***Compositions and Methods for Treatment of Neurological Disease***

**Field of the Disclosure**

Disclosed herein are monomethyl fumarates, pharmaceutical compositions including monomethyl fumarates, and methods of using the monomethyl fumarates and pharmaceutical compositions thereof for treating mitochondrial, neurodegenerative, inflammatory and autoimmune diseases including Leigh's Syndrome, Leber's Hereditary Optic Neuropathy, Mitochondrial Encephalomyopathy Latic Acid (MELAS), Myoclonic Epilepsy Red Ragged Fiber (MERRF), Friedreich's ataxia, multiple sclerosis, various dementias, autism and psychological disorders.

**Background**

Mitochondrial diseases, estimated to impact 1 in 5,000 patients or approximately 66,000 people in the United States in 2020, are devastating inborne error of metabolism defects that result in dysfunctional mitochondria, and are manifested in a range of disease states (Orsucci, Caldarazzo Ienco et al. 2021), and can be the result of nuclear DNA mutations or mitochondrial DNA mutations (Weissig and Edeas 2015, Weissig and Edeas 2015). Many mitochondrial diseases impact the Central Nervous System (CNS), such as Leigh's Syndrome, Myoclonic Epilepsy Red Ragged Fiber (MERRF), and Mitochondrial Encephalomyopathy, Lactic Acid, Stroke (MELAS). Currently, there are no FDA approved therapies for mitochondrial disease (Weissig 2020). Mitochondrial biogenesis (Valero 2014) has been recognized as a potential disease modifying approach that is distinct from attempts to cure mitochondrial disease using gene therapy (Hanaford, Cho et al. 2022). Fumarate acid esters (FAEs) are known to activate the Nrf2 pathway (Linker, Lee et al. 2011), which both activates the Antioxidant Response Element (ARE) and also results in dimethylfumarate triggered mitochondrial biogenesis(Hayashi, Jasoliya et al. 2017). FAEs have previously expressly been proposed as a treatment for mitochondrial diseases (US Pat. No. 6,858,750) but have never demonstrated efficacy in clinical trials, although there is recent evidence from *in vitro* models (Gola, Bierhansl et al. 2023) and *in vivo* models (Hayashi, Jasoliya et al. 2017, Hui, Dedkova et al. 2021) most likely due to insufficient Central Nervous System (CNS) penetration. FAEs have been approved for human treatment of multiple sclerosis (Fox, Miller et al. 2012) as a disease modifying therapy (Dighriri, Aldalbahi et al. 2023) and may have potential utility in a variety of CNS diseases (Lin, Cai et al. 2016, Brandes and Gray 2020, Uruno and Yamamoto 2023). However, FAEs have notable dose limiting side effects including flushing, nausea, vomiting, gastrointestinal disturbances, and lymphopenia (Liang, Chai et al. 2020). Many FAE prodrugs have been prepared and evaluated (see, e.g., U.S. Pat. Nos. 6,436,992; 7,157,426; 7,320,999;

7,432,240; 8,669,281; 9,403,784; 9,409,872; 9,532,968; and 11,142,501). FAE prodrugs such as dimethylfumarate and diroximel fumarate (DRF) are rapidly metabolized to monomethylfumarate (MMF), and MMF is known to penetrate the blood brain barrier (BBB), with calculated Log([Brain]/[Blood]) of -1. However, MMF penetration into human CSF is experimentally only about 11% of the blood concentration (Edwards, Kamath et al. 2021), leaving the high peripheral blood concentration to cause unwanted side effects. In the case of mitochondrial diseases such as MELAS, MERRF and Leigh's Syndrome, it is particularly desirable for the MMF to penetrate the CNS to exert the desired activation of the Nrf2 pathway and subsequent mitochondrial biogenesis with as high a concentration as can be tolerated.

The BBB is a difficult barrier to penetrate even for small molecules. One approach to increasing the CNS penetration of MMF is to utilize a carrier molecule that utilizes active transport proteins, such as glucose transporter 1 (GLUT1), sodium vitamin C transporter 2 (SVCT2) (Nualart, Mack et al. 2014) or large neutral amino acid transporter 1 (LAT1) (Singh and Ecker 2018). Briefly, GLUT1 transports dehydroascorbic acid, but not ascorbic acid. Once transported into the brain, dehydroascorbic acid is reduced to ascorbic acid, and ascorbic acid is retained in the brain at a 10-fold concentration higher than the blood. SVCT2 transports ascorbic acid directly into the brain, while LAT1 transports amino acids into the brain. The same transporters are also involved in intestinal uptake (Fraga, Pinho et al. 2005, Cao, Gibbs et al. 2006, Subramanian, Srinivasan et al. 2017), simultaneously addressing the need for improved intestinal absorption to decrease nausea, vomiting and gastrointestinal disturbances.

CNS penetrating prodrugs utilizing carriers (Rautio, Laine et al. 2008) such as reduced ascorbic acid (Manfredini, Pavan et al. 2002) for transporting Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are known (Zhao, Qu et al. 2014, Wang, Zhang et al. 2018), as are LAT1 utilizing carriers (Gynther, Laine et al. 2008). However, these carriers have not been applied to FAEs. There are examples of a fumarate ester with ascorbic acid for antimicrobial and agricultural applications (see CN Pat. No. 102,442,983), acrylate esters of dehydroascorbic acid (see JP Pat. No. 5,781,983) for agricultural applications, and threonine conjugates to many FDA-approved carboxylic acids (see U.S. Pat. No. 8,173,840). Due to the relatively small size of the FAEs, these are ideal candidates for transport into the brain using GLUT1, SVCT2 and LAT1.

To address the urgent need for new therapeutics in the area of mitochondrial diseases, this disclosure provides enhanced BBB and intestinal penetrating-MMF prodrugs. In some preferred embodiments, these have been designed using dehydroascorbic acid, ascorbic acid and amino acids as carriers that result in a significant increase in brain MMF concentration and mitochondrial biogenesis activity. In some preferred embodiments, bulky functional groups have been added to provide steric hinderance and prevent

unwanted FAE cleavage in the intestine and plasma, but leave GLUT1, SVCT2 and LAT1 substrate transport. These compounds that penetrate the CNS and trigger mitochondrial biogenesis are configured to have broad utility in human disease (Edeas and Weissig 2013). The compounds of the present disclosure, thus, provide a solution to the art-recognized problems discussed above. Other advantages will be clear to those of ordinary skill in the art from this disclosure.

**Summary of Illustrative Embodiments**

The present disclosure captures key innovations created by the inventors to target the underlying molecular mechanisms of mitochondrial dysfunction to manage symptoms, slow disease progression, and ultimately help patients lead healthier lives, however long that life may be.

One of the inventors' primary focuses is in providing improvements to the health and longevity of Leigh Syndrome patients. The inventors are inspired to assist people such as the family in the following illustration.

Like every new parent, Greg and Melissa carefully and lovingly watched their perfect baby girl, Harriet's, every move. Every giggle was music to their ears and every kick was a ballet. They were the typical beaming parents. Then suddenly, there was silence. There was stillness. Harriet stopped eating and had infrequent movements. She showed no visible signs of emotion. They sought answers from doctors who assured them this was a normal growth period where actions seemed suppressed but were merely a phase. But they knew something was terribly wrong. So began their heartbreaking journey.

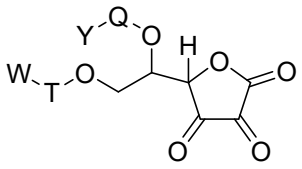
Harriet was diagnosed at 15 months with Leigh syndrome after an exhaustive and debilitating search for the reasons behind their daughter's drastic changes. The doctors told them to enjoy every moment with Harriet as her life would be very short. She passed away at just under 2 years old.

1 in 40,000 children are born with Leigh Syndrome, an inherited neurometabolic (mitochondrial) disorder that affects the central nervous system. Leigh Syndrome presents in infants between the ages of 3 months and 2 years. Symptoms of Leigh syndrome usually progress rapidly and include the inability to take nutrition, hold their head up, poor muscular control, seizures, and inability to speak. There is no cure. It is fatal, with most children dying by age 3. Those who survive into older childhood have severe disabilities that further degenerate in a painful and debilitating manner.

Beyond Leigh syndrome patients, various aspects of the present disclosure are applicable to therapies targeting other mitochondrial diseases as well. In some examples, Leber hereditary optic neuropathy, Kearns-Sayre syndrome, Alpers-Huttenlocher syndrome, ataxia neuropathy syndrome, MELAS syndrome, Red Ragged Fiber syndrome there may be significant improvement in neurological symptoms due to greater drug concentrations penetrating the brain, and positively influencing mitochondrial function, brain metabolism, and human behavior which are all linked together.

Incremental improvement is an enormous improvement in this rare and orphan disease space. Compared with the state of the art which is largely reliant on nutritional supplements, physical therapy and conventional antiepileptic drugs for some disease states, suppressing disease progression will stabilize brain energy, reduce neurological disease progression by keeping brain cells alive and prevent neurological sequalae such as stroke symptoms in MELAS.

In a first aspect, compounds of **Formula 1** are useful for the treatment of human disease:




1

where

Q is a single bond or C(O),

T is a single bond or C(O),

W is hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, or $C(H)=C(H)CO_2R^{50}$,

Y is hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, $C(H)=C(H)CO_2R^{50}$,

when Y is $C(H)=C(H)CO_2R^{50}$, then Q is C(O),

when Y or W is hydrogen, then Q or T is a single bond,

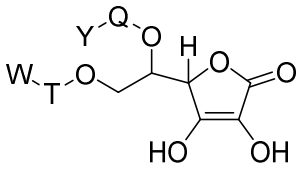
when W is $C(H)=C(H)CO_2R^{50}$, then T is C(O),

$R^{50}$ is $C_1$-$C_6$ alkyl, and,

at least one of W or Y is $C(H)=C(H)CO_2R^{50}$ with (E) regiochemistry;

or a pharmaceutically acceptable salt thereof.

In another aspect, compounds of **Formula 2** are useful for the treatment of human disease:



2

where

Q is a single bond or C(O),

T is a single bond or C(O),

W is hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, or $C(H)=C(H)CO_2R^{50}$,

Y is hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, or $C(H)=C(H)CO_2R^{50}$,

when Y is $C(H)=C(H)CO_2R^{50}$, then Q is C(O),

when Y or W is hydrogen, then Q or T is a single bond,

when W is $C(H)=C(H)CO_2R^{50}$, then S is C(O),

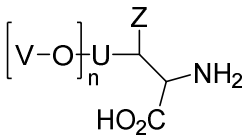
$R^{50}$ is $C_1$-$C_6$ alkyl,

at least one of Y or W is $C(H)=C(H)CO_2R^{50}$ with (E) regiochemistry, and,

if T is C(O) and W is $C(H)=C(H)CO_2R^{50}$ then Y is not hydrogen;

or a pharmaceutically acceptable salt thereof.

In another aspect, compounds of **Formula 3** are useful for the treatment of human disease:



3

where

n is 1 or 2,

U is a single bond or an aryl ring comprised of phenyl or pyridyl,

V is $C(O)C(H)=C(H)CO_2R^{50}$ with (E) regiochemistry,

Z is hydrogen, methyl, trifluoromethyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, or benzyl,

if U is a single bond, Z is hydrogen,

if U is phenyl or pyridyl, Z is hydrogen, methyl or trifluoromethyl, and,

$R^{50}$ is $C_1$-$C_6$ alkyl;

or a pharmaceutically acceptable salt thereof.

Human diseases that can be treated by compounds of **Formulas 1-3** include but are not limited to a mitochondrial disease or a disease resulting from lowered mitochondrial activity. Examples of such diseases include primary mitochondrial diseases, such Leigh's syndrome, Leber hereditary optic neuropathy, Kearns-Sayre syndrome, Alpers-Huttenlocher syndrome, ataxia neuropathy syndrome, MELAS (Mitochondrial Encephalomyopathy, Lactic Acid, Stroke) syndrome, and MERRF (Myoclonic Epilepsy, Red Ragged Fiber) syndrome. Other diseases that can be treated by compounds of Formulas 1-3 include secondary mitochondrial disorders, such as spinal muscular atrophy, Friedreich's ataxia, Charcot-Marie-Tooth syndrome, hereditary spastic paraplegia, or Wilson's disease.

