H, m, CH_2COOH and CH_2 of C-4 and CH_2 of C-3), 2.06 (3H, s, CH_3Ar), 2.03 (3H, s, CH_3Ar), 1.95

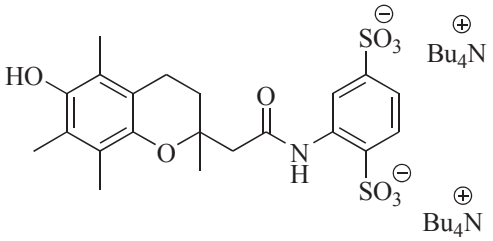
(3H, s, CH_3Ar), 1.34 (3H, s, CH_3C-2). ^{13}C NMR (125 MHz, $DMSOd_6$) δ= 171.47, 145.43, 144.02 , 122.66, 121.19, 120.22 , 116.49 , 72.76 , 56.02, 24.34, 20.06, 18.45, 12.61, 11.66, 11.59 ppm. The ^{13}C NMR data agrees with the literature.[23]

Preparation of target 4: aniline-2, 5-disulfonic acid tetrabutylammonium salt.



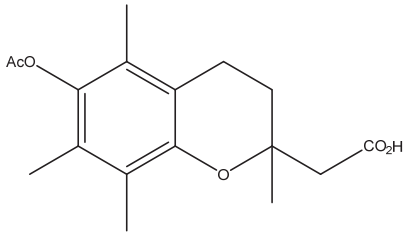
Deionized water (30 mL) was added to (2.00 g, 7.27 mmol) of the aniline-2, 5disulfonic acid monosodium salt from TCI Belgium Company. The mixture was stirred at room temperature for 5 min. A solution of tetrabutylammonium hydroxide (9.5 mL, 14.5 mmol) from Aldrich company in water (30 mL) was added dropwise, and the mixture was stirred under argon at room temperature for 1 h. At the end of the counter-ion exchange, DCM extracts were made and dried with sodium sulphate (Na_2SO_4), filtered and concentrated under reduced pressure to yield a yellow solid (2.05 g, 38 %). ^1H NMR (400 MHz, $CDCl_3$) δ=7.73 (1H, d, J=8 Hz, CH-Ar), 7.15 (1 H, s, CH-Ar), 7.12 (1H, d, J=8 Hz, CH-Ar), 5.03 (2H, s, NH_2), 3.07-3.02 (16 H, m, Bu_4N^+), 1.55-1.31 (32 H, m, Bu_4N^+), 0.97-0.93 (24 H, m, Bu_4N^+). ^{13}C NMR (125 MHz, $CDCl_3$): δ=149.30, 143.81, 131.14, 127.76, 114.17, 114.11, 58.54, 23.85, 19.73, 13.68 ppm. Although this compound is not known in the literature, the characterization of results matches the estimated ^1H ChemNMR programme. Melting point (Mp): 59-60°C. Infrared Spectrophotometer of primary amines shows N-H stretching vibrations in 3500 to 3300 cm^{-1} region. Intramolecular or intermolecular hydrogen bonding broadens the absorption and lowers the frequency. The intensities of O-H bands are stronger than N-H bands. So far the N-H bending as well as C-N stretching absorptions are not strong when corresponding to the alcohol bands. Also the primary amine NH_2 functional group give additional broad band at 900 cm^{-1} to 700 cm^{-1} region caused by out of plane bending[21].

Making target amide 2:



To the stirred solution of carboxylic acid 3 (200 mg, 0.757 mmol, 1 eq) in 4 mL of dry DCM, DCC (187 mg, 0.908 mmol, 1.2 eq) and DMAP (92.2 mg, 0.756 mmol, 1 eq) are added. The resulting brown solution is stirred for 10 min and the amine (557 mg, 1 eq) is dissolved in 1 mL of DCM and transferred to the reaction above. The reaction mixture is stirred at room temperature overnight under argon atmosphere, then diluted with DCM (20 mL), washed with water (2 x 25 mL) and the water layer evaporated in vacuo while the organic layer washed with 25 mL of brine and dried over $MgSO_4$ then evaporated to dryness, to give a yellowish solid, which was not the desired compound.