In another aspect, the human diseases that can be treated by compounds of **Formulas 1-3** can be a neurological disease such as Alzheimer's Disease, Frontotemporal Dementia, Amyotrophic Lateral Sclerosis, Lewy Body Dementia, Vascular dementia, Parkinson's Disease, traumatic brain injury, stroke, multiple sclerosis, Huntington's syndrome. Other neurological diseases that can be treated by compounds of Formulas 1-3 include schizophrenia, obsessive compulsive disorder, bipolar disorder, depressive disorder, drug addiction, psychotic disorders, anxiety disorder, personality disorder, mood disorder, major depressive disorder, post-traumatic stress disorder, panic disorder, autism, Asperger's syndrome, Rett syndrome, childhood disintegrative disorder, Kanner's syndrome, pervasive developmental disorder, high functioning autism, low functioning autism, and classical autism.

Certain syndromes can be treated with a compound of **Formulas 1-3** include Down Syndrome, retinitis pigmentosa, adrenal leukodystrophy, Alexanders Disease, Alper's Disease, Canavan Disease, Childhood Ataxia with Central Nervous System Hypomyelination, Globoid Cell Leukodystrophy, and Aicardi-Goutieres syndrome.

In another aspect, a proliferative disease such as brain cancer, glioblastoma multiforme, neuroblastoma, leukemia, lymphoma, sarcoma, or psoriasis can be treated using a compound of **Formula 1-3**.

The foregoing general description of the illustrative implementations and the following detailed description thereof are merely exemplary aspects of the teachings of this disclosure, and are not restrictive.

**Brief Description of the Drawings**

The accompanying drawings, which are incorporated in and constitute a part of the specification, illustrate one or more embodiments and, together with the description, explain these embodiments. The accompanying drawings have not necessarily been drawn to scale. Any values dimensions illustrated in the accompanying graphs and figures are for illustration purposes only and may or may not represent actual or preferred values or dimensions. Where applicable, some or all features may not be illustrated to assist in the description of underlying features. In the drawings:

Figure 1: Synthesis of key compounds from **Formula 1**.

Figure 2: Synthesis of key intermediates from **Formula 1**.

Figure 3: Synthesis of key compounds from **Formula 1**.

Figure 4: Synthesis of key compounds utilizing the benzyl functional group.

Figure 5: Synthesis of key compounds from **Formula 1**.

Figure 6: Synthesis of key intermediates from **Formula 1**.

Figure 7: Synthesis of key compounds from **Formula 1**.

Figure 8: Synthesis of precursor compounds from **Formula 2**.

Figure 9: Synthesis of key intermediates from **Formula 2**.

Figure 10: Synthesis of key compounds from **Formula 2**.

Figure 11: Synthesis of key intermediates from **Formula 2**.

Figure 12: Synthesis of key intermediates from **Formula 2**.

Figure 13: Synthesis of key compounds from **Formula 2**.

Figure 14: Synthesis of key intermediates from **Formula 2**.

Figure 15: Synthesis of key compounds from **Formula 2**.

Figure 16: Synthesis of key intermediates from **Formula 2**.

Figure 17: Synthesis of key intermediates from **Formula 2**.

Figure 18: Synthesis of key compounds from **Formula 2**.

Figure 19: Synthesis of key compounds utilizing the phenyl functional group.

Figure 20: Synthesis of key compounds from **Formula 3**.

Figure 21: Synthesis of key compounds from **Formula 3**.

Figure 22: Synthesis of key compounds from **Formula 3**.

Figure 23: Synthesis of key compounds from **Formula 3**.

Figure 24: Synthesis of key compounds from **Formula 3**.

**Terms and definitions**

The following is a list of abbreviations, plus terms and their definitions, used throughout the specification and the claims are described below.

General abbreviations and their corresponding meanings include: AA = ascorbic acid; DHAA = dehydroascobic acid; mg = milligram(s); ml or mL = milliliter(s); mM = millimolar; nmol = nanomole(s); pmol = picomole(s); ppm = parts per million; RT = room temperature; U = unit(s); ug, µg = microgram(s); ul, µl = microliter(s); uM, µM = micromolar; AD = Alzheimer's Disease; TEA = triethylamine; LDA = lithium diisopropyl amide; THF = tetrahydrofuran; DMAP = 4-dimethylaminopyridine; DMF = N,N'-dimethylformamide; MMF = monomethyl fumarate; MMF-Cl = monomethyl fumarate acid chloride; DRF = diroximel fumarate; GLUT1 = glucose transporter 1; SVCT2 = sodium vitamin C transporter 2; LAT1 = large amino acid transporter 1; MS = multiple sclerosis,

Chemical abbreviations include: Me = methyl; Et = ethyl; n-Pr = n-propyl; i-Pr = isopropyl; n-Bu = n-butyl; i-Bu = isobutyl; t-Bu = t-butyl; Ph = phenyl; Bn = benzyl; Ms = mesyl; Ts = tosyl; THF = tetrahydrofuran; TEA = triethylamine; TFA = trifluoroacetic acid; MMF-Cl = monomethyl fumarate chloride; NaH = sodium hydride; TBDPS = tert-butyl diphenyl silyl.

The terms "cell" and "cells", which are meant to be inclusive, refer to one or more cells which can be in an isolated or cultured state, as in a cell line including a homogeneous or heterogeneous population of cells, or in a tissue sample, or as part of an organism, such as a transgenic mammal.

The term "disease" refers to a disease, disorder, condition, or symptom of any of the foregoing.

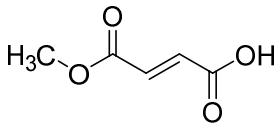
The term "drug" as used herein is as defined under 21 U.S.C. § 321(g)(1), meaning "(A) articles recognized in the official United States Pharmacopoeia, official Homeopathic Pharmacopoeia of the United States, or official National Formulary, or any supplement to any of them; and (B) articles intended for use in the

diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; and (C) articles (other than food) intended to affect the structure or any function of the

body of man or other animals ...”

The terms “MHF”, “MMF”, “monomethyl fumarate” and “methyl hydrogen fumarate”

are synonymous, all referring to a compound having the following chemical structure:




The term "amino acid" encompasses both naturally occurring and non-naturally occurring amino acids unless otherwise designated.

The term "an effective amount" means an amount of the substance in question which produces a statistically significant effect. For example, an "effective amount" for therapeutic uses is the amount of the composition including an active compound herein required to provide a clinically significant alteration in a measurable trait. Such effective amounts will be determined using routine optimization techniques and are dependent on the particular condition to be treated, the condition of the patient, the route of administration, the formulation, and the judgment of the practitioner and other factors evident to those skilled in the art. The dosage required for the compounds of certain embodiments is manifested as that which induces a statistically significant difference between treatment and control groups.

A "therapeutically-effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically-effective amount of a compound of this disclosure (e.g., a modulator) may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the modulator to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically-effective amount is also one in which any toxic or detrimental effects of the modulator are outweighed by the therapeutically beneficial effects.

A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. A prophylactically effective amount can be determined as described above for the therapeutically-effective amount. Typically, since a prophylactic

dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically-effective amount.

The term "treatment" includes both prophylaxis and therapy.

The term "animal" includes human beings.

The term “patient” includes human beings that are treated for a given disease state.

The term “mitochondrial biogenesis modulator” includes compounds that can increase or decrease the formation of mitochondria in a cell, animal or patient.

The term “substituted aromatic or heteroaromatic” refers to aromatic or heteroaromatic rings may contain one or more substituents such as -OH, SH, -CN, -F, -Cl, -Br, -R, $-NO_2$ -NO, $-NH_2$, -NHR, -NRR, -C(O)R, -C(O)OH, -C(O)OR, $-C(O)NH_2$, -C(O)NHR, -C(O)NRR, and the like where each R is independently ($C_1$-$C_5$) alkyl, substituted ($C_1$-$C_6$) alkyl, ($C_2$-$C_6$) alkenyl, substituted ($C_2$-$C_6$) alkenyl, ($C_2$-$C_6$) alkynyl, substituted ($C_2$-$C_6$) alkynyl, ($C_5$-$C_{20}$) aryl, substituted ($C_5$-$C_{20}$) aryl, ($C_6$-$C_{26}$) arylalkyl, substituted ($C_6$-$C_{26}$) arylalkyl, 5-20 membered heteroaryl, substituted 5-20 membered heteroaryl, 6-26 membered heteroarylalkyl or substituted 6-26 membered heteroarylalkyl.

For the purposes of the present disclosure the terms "compound," "analog," and "composition of matter" stand equally well for the mitochondrial biogenesis modulators described herein, including all enantiomeric forms, diastereomeric forms, salts, and the like.

The term “pharmaceutically acceptable salt” herein includes all salt forms, for example, salts of both basic groups, inter alia, amines, as well as salts of acidic groups, inter alia, carboxylic acids. The following are non-limiting examples of anions that can form pharmaceutically acceptable salts with basic groups: chloride, bromide, iodide, sulfate, bisulfate, carbonate, bicarbonate, phosphate, formate, acetate, propionate, butyrate, pyruvate, lactate, oxalate, malonate, maleate, succinate, tartrate, fumarate, citrate, and the like. The following are non-limiting examples of cations that can form pharmaceutically-acceptable salts of the anionic form of acidic substituent groups on the compounds described herein: sodium, lithium, potassium, calcium, magnesium, zinc, bismuth, and the like.

The term "pharmaceutically acceptable carrier" refers to any suitable adjuvants, carriers, excipients, or stabilizers, and can be in solid or liquid form such as, tablets, capsules, powders, solutions, suspensions, emulsions, or implantable disc.

**Detailed Description of Illustrative Embodiments**

The description set forth below in connection with the appended drawings is intended to be a description of various, illustrative embodiments of the disclosed subject matter. Specific features and functionalities are described in connection with each illustrative embodiment; however, it will be apparent to those skilled in the art that the disclosed embodiments may be practiced without each of those specific features and functionalities.

Reference throughout the specification to "one embodiment" or "an embodiment" means that a particular feature, structure, or characteristic described in connection with an embodiment is included in at least one embodiment of the subject matter disclosed. Thus, the appearance of the phrases "in one embodiment" or "in an embodiment" in various places throughout the specification is not necessarily referring to the same embodiment. Further, the particular features, structures or characteristics may be combined in any suitable manner in one or more embodiments. Further, it is intended that embodiments of the disclosed subject matter cover modifications and variations thereof.

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context expressly dictates otherwise. That is, unless expressly specified otherwise, as used herein the words "a," "an," "the," and the like carry the meaning of "one or more." Additionally, it is to be understood that terms such as "left," "right," "top," "bottom," "front," "rear," "side," "height," "length," "width," "upper," "lower," "interior," "exterior," "inner," "outer," and the like that may be used herein merely describe points of reference and do not necessarily limit embodiments of the present disclosure to any particular orientation or configuration. Furthermore, terms such as "first," "second," "third," etc., merely identify one of a number of portions, components, steps, operations, functions, and/or points of reference as disclosed herein, and likewise do not necessarily limit embodiments of the present disclosure to any particular configuration or orientation.

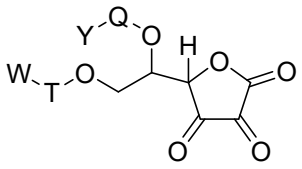
Furthermore, the terms "approximately," "about," "proximate," "minor variation," and similar terms generally refer to ranges that include the identified value within a margin of 20%, 10% or preferably 5% in certain embodiments, and any values therebetween.

All of the functionalities described in connection with one embodiment are intended to be applicable to the additional embodiments described below except where expressly stated or where the feature or function is incompatible with the additional embodiments. For example, where a given feature or function is expressly described in connection with one embodiment but not expressly mentioned in connection

with an alternative embodiment, it should be understood that the inventors intend that that feature or function may be deployed, utilized or implemented in connection with the alternative embodiment unless the feature or function is incompatible with the alternative embodiment.

Monomethyl fumarate prodrugs having high gastrointestinal and plasma stability, high gastrointestinal and brain permeability and preferential cleavage of promoieties in the brain are desirable. Such monomethyl fumarate prodrugs which provide higher oral bioavailability and BBB penetration may enhance the efficacy of mitochondrial biogenesis compared to present fumaric acid esters; facilitate the use of lower doses, reduce dosing frequency and standardize dosing regimens; reduce food effects; reduce gastrointestinal side effects; and enhance neurological treatment. This disclosure provides monomethyl fumarate prodrugs having enhanced brain penetration and reduced gastrointestinal side effects, as well as methods for preparing, formulating and using the same to treat disease.

In a first aspect, compounds of **Formula 1** are useful for the treatment of human disease:




1

where

Q is a single bond or C(O),

T is a single bond or C(O),

W is hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, $C(H)=C(H)CO_2R^{50}$,

Y is hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, $C(H)=C(H)CO_2R^{50}$,

when Y is $C(H)=C(H)CO_2R^{50}$, then Q is C(O),

when Y or W is hydrogen, then Q or T is a single bond,

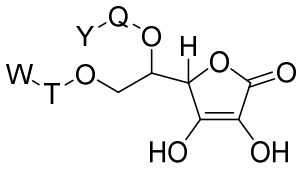
when W is $C(H)=C(H)CO_2R^{50}$, then T is C(O),

$R^{50}$ is $C_1$-$C_6$ alkyl, and,

where at least one of W or Y is $C(H)=C(H)CO_2R^{50}$ with (E) regiochemistry;

or a pharmaceutically acceptable salt thereof.

In another aspect, compounds of **Formula 2** are useful for the treatment of human disease:



2

where

Q is a single bond or C(O),

T is a single bond or C(O),

W is hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, or $C(H)=C(H)CO_2R^{50}$,

Y is hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, or $C(H)=C(H)CO_2R^{50}$,

when Y is $C(H)=C(H)CO_2R^{50}$, then Q is C(O),

when Y or W is hydrogen, then Q or T is a single bond,

when W is $C(H)=C(H)CO_2R^{50}$, then S is C(O),

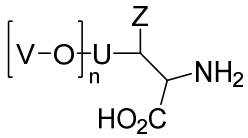
$R^{50}$ is $C_1$-$C_6$ alkyl,

at least one of Y or W is $C(H)=C(H)CO_2R^{50}$ with (E) regiochemistry, and,

if T is C(O) and W is $C(H)=C(H)CO_2R^{50}$ then Y is not hydrogen;

or a pharmaceutically acceptable salt thereof.

In another aspect, compounds of **Formula 3** are useful for the treatment of human disease:



3

where

n is 1 or 2,

U is a single bond or an aryl ring comprised of phenyl or pyridyl,

V is $C(O)C(H)=C(H)CO_2R^{50}$ with (E) regiochemistry,

Z is hydrogen, methyl, trifluoromethyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, or benzyl,

if U is a single bond, Z is hydrogen,

if U is phenyl or pyridyl, Z is hydrogen, methyl, or trifluoromethyl, and,

$R^{50}$ is $C_1$-$C_6$ alkyl;

or a pharmaceutically acceptable salt thereof.

Related aspects of this disclosure are directed to compositions, including pharmaceutical compositions, including the compounds of **Formulas 1, 2** and **3** ("**Formulas 1-3**"), shown above and elsewhere herein, including methods for pharmaceutical formulation of the compounds disclosed herein for administration to a human being (e.g., preferably use in oral and/or intravenous applications, and/or in implantable materials).

In one aspect, this disclosure provides methods of preventing, ameliorating or treating at least one mitochondrial related condition or disease in a subject, by administering to the subject a therapeutically effective amount of the compound or salt described above and encompassed by any of **Formulas 1-3**. In preferred embodiments, the condition or disease a primary mitochondrial disease, secondary mitochondrial disorder, neurological disorder, degenerative central nervous system disorder, metabolic disorder and/or proliferative disorder.

One preferred aspect of such methods includes modulating the level or activity of mitochondrial biogenesis in a cell, including contacting the cell with an effective amount of the compound or salt described above and encompassed by any of **Formulas 1-3**.

One preferred aspect of such methods includes modulating mitochondrial biogenesis in a subject, which includes administering a therapeutically effective amount of a compound or salt described above and encompassed by any of **Formulas 1-3** to the subject.

One preferred aspect of such methods includes treatment of CNS diseases by modulating Nrf2 pathway activation, another aspect is through modulation of the Antioxidant Response Element (ARE), and another aspect is through the modulation of mRNA expression.

Other embodiments, methods for manufacturing and using a compound of any of one of **Formulas 1-3** would be understood by those of ordinary skill in the art, and/or are further disclosed herein.

Exemplary Compounds

In preferred embodiments, the compounds from **Formulas 1-3** can be any of the following named compounds:

2-hydroxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;
2-methoxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;
2-acetoxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;
2-(benzoyloxy)-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;
methyl (2-(2-phenylacetoxy)-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl) fumarate;
methyl (2-(pivaloyloxy)-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl) fumarate;
2-hydroxy-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;
2-acetoxy-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;
methyl (2-(pivaloyloxy)-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl) fumarate;
2-(benzoyloxy)-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;
methyl (2-(2-phenylacetoxy)-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl) fumarate;
2-(tert-butoxy)-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;
dimethyl O,O'-((1S)-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethane-1,2-diyl) difumarate;
O,O'-(1-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)ethane-1,2-diyl) dimethyl difumarate;
2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-methoxyethyl methyl fumarate;
2-acetoxy-2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)ethyl methyl fumarate;
2-(benzoyloxy)-2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)ethyl methyl fumarate;
2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-(2-phenylacetoxy)ethyl methyl fumarate;
2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-(pivaloyloxy)ethyl methyl fumarate;
1-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-hydroxyethyl methyl fumarate;
1-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-(pivaloyloxy)ethyl methyl fumarate;
2-(benzoyloxy)-1-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)ethyl methyl fumarate;
1-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-(2-phenylacetoxy)ethyl methyl fumarate;
2-(tert-butoxy)-1-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)ethyl methyl fumarate;
(E)-2-amino-3-((4-methoxy-4-oxobut-2-enoyl)oxy)butanoic acid;
(E)-2-amino-3-((4-methoxy-4-oxobut-2-enoyl)oxy)-4,4-dimethylpentanoic acid;
(E)-2-amino-4,4,4-trifluoro-3-((4-methoxy-4-oxobut-2-enoyl)oxy)butanoic acid;
(E)-2-amino-3-((4-methoxy-4-oxobut-2-enoyl)oxy)-3-phenylpropanoic acid;
(E)-2-amino-3-((4-methoxy-4-oxobut-2-enoyl)oxy)-4-phenylbutanoic acid;
(E)-2-amino-3-(4-((4-methoxy-4-oxobut-2-enoyl)oxy)phenyl)propanoic acid;
(E)-2-amino-3-(3-((4-methoxy-4-oxobut-2-enoyl)oxy)phenyl)propanoic acid;
(E)-2-amino-3-(2-((4-methoxy-4-oxobut-2-enoyl)oxy)phenyl)propanoic acid; or,
2-amino-3-(3,5-bis(((E)-4-methoxy-4-oxobut-2-enoyl)oxy)phenyl)propanoic acid.

In preferred embodiments, the human diseases that can be treated by administration of one or more compounds of **Formulas 1-3** include but are not limited to a mitochondrial disease or a disease of lowered mitochondrial activity. Some examples of such diseases include primary mitochondrial diseases, such Leigh's syndrome, Leber hereditary optic neuropathy, Kearns-Sayre syndrome, Alpers-Huttenlocher

syndrome, ataxia neuropathy syndrome, MELAS (Mitochondrial Encephalomyopathy, Lactic Acid, Stroke) syndrome, and MERRF (Myoclonic Epilepsy, Red Ragged Fiber) syndrome. Another type of disease that can be treated by compounds of **Formula 1-3** include secondary mitochondrial disorders, such as spinal muscular atrophy, Friedreich's ataxia, Charcot-Marie-Tooth syndrome, hereditary spastic paraplegia, Wilson's disease.

In another preferred aspect, the human diseases that can be treated by compounds of **Formula 1-3** can be a neurological disease such as Alzheimer's Disease, Frontotemporal Dementia, Amyotrophic Lateral Sclerosis, Lewy Body Dementia, Vascular dementia, Parkinson's Disease, traumatic brain injury, stroke, multiple sclerosis, Huntington's syndrome. Other neurological diseases that can be treated by compounds of **Formula 1-3** include schizophrenia, obsessive compulsive disorder, bipolar disorder, depressive disorder, drug addiction, psychotic disorders, anxiety disorder, personality disorder, mood disorder, major depressive disorder, post-traumatic stress disorder, panic disorder, autism, Asperger's syndrome, Rett syndrome, childhood disintegrative disorder, Kanner's syndrome, pervasive developmental disorder, high functioning autism, low functioning autism, classical autism.

Certain syndromes can be preferably treated with a compound of **Formula 1-3** include Down Syndrome, retinitis pigmentosa, adrenal leukodystrophy, Alexanders Disease, Alper's Disease, Canavan Disease, Childhood Ataxia with Central Nervous System Hypomyelination, Globoid Cell Leukodystrophy, and Aicardi-Goutieres syndrome.

In another preferred aspect, aproliferative disease such as brain cancer, glioblastoma multiforme, neuroblastoma, leukemia, lymphoma, sarcoma, psoriasis can be treated using a compound of **Formula 1-3**.

Therapeutic Uses of Compositions Having Compounds of the Present Disclosure

Mitochondrial disease can result in dysfunctional brain metabolism. Preventing neuronal death by modulating mitochondrial biogenesis can be accomplished by activating the Nrf2 pathway, resulting in a neuroprotective effect.

One aspect is directed to a method of preventing, ameliorating, or treating mitochondrial dysfunction or disease in a subject, including administering to the subject a therapeutically-effective amount of the compound or salt described above and encompassed by any of **Formulas 1-3**, where the condition or disease is selected from the group consisting of primary mitochondrial diseases, secondary mitochondrial

disorders, neurological disorders, degenerative central nervous system disorders, metabolic disorders, and proliferative cell disorders. Another aspect is directed to the methods noted above, where the condition or disease is a degenerative disorder selected from the group consisting of Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis, traumatic brain injury, and multiple sclerosis. Another aspect is related to the methods noted above, where the condition or disease is a neurological disorder. Still another aspect relates to a method where the neurological disorder is selected from the group consisting of epilepsy, epileptogenesis, depression, and stroke. Another aspect is related to the methods noted above, where the condition or disease is a metabolic disorder, such as obesity, diabetes, anemia, and renal failure-associated anemia. Another aspect is related to the methods noted above where the condition or disease is a proliferative disease, such as cancer, and specific types of cancer, such as brain cancer. Another aspect of the present disclosure is directed to the treatment of immunological diseases by modulating immune cell activity.

In one aspect, the present disclosure is directed to a method of treating, suppressing, reducing the severity of a neurological disease including administering a compound as described in certain embodiments to a subject suffering from a mitochondrial disorder or dysfunction under conditions effective to trigger mitochondrial biogenesis.

Moreover, based upon the designed mode of action of Nrf2 activators, it is believed that other CNS disease states, such as depression, schizophrenia, stroke, traumatic brain injury, Alzheimer's and Parkinson's disease will likewise be treatable or preventable upon administration of the compounds or compositions to a patient. Preferred compounds of the present disclosure are able to preserve neurons or astrocytes at risk of death thereby preventing subsequent CNS pathological states due to the death of neurons or astrocytes, and also disrupt the process of disease progression.

Another aspect provides for the use of a compound as herein described, or its isomer, metabolite, tautomer, pharmaceutically acceptable salt, pharmaceutical product, polymorph, crystal, N-oxide, hydrate, or any combination thereof, for treating, suppressing, preventing, reducing the severity, of mitochondrial disorder or dysfunction.

Another aspect provides for the post-operative recovery and suppression of mitochondrial disease or dysfunction following brain surgery. Treatment with a pharmaceutical composition including a compound and a pharmaceutically acceptable carrier under conditions to treat mitochondrial disease or dysfunction is useful by one skilled in the art.

Pharmaceutical Compositions

Related aspects are directed to compositions, including pharmaceutical compositions, including the compounds of various embodiments of the present disclosure, noted above. In one aspect, the present disclosure is directed to a pharmaceutical composition including at least one pharmaceutically acceptable excipient and a therapeutically effective amount of the compound or salt disclosed above. Still another aspect relates to a method for pharmaceutical formulation of previously described compounds for use in oral and intravenous applications, and in implantable materials.

Another aspect relates to a pharmaceutical composition including a pharmaceutically acceptable carrier and a compound according to the various embodiments of the present disclosure. The pharmaceutical composition can contain one or more of the above-identified compounds.