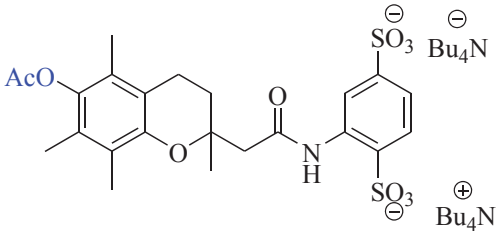
Preparation of compound 14:2-(6-acetoxy-2, 5, 7, 8-tetramethylchroman-2-yl) acetic acid.



A mixture of carboxylic acid 3 (1.00 g, 3.78 mmol) in 4 mL of each of pyridine and acetic anhydride was stirred at 25°C under argon for 20 hours. Then stripped of solvent at 40°C to give an orange brown oil. To a suspension of this material in 7.5 mL of H_2O and 1.5 mL of tetrahydrofuran (THF) was added 2.50 g of $NaHCO_3$. The suspension was stirred at RT for 4 hours. Then the water layer washed with diethyl ether, acidified with 2N-aqueous HCl and extracted with ether. The ether solutions were washed with saturated brine, dried over sodium sulfate and stripped of solvent to give (329 mg, 28%) as an orangish resin. This crude is sufficiently

pure for the next step referred to Scott *et al.*[22] ^{1}H NMR (400 MHz, $CDCl_3$) δ=10.25 (1H, broad, OH), 2.63 (2H, dd, J= 4.49 and 2.74 Hz, CH_2COOH), 2.58-2.79 (2H, m, CH_2 of C-4), 2.30 (3H, s, CH_3COO), 2.07 (3H, s, CH_3Ar), 2.05-2.03 (1H, m, H_a of C-3), 2.01 (3H, s, CH_3Ar), 1.99 (3H, s, CH_3Ar), 1.97-1.87 (1H, m, H_b of C-3), 1.44 (3H, s, CH_3C-2). ^{13}C NMR (125 MHz, $CDCl_3$) δ= 175.95, 169.59, 148.54, 141.40, 127.23 , 125.18, 123.39, 117.11, 73.73, 44.13, 31.01, 24.70, 20.49, 20.45, 12.91, 12.05,11.75, as given in (appendices 1 and 2). IR spectrum as given in (appendix 3 (soso-15)) is dominated by the intense broad absorption from 3500 to 2500 cm^{-1} which corresponds to the O-H stretch of the carboxyl group. Strong peak in the 1700 cm^{-1} region corresponding to the C=O. In this compound the strong C-H stretching absorptions are seen at 2931 and 2677 cm^{-1}. According to (appendix 4) mass spectrum is given m/z: 264.1, 100% and thus give the molecular ion 306.1 (32 %) which is similar to the molecular weight (MW) 306.3587 of the compound. The procedure is described by Scott et al.[21] and the ^{1}H and ^{13}C NMR data correspond with the findings reported in the literature, although they were synthesized through a different route[24].

Preparation of 15: tetrabutylammonium 2-(2-(6-acetoxy-2, 5, 7, 8-tetramethylchroman-2-yl) acetamido) benzene, 1,4-disulfonate.



To a stirred solution of acetate 14 (125 mg, 0.392 mmol) in dry toluene (1.2 mL), thionyl chloride (0.05 mL, 0.666 mmol) and 2 to 3 drops of dry DMF were added. The mixture was heated by refluxing for 90 min and then cooled to room temperature. Excess solvent and thionyl chloride were evaporated in vacuo and the residue was dissolved in dry dichloromethane (DCM) (1 mL). A solution of amine 4 (aniline-2,5disulfonic acid tetrabutylammonium salt) (480 mg, 0283 mmol) and triethylamine (0.3 mL) in 1 mL of DCM were then added drop-wise to the mixture. The reaction mixture was stirred at room temperature overnight until the disappearance of the reactant among check TLC plate. The mixture was washed first with 10% sodium bicarbonate solution and then with water. The organic layer was dried with sodium sulfate and the solvent was evaporated in vacuo until formation of a brown oil (106 mg, 35%). This procedure was taken from López *et al.*[25] The product was purified by column chromatographic elution with hexane-ethyl acetate (4:1) to remove the starting materials and then by elution with EtOAc-MeOH (4:1) to give amide 15 (36 mg, 11%) as a yellow solid. The ^{1}H NMR peaks of this pure compound are as follows: (400 MHz, $CDCl_3$) δ= 10.49 (1H, broad, CONH), 8.71 (1 H, s, CH-Ar), 7.85 (1H, d, J= 8 Hz, CH-Ar), 7.52 (1H, d, J= 8 Hz, CH-Ar), 3.02 (3H, s, OCH_3), 2.6-2.0 (4H, m,CH_2C-3 and CH_2C-4) 2.322.25 (16 H, m, Bu_4N^+), 2.00 (3H, s, CH_3Ar), 1.90 (3H, s, CH_3Ar), 1.89 (3H, s, CH_3Ar), 1.45 (3H, s, CH_3C-2), 1.38-1.26 (32 H, m, Bu_4N^+), 0.88-0.85 (24 H, m, Bu_4N^+) as shown in appendix 5.