Typically, the pharmaceutical composition will include a compound or its pharmaceutically acceptable salt, as well as a pharmaceutically acceptable carrier.

Typically, the composition will contain from about 0.01 to 99 percent, preferably from about 20 to 75 percent of active compound(s), together with the adjuvants, carriers and/or excipients. While individual needs may vary, determination of optimal ranges of effective amounts of each component is within the skill of the art. Typical dosages include about 0.01 to about 100 mg/kg body wt. The preferred dosages include about 0.1 to about 100 mg/kg body wt. The most preferred dosages include about 1 to about 100 mg/kg body wt. Treatment regimen for the administration of the compounds of the present disclosure can also be determined readily by those with ordinary skill in art. That is, the frequency of administration and size of the dose can be established by routine optimization, preferably while minimizing any side effects.

Granules of a pharmaceutical composition can be coated either one or multiple times, to result in a time release or enteric coating for intestinal release as opposed to gastric release.

Dosage Forms

The solid unit dosage forms can be of the conventional type. The solid form can be a capsule and the like, such as an ordinary gelatin type containing the compounds of the present disclosure and a carrier, for example, lubricants and inert fillers such as, lactose, sucrose, or cornstarch. In another embodiment, these compounds are tabulated with conventional tablet bases such as lactose, sucrose, or cornstarch in

combination with binders like acacia, cornstarch, or gelatin, disintegrating agents, such as cornstarch, potato starch, or alginic acid, and a lubricant, like stearic acid or magnesium stearate.

The tablets, capsules, and the like can also contain a binder such as gum tragacanth, acacia, com starch, or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as com starch, potato starch, alginic acid; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose, or saccharin. When the dosage unit form is a capsule, it can contain, in addition to materials of the above type, a liquid carrier such as a fatty oil.

Optional Coatings

Various other materials may be present as coatings or to modify the physical form of the dosage unit. For instance, tablets can be coated with shellac, sugar, or both. A syrup can contain, in addition to active ingredient, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye, and flavoring such as cherry or orange flavor. Still other materials can be used to coat a tablet or capsule that are resistant to gastric pH, but readily dissolve at higher pH found in the intestine.

Excipients

For oral therapeutic administration, these active compounds can be incorporated with excipients and used in the form of tablets, capsules, elixirs, suspensions, syrups, and the like. Such compositions and preparations should contain at least 0.1 % of active compound. The percentage of the compound in these compositions can, of course, be varied and can conveniently be between about 2% to about 60% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions according to the present disclosure are prepared so that an oral dosage unit contains between about 1 mg and 800 mg of active compound.

Modes of administration

The active compounds may be orally administered, for example, with an inert diluent, or with an assailable edible carrier, or they can be enclosed in hard or soft shell capsules, or they can be compressed into tablets, or they can be incorporated directly with the food of the diet.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form should be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and should be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

The compounds or pharmaceutical compositions may also be administered in injectable dosages by solution or suspension of these materials in a physiologically acceptable diluent with a pharmaceutical adjuvant, carrier or excipient. Such adjuvants, carriers and/or excipients include, but are not limited to, sterile liquids, such as water and oils, with or without the addition of a surfactant and other pharmaceutically and physiologically acceptable components. Illustrative oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, or mineral oil. In general, water, saline, aqueous dextrose and related sugar solution, and glycols, such as propylene glycol or polyethylene glycol, are preferred liquid carriers, particularly for injectable solutions.

The pharmaceutical forms suitable for implantable use include sterile wafers of polycarboxyphenoxypropane-sebacic-acid (pCPP:SA) polymers, poly(D,L-lactic acid), polyhydroxybutyrate, lysine diisocyanate (LDI)-glycerol polyurethane, and poly(D-L lactide-co-glycolide). In all cases, the form should be sterile and should be a wafer or disc of suitable dimensions for surgical implantation in the brain. The polymers should be stable under the conditions of manufacture and storage and should be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The wafers should be biodegradable in the central nervous system, and should permit the slow release of the above mentioned compounds, ranging from 24 hours up to 6 months. Such wafers may be of particular value in enhancing the success of temporal lobe epilepsy surgery by suppressing persistent epileptogenic structures.

Combination Therapy

In one aspect, a compound or a composition will have utility in inhibiting, suppressing, enhancing or stimulating a desired response in a subject, as will be understood by one skilled in the art. In certain embodiments, the compositions further include additional active ingredients, whose activity is useful for the particular application such as primary mitochondrial diseases and secondary mitochondrial disorders

for which the compound is being administered, in particular monomethyl fumarate and dimethylfumarate, clofibrate, bezofibrate, gemfibrizil, fenofibrate and meclofenamic acid.

## EXAMPLES

The foregoing discussion may be better understood in connection with the following representative examples which are presented for purposes of illustrating the principle methods and compositions of the present disclosure, and not by way of limitation. Various other examples will be apparent to the person skilled in the art after reading the present disclosure without departing from the spirit and scope of the present disclosure. It is intended that all such other examples be included within the scope of the appended claims.

### General Materials and Methods

All parts are by weight (e.g., % w/w), and temperatures are in degrees centigrade (°C), unless otherwise indicated.

Melting points are determined with a Hoover melting point apparatus and are uncorrected. Infrared (IR) spectra for the compounds are recorded in KBr discs on a Mattson Satellite FTIR in $cm^{-1}$. $^{1}H$ and $^{13}C$ spectra are recorded in DMSO-$d_6$ on a Bruker Avance III DPX 300 MHz instrument. $^{19}F$ spectra are recorded in DMSO $d_6$ on a Bruker Avance III 600 (564.6 mHz). Chemical shifts are expressed in parts per million (δ) with tetramethylsilane as internal standard. Mass spectrometry is performed on a Thermo Scientific LTQ-FT at the University of Cincinnati Mass Spectrometry facility. The purity of the compounds is monitored by HPLC using a Waters 2695 separation module and a 2487 dual λ absorbance detector with a NovaPak C18 4µm 3.9x150mm column. The mobile phases consisting of acetonitrile/$H_2O$ using a 30 minute gradient. All compounds are ≥95%. Microanalysis is performed by Atlantic Microlab Inc., and all compounds are found to be ±0.4%. All reagents are sourced from Chemical Abstracts service. LogS, LogP, Log BBB, human intestinal absorption, p-glycoprotein category, CYP 2C9 pKi, hERG pIC50, CYP 2D6 affinity category, oral CNS score, IV CNS score, MW, flexibility, and total polar surface area are calculated using StarDrop 7.3.1.33573.

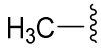
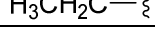
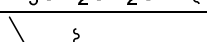
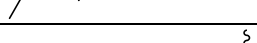
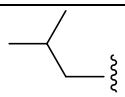
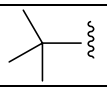
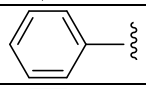
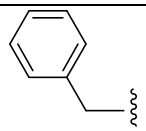
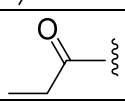
General Chemical Procedures

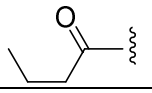
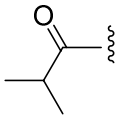
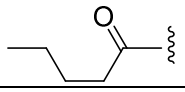
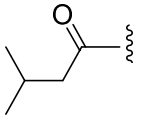
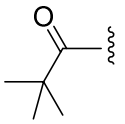
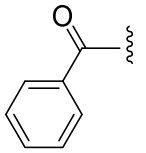
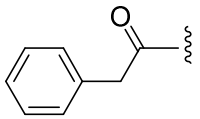
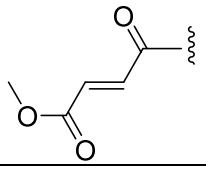
Dry THF preparation: THF is refluxing with molten sodium under nitrogen gas with acetophenone. Increasing temperature results in distilling, anhydrous THF for use in chemical reactions.

MMF-Cl Preparation: Oxalyl chloride (2.0 M in $CH_2Cl_2$; 15.2 mL, 30.3 mmol) is adding dropwise over 2-3 min to a stirring suspension of monomethyl fumarate (2.63 g, 20.2 mmol) in $CH_2Cl_2$ (80 mL) at 0° C under nitrogen. After complete addition, the mixture is stirring at 0° C for 5 min then allowed to warm to room temperature and stirring for 3 hours (a yellow solution developed). A small aliquot is being removed and quenched with MeOH-TLC indicating no acid remaining. The mixture is concentrating under vacuum and $CH_2Cl_2$ (50 mL) is being added to the residue and the mixture is being concentrated under vacuum once more to leave the acid chloride, which is being used directly in the subsequent reactions.

The symbols used in the nomenclature of each of Formulas 1-3, unless otherwise indicated, are preferably those shown in **Table 1** below.

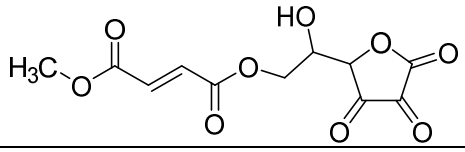
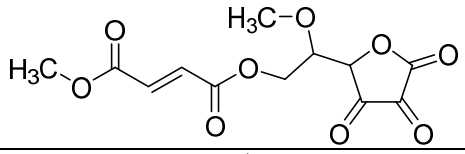
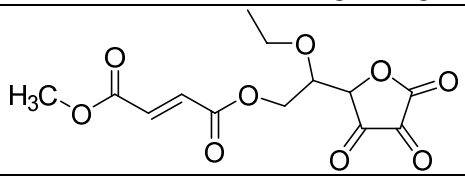
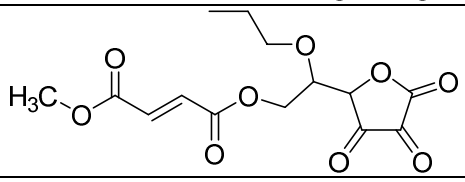
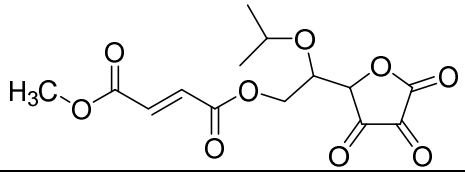
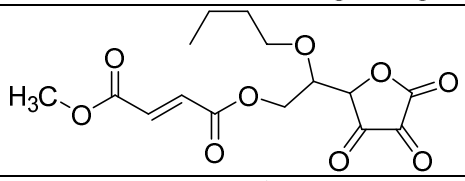
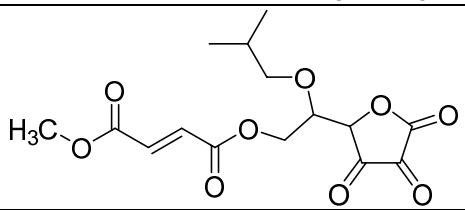
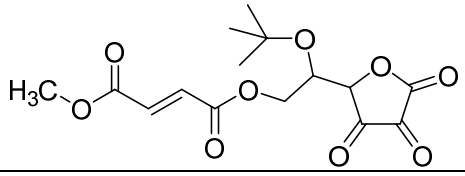
**Table 1**

| Formula | Structure |
|---|---|
| Y or W = hydrogen; Q or T = single bond | |
| Y or W = methyl; Q or T = single bond |  |
| Y or W = ethyl; Q or T = single bond |  |
| Y or W = n-propyl; Q or T = single bond |  |
| Y or W = i-propyl; Q or T = single bond | |
| Y or W = n-butyl; Q or T = single bond |  |
| Y or W = i-butyl; Q or T = single bond |  |
| Y or W = t-butyl; Q or T = single bond |  |
| Y or W = phenyl; Q or T = single bond |  |
| Y or W = benzyl; Q or T = single bond |  |
| Y or W = methyl; Q or T = C(O) | |
| Y or W = ethyl; Q or T = C(O) |  |

| | |
|---|---|
| Y or W = n-propyl; Q or T = C(O) |  |
| Y or W = i-propyl; Q or T = C(O) |  |
| Y or W = n-butyl; Q or T = C(O) |  |
| Y or W = i-butyl; Q or T = C(O) |  |
| Y or W = t-butyl; Q or T = C(O) |  |
| Y or W = phenyl; Q or T = C(O) |  |
| Y or W = benzyl; Q or T = C(O) |  |
| Y or W = C(H)=C(H)$CO_2CH_3$; Q or T = C(O) |  |

Preferred exemplary compounds from **Formula 1** are shown in **Table 2** below.