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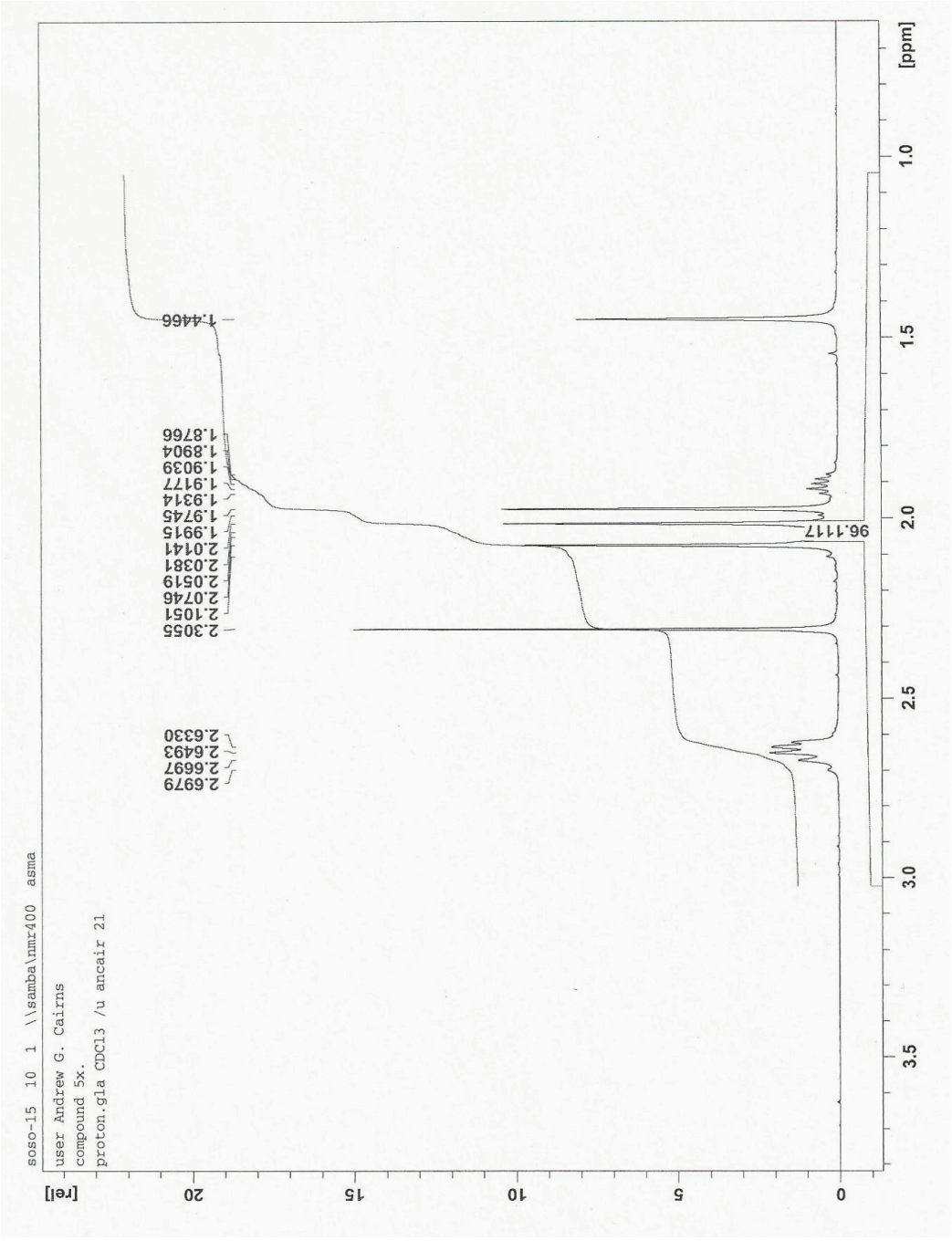