**Table 2**

| Compound Number | Substituents in **Formula 1** | Structure |
|---|---|---|
| 1 | Formula 1<br>Y = H; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 2 | Formula 1<br>Y = Me; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 3 | Formula 1<br>Y = Et; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 4 | Formula 1<br>Y = n-Pr; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 5 | Formula 1<br>Y = i-Pr; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 6 | Formula 1<br>Y = n-Bu; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 7 | Formula 1<br>Y = i-Bu; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 8 | Formula 1<br>Y = t-Bu; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |

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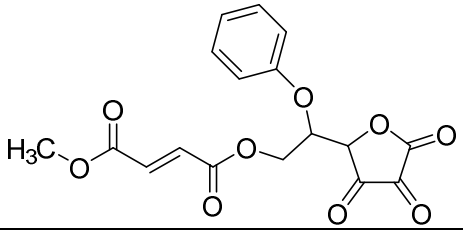
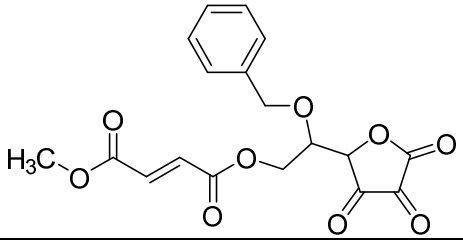
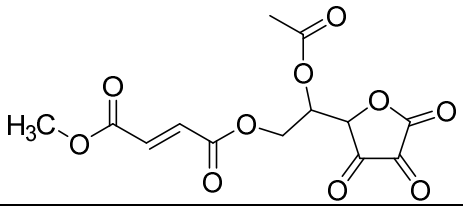
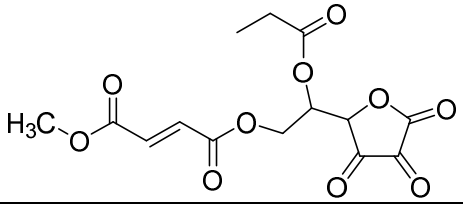
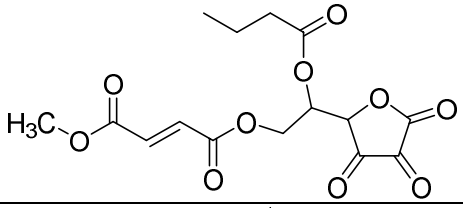
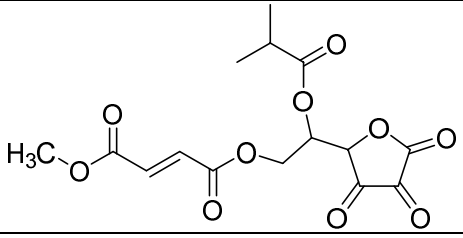
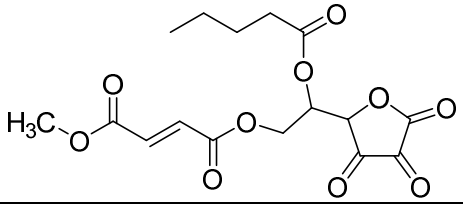
|---|---|---|
| 9 | Formula 1<br>Y = Ph; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 10 | Formula 1<br>Y = Bn; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 11 | Formula 1<br>Y = Me; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 12 | Formula 1<br>Y = Et; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 13 | Formula 1<br>Y = n-Pr; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 14 | Formula 1<br>Y = i-Pr; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 15 | Formula 1<br>Y = n-Bu; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |

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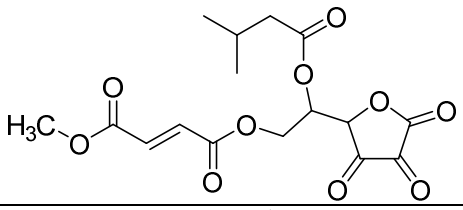
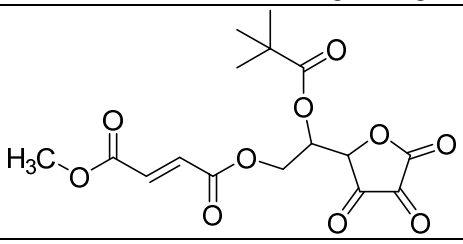
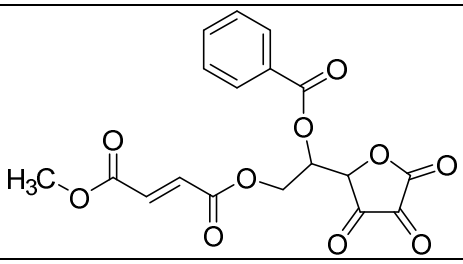
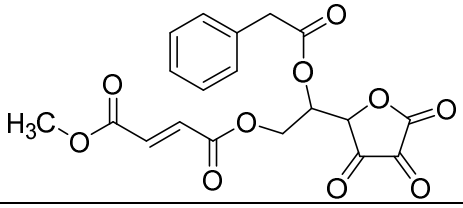
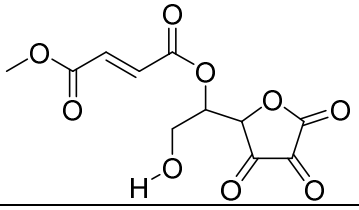
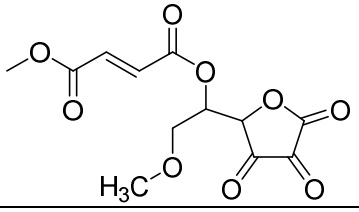
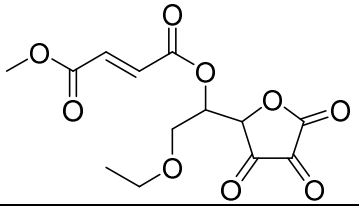
|---|---|---|
| 16 | Formula 1<br>Y = i-Bu; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 17 | Formula 1<br>Y = t-Bu; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 18 | Formula 1<br>Y = Ph; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 19 | Formula 1<br>Y = Bn; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 20 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = H;<br>T = single bond |  |
| 21 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = Me;<br>T = single bond |  |
| 22 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = Et;<br>T = single bond |  |

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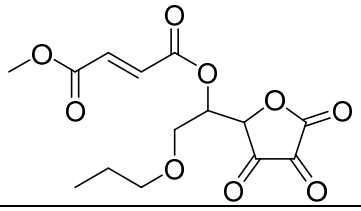
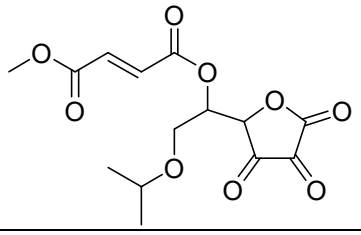
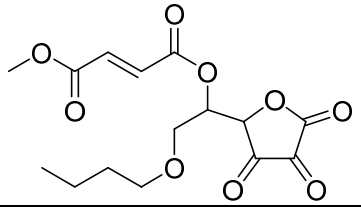
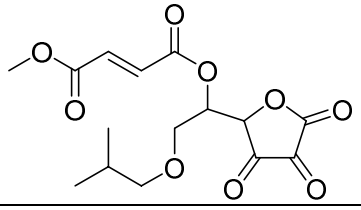
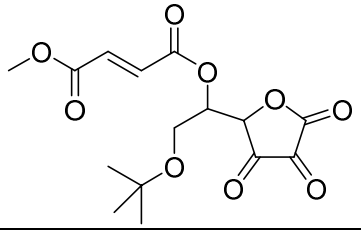
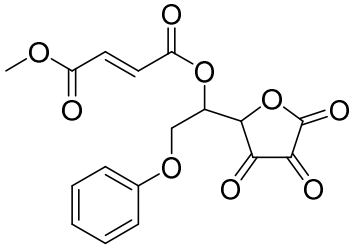
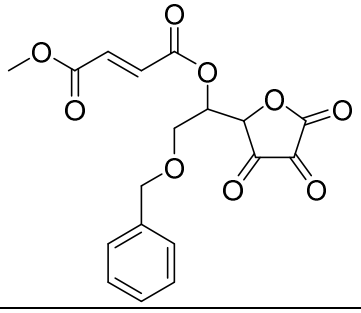
|---|---|---|
| 23 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = n-Pr;<br>T = single bond |  |
| 24 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = i-Pr;<br>T = single bond |  |
| 25 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = n-Bu;<br>T = single bond |  |
| 26 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = i-Bu;<br>T = single bond |  |
| 27 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = t-Bu;<br>T = single bond |  |
| 28 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = Ph;<br>T = single bond |  |
| 29 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = Bn;<br>T = single bond |  |

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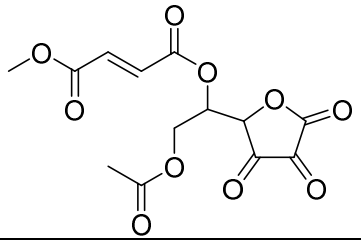
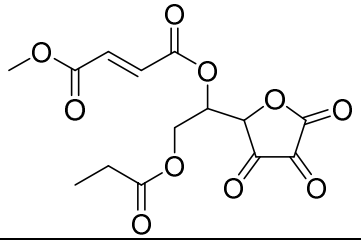
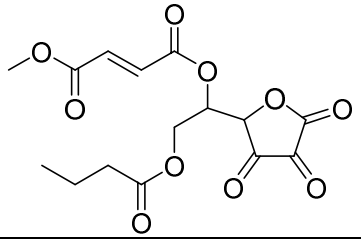
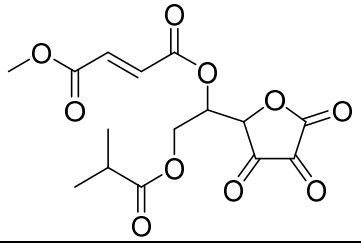
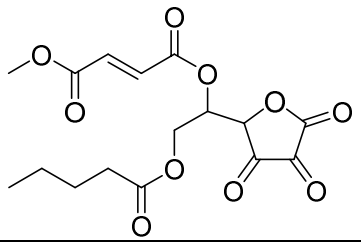
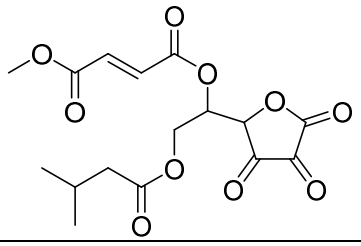
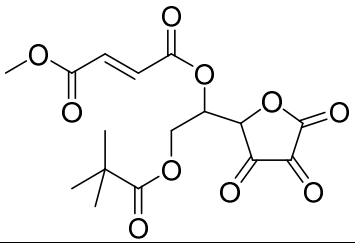
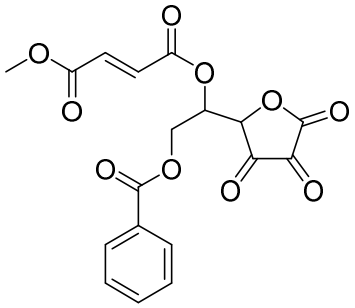
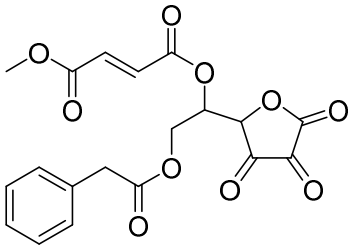
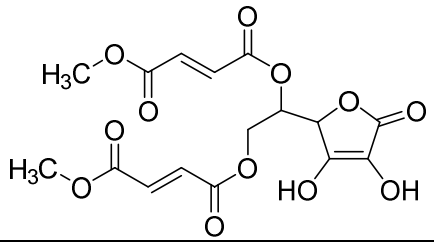
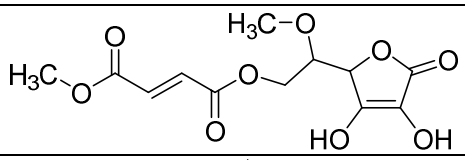
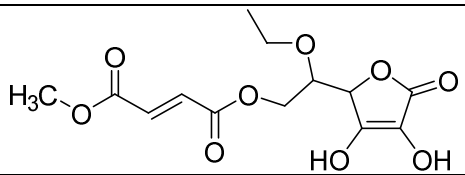
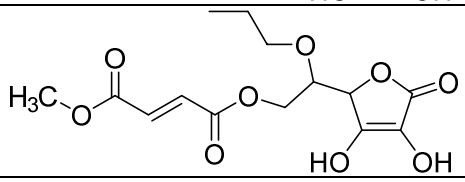
|---|---|---|
| 30 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = Me;<br>T = C(O) |  |
| 31 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = Et;<br>T = C(O) |  |
| 32 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = n-Pr;<br>T = C(O) |  |
| 33 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = i-Pr;<br>T = C(O) |  |
| 34 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = n-Bu;<br>T = C(O) |  |
| 35 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = i-Bu;<br>T = C(O) |  |
| 36 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = i-Bu;<br>T = C(O) |  |

| | | |
|---|---|---|
| 37 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = Ph;<br>T = C(O) |  |
| 38 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = Bn;<br>T = C(O) |  |
| 39 | Formula 1<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |

Preferred exemplary compounds from **Formula 2** are shown in **Table 3** below.