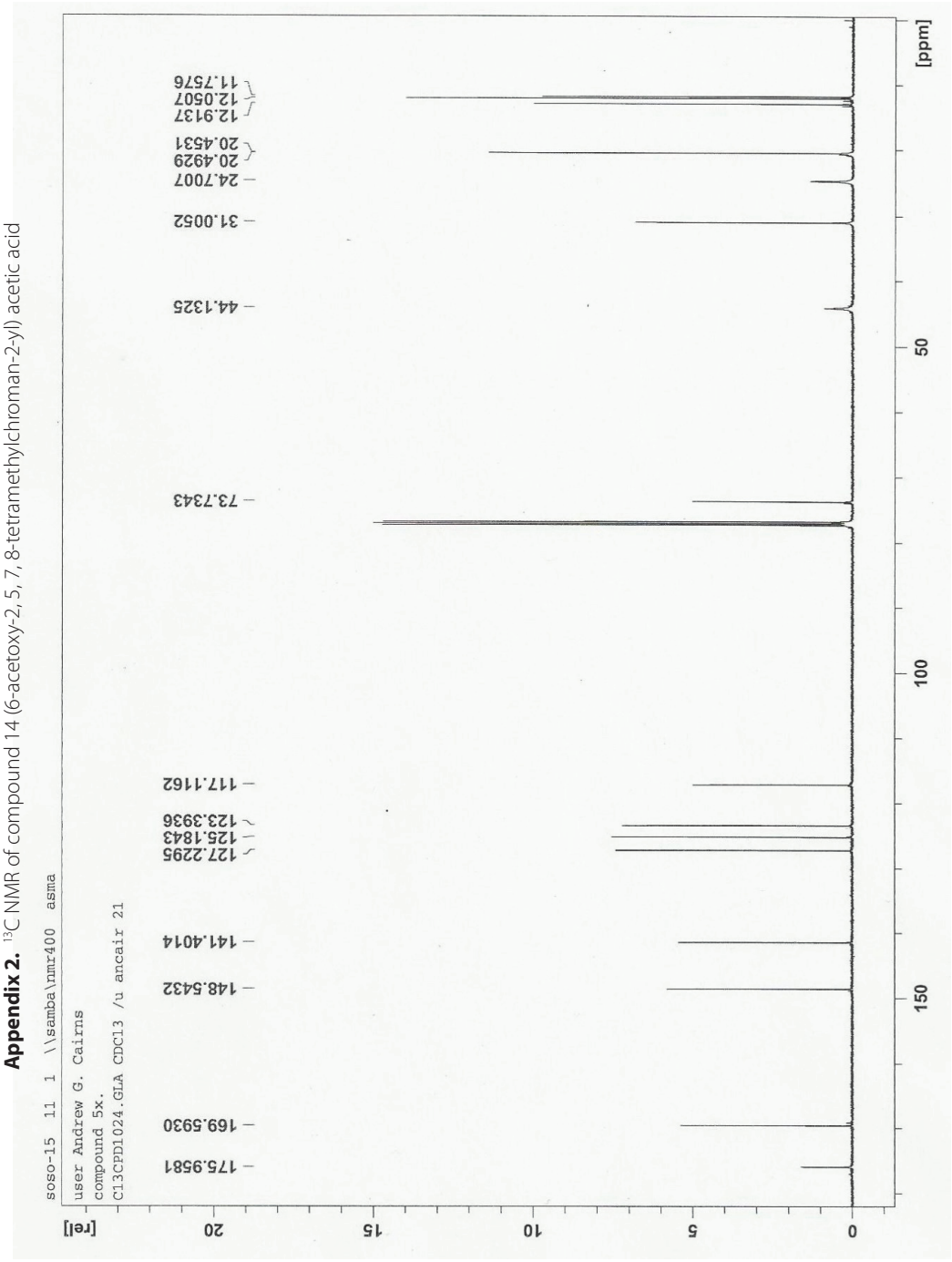
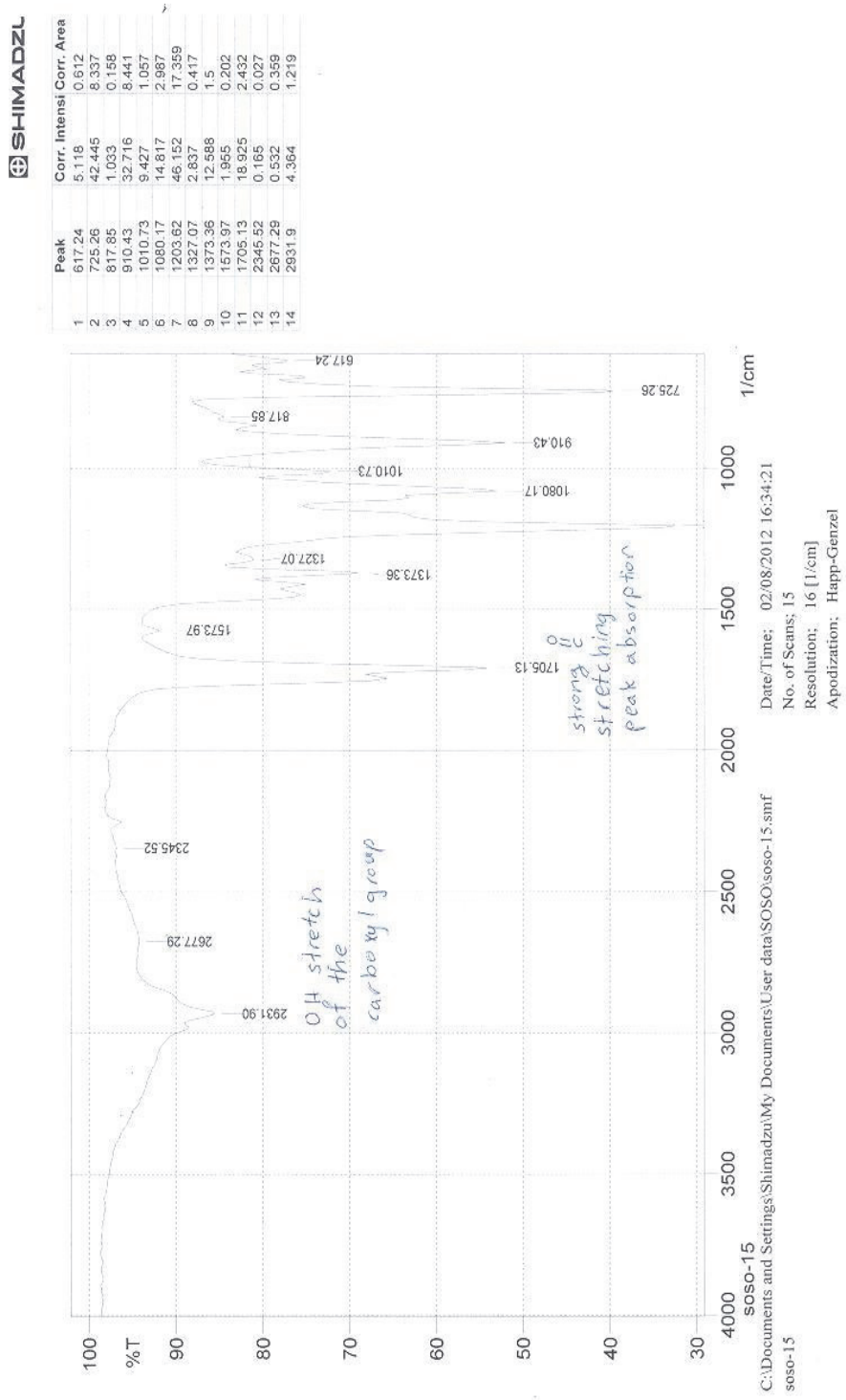