**Table 3**

| Compound Number | Substituents in Formula 2 | Structure |
|---|---|---|
| 40 | Formula 2<br>Y = Me; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 41 | Formula 2<br>Y = Et; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 42 | Formula 2<br>Y = n-Pr; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |

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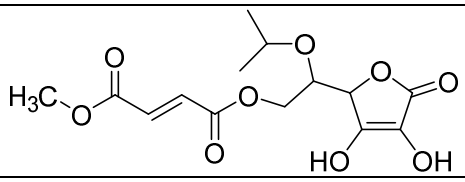
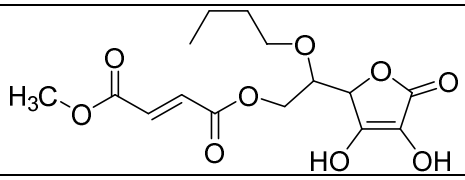
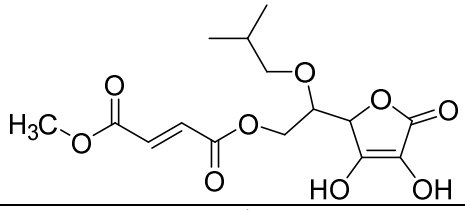
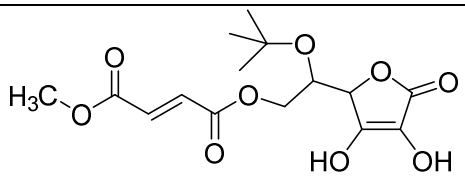
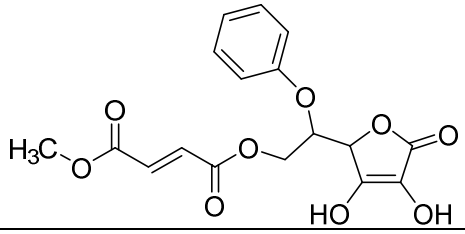
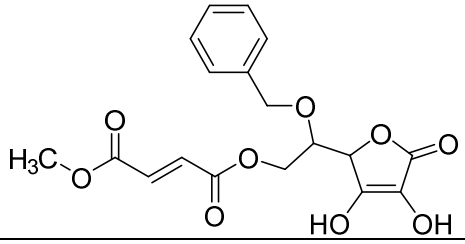
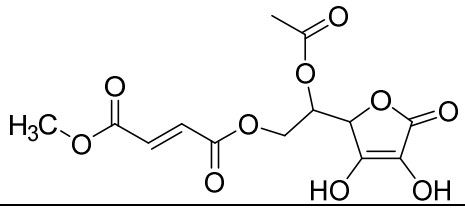
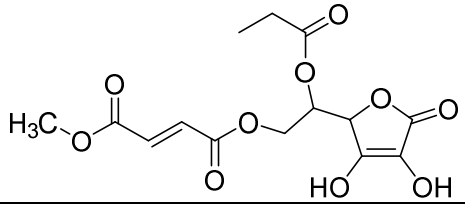
|---|---|---|
| 43 | Formula 2<br>Y = i-Pr; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 44 | Formula 2<br>Y = n-Bu; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 45 | Formula 2<br>Y = i-Bu; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 46 | Formula 2<br>Y = t-Bu; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 47 | Formula 2<br>Y = Ph; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 48 | Formula 2<br>Y = Bn; Q = single bond;<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 49 | Formula 2<br>Y = Me; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 50 | Formula 2<br>Y = Et; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |

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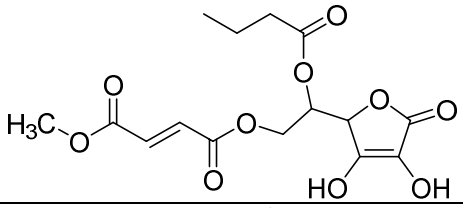
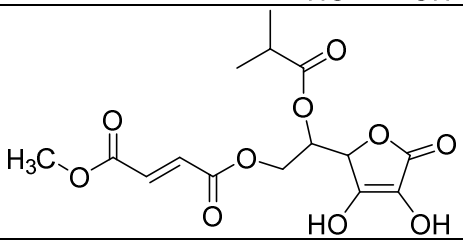
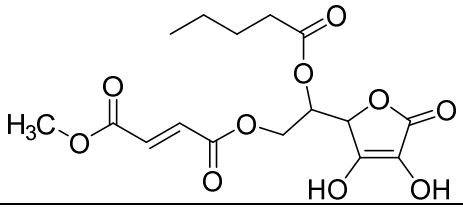
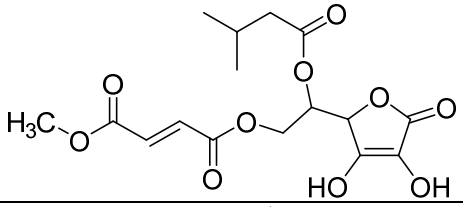
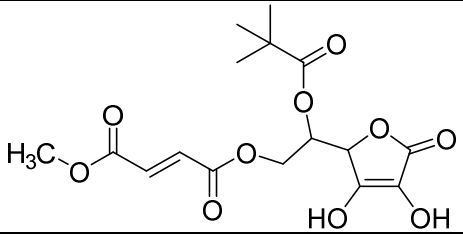
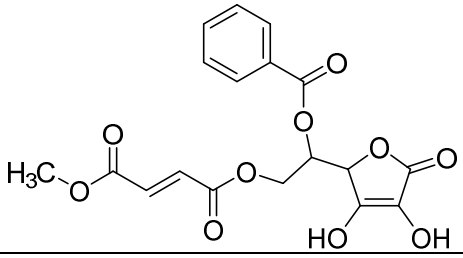
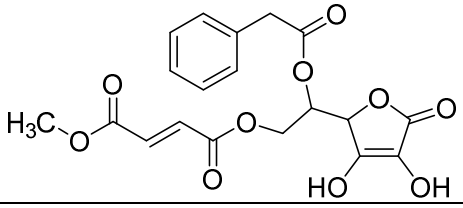
|---|---|---|
| 51 | Formula 2<br>Y = n-Pr; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 52 | Formula 2<br>Y = i-Pr; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 53 | Formula 2<br>Y = n-Bu; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 54 | Formula 2<br>Y = i-Bu; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 55 | Formula 2<br>Y = t-Bu; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 56 | Formula 2<br>Y = Ph; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |
| 57 | Formula 2<br>Y = Bn; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |

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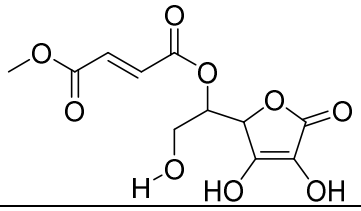
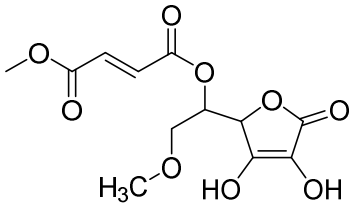
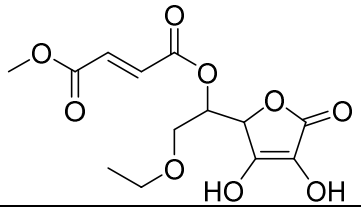
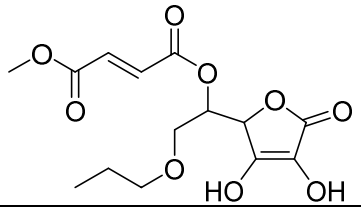
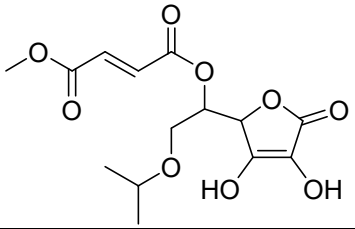
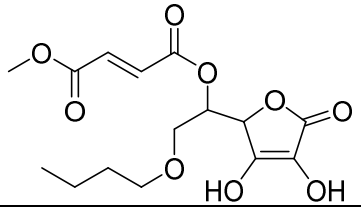
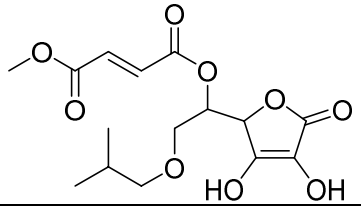
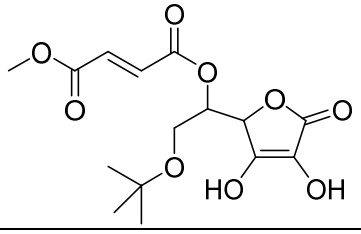
|---|---|---|
| 58 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = H;<br>T = single bond |  |
| 59 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = Me;<br>T = single bond |  |
| 60 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = Et;<br>T = single bond |  |
| 61 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = n-Pr;<br>T = single bond |  |
| 62 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = i-Pr;<br>T = single bond |  |
| 63 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = n-Bu;<br>T = single bond |  |
| 64 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = i-Bu;<br>T = single bond |  |
| 65 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = t-Bu;<br>T = single bond |  |

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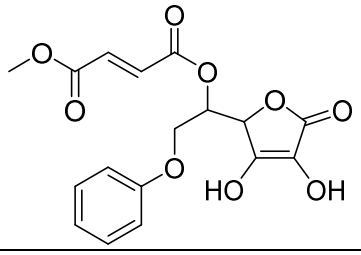
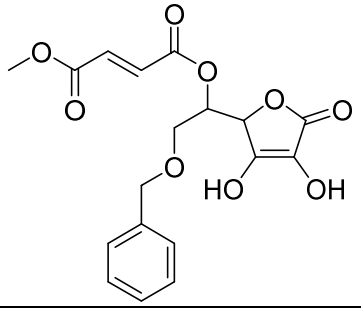
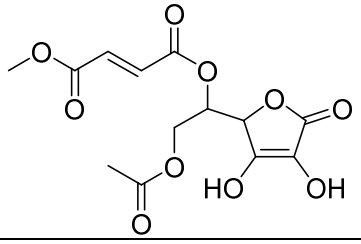
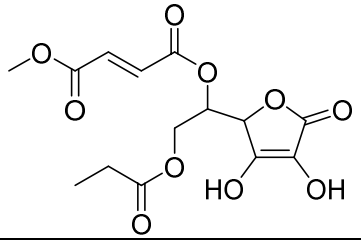
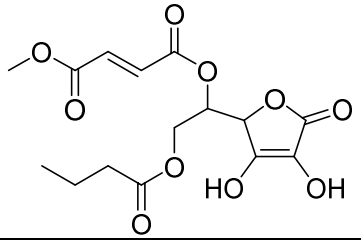
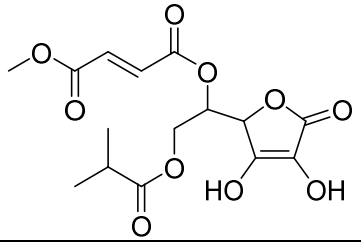
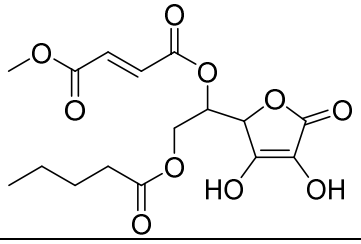
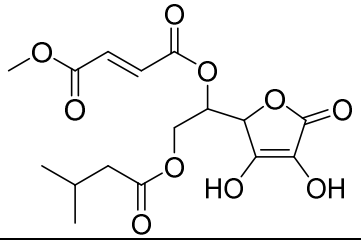
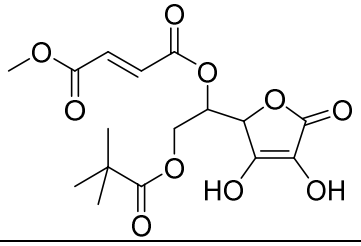
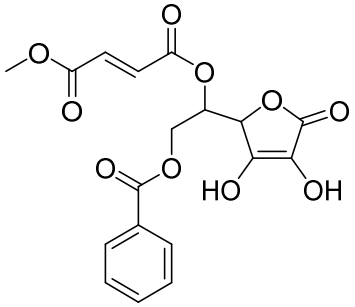
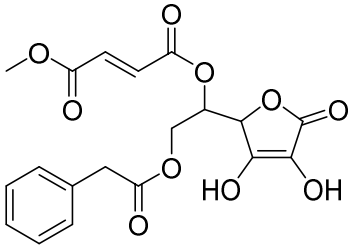
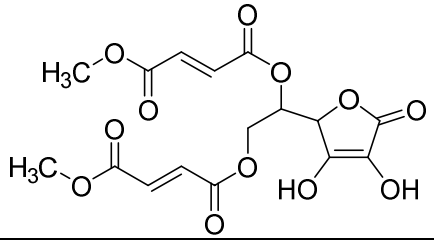
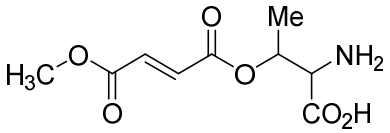
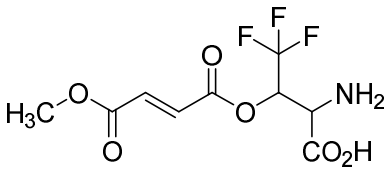
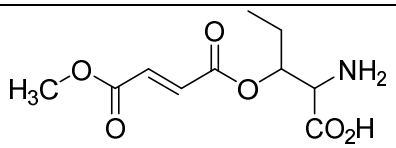
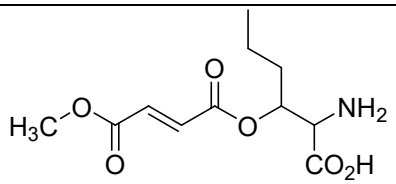
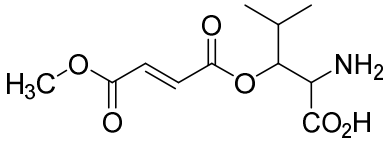
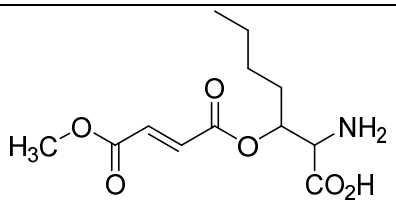
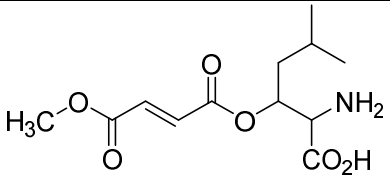
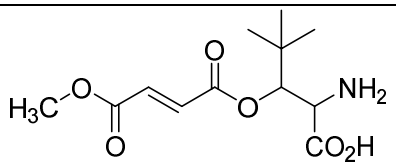
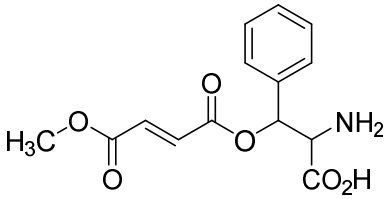
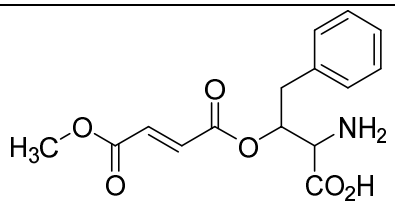
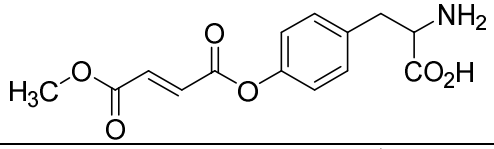
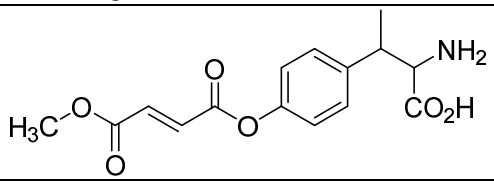
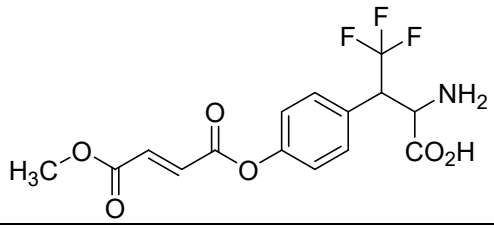
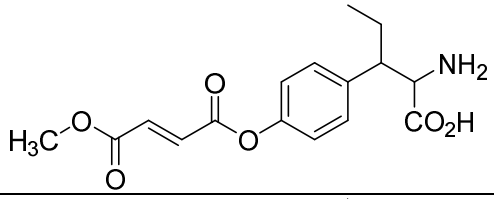
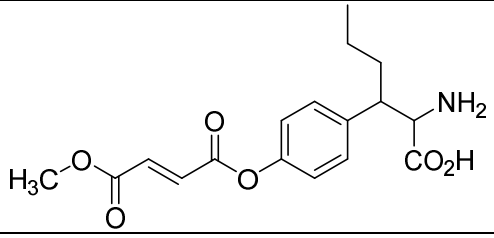
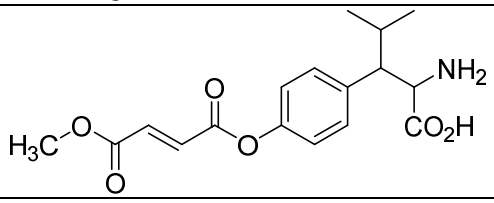
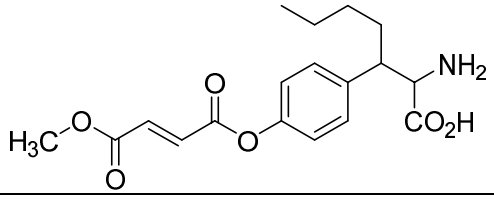
|---|---|---|
| 66 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = Ph;<br>T = single bond |  |
| 67 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = Bn;<br>T = single bond |  |
| 68 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = Me;<br>T = C(O) |  |
| 69 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = Et;<br>T = C(O) |  |
| 70 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = n-Pr;<br>T = C(O) |  |
| 71 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = i-Pr;<br>T = C(O) |  |

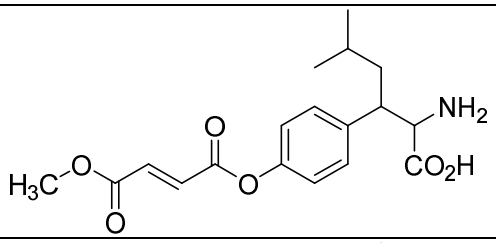
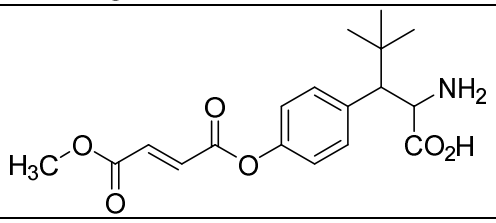
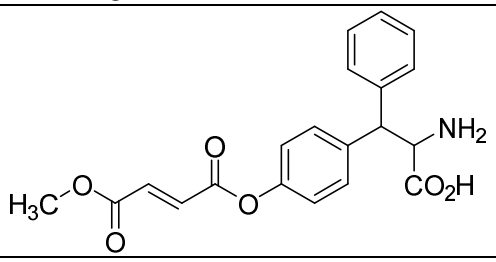
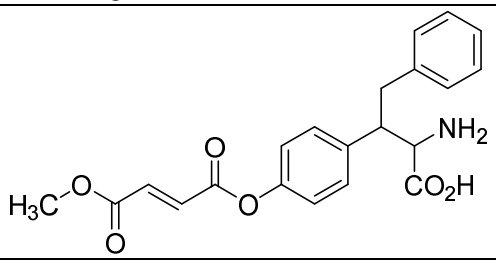
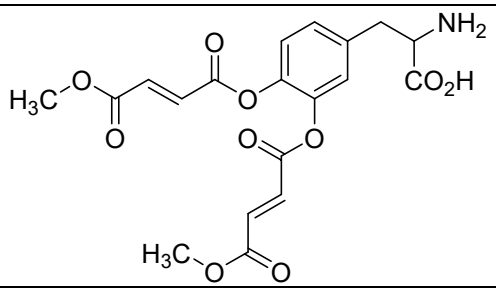
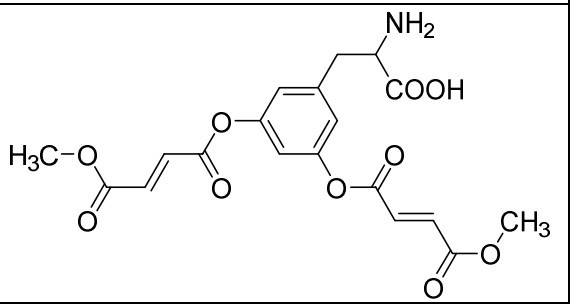
| | | |
|---|---|---|
| 72 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = n-Bu;<br>T = C(O) |  |
| 73 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = i-Bu;<br>T = C(O) |  |
| 74 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = i-Bu;<br>T = C(O) |  |
| 75 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = Ph;<br>T = C(O) |  |
| 76 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = Bn;<br>T = C(O) |  |
| 77 | Formula 2<br>Y = C(H)=C(H)$CO_2CH_3$; Q = C(O);<br>W = C(H)=C(H)$CO_2CH_3$;<br>T = C(O) |  |

Preferred exemplary compounds of **Formula 3** are shown in **Table 4**.

**Table 4**

| Compound Number | Substituents in Formula 3 | Structure |
|---|---|---|
| 78 | U = single bond; V = $C(O)=C(H)CO_2CH_3$; Z = Me; n = 1. |  |
| 79 | U = single bond; V = $C(O)=C(H)CO_2CH_3$; Z = $F_3C$; n = 1. |  |
| 80 | U = single bond; V = $C(O)=C(H)CO_2CH_3$; Z = Et; n = 1. |  |
| 81 | U = single bond; V = $C(O)=C(H)CO_2CH_3$; Z = n-Pr; n = 1. |  |
| 82 | U = single bond; V = $C(O)=C(H)CO_2CH_3$; Z = i-Pr; n = 1. |  |
| 83 | U = single bond; V = $C(O)=C(H)CO_2CH_3$; Z = n-Bu; n = 1. |  |
| 84 | U = single bond; V = $C(O)=C(H)CO_2CH_3$; Z = i-Bu; n = 1. |  |
| 85 | U = single bond; V = $C(O)=C(H)CO_2CH_3$; Z = t-Bu; n = 1. |  |
| 86 | U = single bond; V = $C(O)=C(H)CO_2CH_3$; Z = Ph; n = 1. |  |

| | | |
|---|---|---|
| 87 | U = single bond; V = $C(O)=C(H)CO_2CH_3$; Z = Bn; n = 1. |  |
| 88 | U = Ph; V = $C(O)=C(H)CO_2CH_3$; Z = H; n = 1. |  |
| 89 | U = Ph; V = $C(O)=C(H)CO_2CH_3$; Z = Me; n = 1. |  |
| 90 | U = Ph; V = $C(O)=C(H)CO_2CH_3$; Z = $CF_3$; n = 1. |  |
| 91 | U = Ph; V = $C(O)=C(H)CO_2CH_3$; Z = Et; n = 1. |  |
| 92 | U = Ph; V = $C(O)=C(H)CO_2CH_3$; Z = n-Pr; n = 1. |  |
| 93 | U = Ph; V = $C(O)=C(H)CO_2CH_3$; Z = i-Pr; n = 1. |  |
| 94 | U = Ph; V = $C(O)=C(H)CO_2CH_3$; Z = n-Bu; n = 1. |  |

| | | |
|---|---|---|
| 95 | U = Ph; V = $C(O)=C(H)CO_2CH_3$; Z = i-Bu; n = 1. |  |
| 96 | U = Ph; V = $C(O)=C(H)CO_2CH_3$; Z = t-Bu; n = 1. |  |
| 97 | U = Ph; V = $C(O)=C(H)CO_2CH_3$; Z = Ph; n = 1. |  |
| 98 | U = Ph; V = $C(O)=C(H)CO_2CH_3$; Z = Bn; n = 1. |  |
| 99 | U = Ph; V = $C(O)=C(H)CO_2CH_3$; Z = H; n = 2. |  |
| 100 | U = Ph; V = $C(O)=C(H)CO_2CH_3$; Z = H; n = 2. |  |

**Example 1**

Compound 1: 1 gram of Compound 1001 purchased commercially is dried *en vacuo*. The compound is dissolved in 80mL of fresh, dry THF with stirring under nitrogen, and 1 equivalent of TEA is added dropwise. 1 equivalent of MMF-Cl is freshly prepared and added dropwise in 20mL of dry THF with stirring in an ice bath. The reaction is allowed to warm up to room temperature and then stirred for 12 hours, followed by the addition of 10mL of aqueous saturated $Na_2CO_3$ and is stirred for 1 hour. The reaction is extracted in 3x20mL with diethyl ether, the combined ether extracts are washed with aqueous $NaHCO_3$, 1N HCl and brine, and the ether extract is then dried over sodium sulfate and concentrated *en vacuo*.