Appendix 1. ^{1}H NMR of compound 14 (6-acetoxy-2, 5, 7, 8-tetramethylchroman-2-yl) acetic acid.

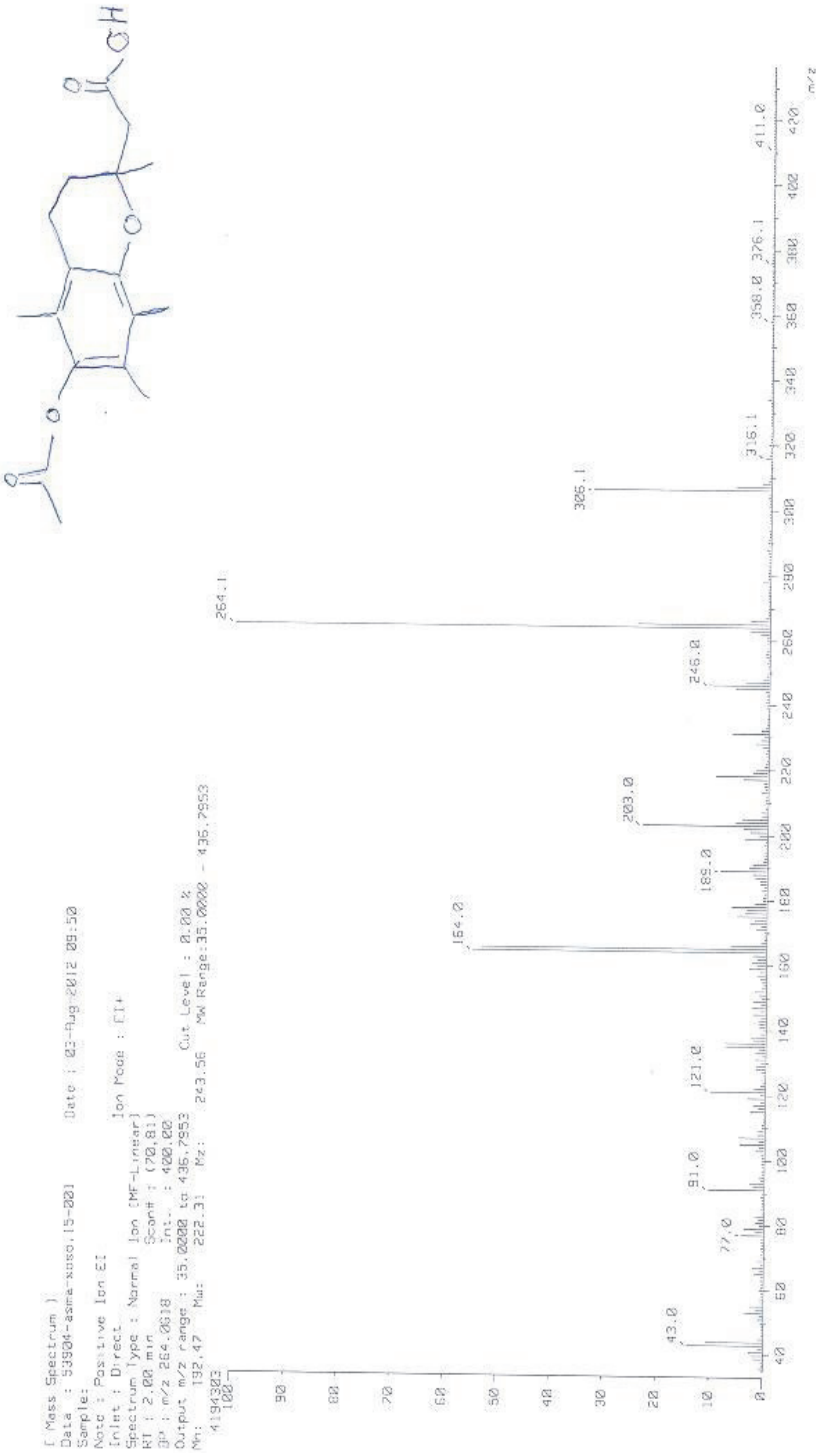


Appendix 2. ^{13}C NMR of compound 14 (6-acetoxy-2, 5, 7, 8-tetramethylchroman-2-yl) acetic acid

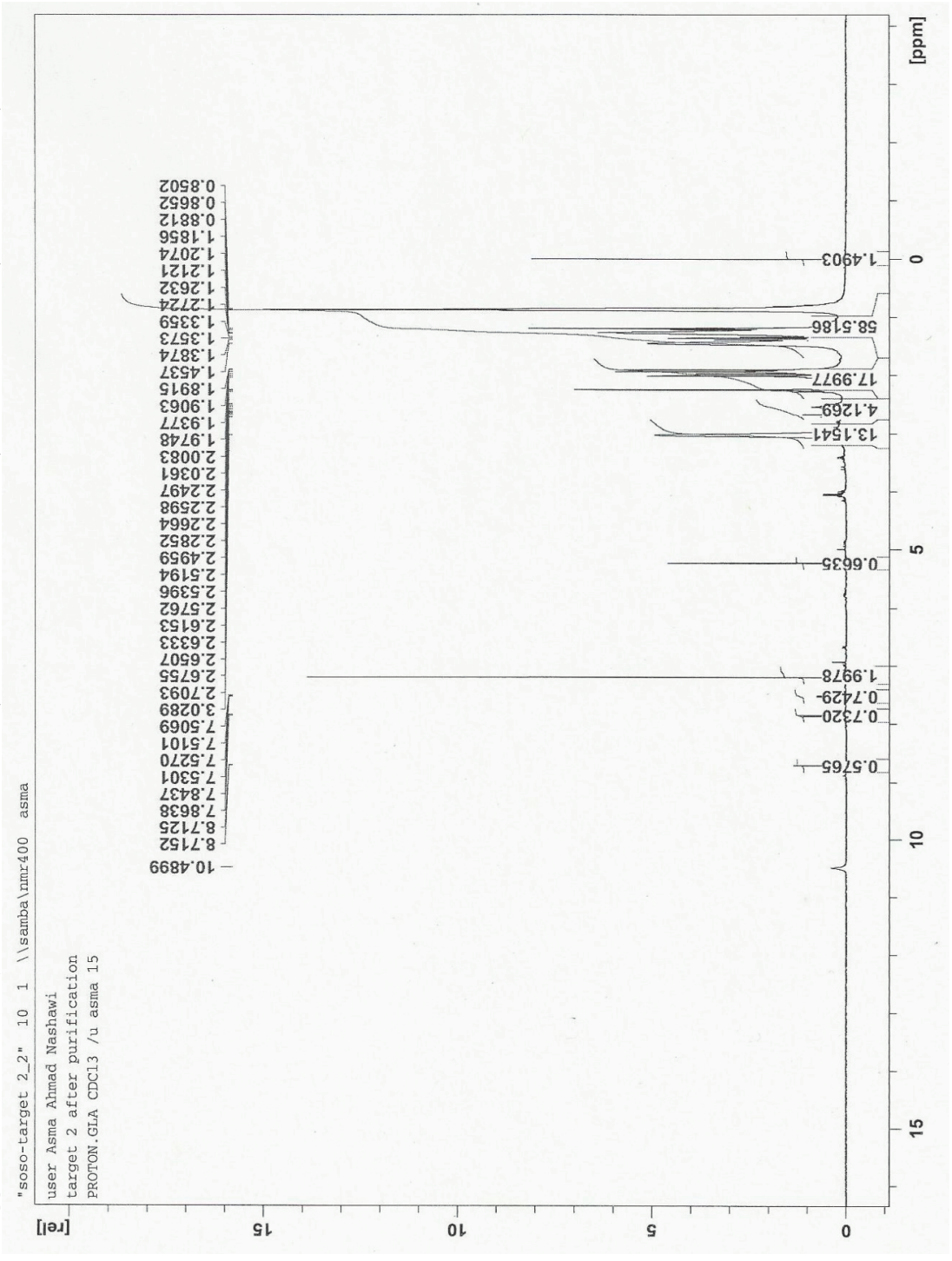
Appendix 3. IR spectrum of compound 14 (6-acetoxy-2, 5, 7, 8-tetramethylchroman-2-yl) acetic acid.



Appendix 4. Mass spectrum m/z: of compound 14 (6-acetoxy-2, 5, 7, 8-tetramethylchroman-2-yl) acetic acid



Appendix 5. ^{1}H NMR of compound of 15: 2, 5, 7, 8-tetramethyl (2, 5-disulfonic acid tetrabutylammonium carbamoyl salt) chroman-6-yl acetate



أشباه فيتامين إ

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جلاسكو – المملكة المتحدة

المستخلص. وسيط العديد من الأحداث المرضية مثل تصلب الشرايين، وأمراض الاعصابوغيرها هو بيروكسيد الدهون، والتي يسببها أنواع الاكسجين التفاعلية التي تتفاعلمع ركائز البيولوجية، مما يؤدي إلى تلف الخلايا. ويعتقد أن انزيمنيكوتيناميد الادنين ثنائي الفوسفات اوكسيديز، فضلا عن ضعفالميتوكندريا وغيرها من المصادر،فهو يعتبر هدفا علاجيا هاما. من أجل ذلكمضادات الأكسدة فيتامين إالطبيعية والاصطناعية تدرس بشكل مكثف. في هذهالدراسة نختصر المعلومات المعروفة عن وظيفة فيتامين إ والتأكيد على مضاداتالاكسدةومقارنتها بخصائص اخرى.والغرض من هذه الدراسة هو تصميم وتخليقاشباه فيتامين إ التي يتم وضعها خارج الخلاياوبالتالي بيروكسيد الدهون يدرسوبالتالي في الأشخاص الاصحاء والمرضى كذلك.ختاما الدراسة الحالية في المعمل تشير الى مركب الاميد يعرف ب اشباهفيتامين إ تم تحضيره في المعمل عن طريق تفاعل (٦ استوكسي -٢,٥,٧,٨ رباعي الميثايل الكرومان ٢اسيل كلوريد) مع الأنيلين٢,٥سلفونك تيترابيوتيل الأمونيوم وهذا الأخير تكو فيرول هو مركب أعد حديثا،من المتوقع أن يعمل حماية الغشاء الدهني من سلوك الضرر التأكسدي.