**Example 2**

Compound 39: 1 gram of Compound 1001 purchased commercially is dried *en vacuo*. The compound is dissolving in 80mL of fresh, dry THF with stirring under nitrogen, and 2 equivalents of TEA is added dropwise. 2.2 equivalents of MMF-Cl is freshly prepared and is added dropwise in 20mL of dry THF with stirring with an ice bath. The reaction is allowed to warm up to room temperature and is stirred for 24 hours, and 10mL of aqueous saturated $Na_2CO_3$ is added and is stirred for 1 hour. The reaction is extracted 3x20mL with diethyl ether, the combined ether extracts are washed with aqueous saturated $NaHCO_3$, 1N HCl and brine, the ether extract is dried over sodium sulfate and concentrated *en vacuo*.

**Example 3**

Compound 8: 1 gram of Compound 1 is dried *en vacuo*. The compound is dissolved in 80mL of fresh, dry THF with stirring under nitrogen, and 1 equivalent of TEA is added dropwise. 1 equivalent of pivaloyl chloride is added dropwise in 20mL of dry THF with stirring with an ice bath. The reaction is allowed to warm up to room temperature and is stirred for 12 hours, and 10mL of aqueous saturated $Na_2CO_3$ is added and is stirred for 1 hour. The reaction is extracted 3x20mL with diethyl ether, the combined ether extracts are washed with aqueous $NaHCO_3$, 1N HCl and brine, the ether extract is dried over sodium sulfate and concentrated *en vacuo*.

**Example 4**

Compound 1021: 1 gram of Compound 1001 purchased commercially is dried *en vacuo*. The compound is dissolved in 80mL of fresh, dry THF with stirring under nitrogen, and 1 equivalent of TEA is added dropwise. 1 equivalent of pivaloyl chloride is added dropwise in 20mL of dry THF with stirring with an ice bath. The reaction is allowed to warm up to room temperature and is stirred for 12 hours, and 10mL of aqueous saturated $Na_2CO_3$ is added and is stirred for 1 hour. The reaction is extracted 3x20mL with diethyl ether, the combined ether extracts are washed with aqueous $NaHCO_3$, 1N HCl and brine, the ether extract is dried over sodium sulfate and concentrated *en vacuo*.

**Example 5**

Compound 36: 1 gram of Compound 1021 is dried *en vacuo*. The compound is dissolved in 80mL of fresh, dry THF with stirring under nitrogen, and 1 equivalent of TEA is added dropwise. 1 equivalent of MMF-Cl is freshly prepared and is added dropwise in 20mL of dry THF with stirring with an ice bath. The reaction is allowed to warm up to room temperature and is stirred for 24 hours, and 10mL of aqueous saturated $Na_2CO_3$ is added and is stirred for 1 hour. The reaction is extract 3x20mL with diethyl ether, the combined ether extracts are washed with aqueous saturated $NaHCO_3$, 1N HCl and brine, the ether extract is then dried over sodium sulfate and concentrated *en vacuo*.

**Example 6**

Compound 1067: 1 gram of Compound 1001 purchased commercially is dried *en vacuo*. The compound is dissolved in 80mL of fresh, dry THF with stirring under nitrogen, and 1 equivalent of TEA is added dropwise. 1 equivalent of benzoyl chloride is added dropwise in 20mL of dry THF with stirring with an ice bath. The reaction is allowed to warm up to room temperature and is stirred for 12 hours, and 10mL of aqueous saturated $Na_2CO_3$ is added and is stirred for 1 hour. The reaction is extract 3x20mL with diethyl ether, the combined ether extracts are washed with aqueous $NaHCO_3$, 1N HCl and brine, the ether extract is then dried over sodium sulfate and concentrated *en vacuo*.

**Example 7**

Compound 37: 1 gram of Compound 1067 is dried *en vacuo*. The compound is dissolved in 80mL of fresh, dry THF with stirring under nitrogen, and 1 equivalent of TEA is added dropwise. 1 equivalent of MMF-Cl is freshly prepared and is added dropwise in 20mL of dry THF with stirring with an ice bath. The reaction is allowed to warm up to room temperature and is stirred for 24 hours, and 10mL of aqueous saturated $Na_2CO_3$ is added and is stirred for 1 hour. The reaction is extract 3x20mL with diethyl ether, the combined ether extracts are washed with aqueous saturated $NaHCO_3$, 1N HCl and brine, the ether extract is then dried over sodium sulfate and concentrated *en vacuo*.

**Example 8**

Compound 1008: 1 gram of Compound 1001 purchased commercially is dried *en vacuo*. The compound is dissolved in 80mL of fresh, dry $CH_2Cl_2$ with stirring under nitrogen, and 0.1 equivalent of tosic acid is added. 1 equivalent of isobutylene is bubbled through the reaction mixture. The reaction is stirred at room temperature for 16 hours. The reaction is washed with aqueous $NaHCO_3$ and brine, and the $CH_2Cl_2$ layer is dried over sodium sulfate and concentrated *en vacuo*.

**Example 9**

Compound 27: 1 gram of Compound 1008 is dried *en vacuo*. The compound is dissolved in 80mL of fresh, dry THF with stirring under nitrogen, and 1 equivalent of TEA is added dropwise. 1 equivalent of MMF-Cl is freshly prepared and is added dropwise in 20mL of dry THF with stirring with an ice bath. The reaction is allowed to warm up to room temperature and is stirred for 24 hours, and 10mL of aqueous saturated $Na_2CO_3$ is added and is stirred for 1 hour. The reaction is extracted 3x20mL with diethyl ether, the combined ether extracts are washed with aqueous saturated $NaHCO_3$, 1N HCl and brine, the ether extract is dried over sodium sulfate and concentrated *en vacuo*.

**Example 10**

Compound 1072: 1 gram of Compound 1071 purchased commercially is dried *en vacuo*. The compound is dissolved in 80mL of fresh, dry THF with stirring under nitrogen, and 1 equivalent of TEA is added

dropwise. 1 equivalent of MMF-Cl is freshly prepared and is added dropwise in 20mL of dry THF with stirring with an ice bath. The reaction is allowed to warm up to room temperature and is stirred for 12 hours, and 10mL of aqueous saturated $Na_2CO_3$ is added and is stirred for 1 hour. The reaction is extracted 3x20mL with diethyl ether, the combined ether extracts are washed with aqueous $NaHCO_3$, 1N HCl and brine, the ether extract is dried over sodium sulfate and concentrated *en vacuo*.

**Example 11**

Compound 78: 1 gram of Compound 1072 is dissolved in 20mL of TFA and is left at room temperature for 10 minutes. The flask is chilled at -78°C until frozen, and concentrated *en vacuo*.

**Example 12**

Compound 1084: 1 gram of Compound 1083 purchased commercially is dried *en vacuo*. The compound is dissolved in 80mL of fresh, dry THF with stirring under nitrogen, and 1 equivalent of TEA is added dropwise. 1 equivalent of MMF-Cl is freshly prepared and is added dropwise in 20mL of dry THF with stirring with an ice bath. The reaction is allowed to warm up to room temperature and is stirred for 12 hours, and 10mL of aqueous saturated $Na_2CO_3$ is added and is stirred for 1 hour. The reaction is extracted 3x20mL with diethyl ether, the combined ether extracts are washed with aqueous $NaHCO_3$, 1N HCl and brine, the ether extract is dried over sodium sulfate and concentrated *en vacuo*.

**Example 13**

Compound 88: 1 gram of Compound 1084 is dissolved in 20mL of TFA and is left at room temperature for 10 minutes. The flask is chilled at -78°C until frozen, and concentrated *en vacuo*.

**Example 14**

***In vitro* drug evaluation**

For these MMF prodrugs, it is desirable that the prodrug remains intact while in the gastrointestinal tract and in systemic circulation and be cleaved to MMF in the target tissue, the brain. A useful level of stability

can at least in part be determined by the mechanism and pharmacokinetics of the prodrug or active compound. In general, prodrugs or active compounds that are more stable in pancreatin or colonic wash assay and in mouse plasma, human plasma, mouse liver S9, and/or human liver S9 preparations, can be useful as an orally administered prodrugs. In particular, prodrugs that are stable in mouse plasma, human plasma, mouse liver S9, and/or human liver S9 preparations, and which are more labile in cell homogenate preparations such CaCo2 S9 preparations or brain homogenate, can be useful as systemically administered prodrugs and can be more effective in delivering a prodrug or active compound to a target tissue, such as the brain. In general, prodrugs that are more stable in a range of pH physiological buffers (pH 6.0 to pH 8.5) can be more useful as orally administered. In general, prodrugs that are more labile in cell homogenate preparations, such CaCo2 S9 preparations or mouse brain lysate, can be intracellularly metabolized. The results of tests, such as those described in this example, for determining the enzymatic or chemical cleavage of compounds *in vitro* can be used to select prodrugs for *in vivo* testing.

The stabilities of MMF prodrugs can be evaluated in one or more *in vitro* systems using a variety of preparations following methods known in the art. For example, methods used to determine the stability of prodrugs in Caco2 S9 homogenate, mouse brain lysate, rat liver S9, mouse plasma, porcine pancreatin, rat colonic wash, and pH 8.0 buffer are described herein.

$CaCo_2$ S9 homogenate is prepared using the following procedure. CaCo2 cells are grown in culture for 21 days prior to harvesting. Culture medium is removed from the culture vessel and the monolayer is rinsed twice with 10-15 mL chilled phosphate buffered saline (PBS) buffer. PBS buffer (7-10 mL) is added to the flask and the cells scraped from the growth surface and transferred to a centrifuge tube. The cells are pelleted by centrifugation at 1,500 rpm for 5 min at 4°C. The supernatant is removed and the cell pellet washed with ice cold PBS and re-pelleted by centrifugation. The supernatant is removed and the pellet re-suspended in cell lysis buffer (0.15M KCl and 10 mM sodium phosphate buffer, pH 7.4). Cells are lysed by sonication at 4°C using a probe sonicator. The lysed cells are then transferred to vials and centrifuged at 1,600 rpm for 10 min at 4°C to remove intact cells, nuclei, and large cellular debris. The supernatant is removed and transferred to a tube for centrifugation at 8,600 rpm for 20 min at 4°C. After centrifugation, the resulting supernatant, representing the CaCo2 cell homogenate S9 fraction, is carefully removed and aliquoted into vials for storage at -80°C until the time of use. At the time of use, CaCo2 S9 lysate is diluted to 0.5 mg/mL in 0.1M Tris buffer, pH 7.4.

Rat liver S9 (Sigma Aldrich, St. Louis, MO; S2067) is diluted to 0.5 mg/mL in 0.1 M potassium phosphate buffer at pH 7.4 and ImM NADPH cofactor. Mouse plasma (Creative Biolabs, Shirley, NY; SPS9364) is used

as obtained from the supplier. Mouse liver S9 (Sigma Aldrich, St. Louis, MO; S2192) S9 is diluted to 0.5 mg/mL in 0.1 M potassium phosphate buffer at pH 7.4 and ImM NADPH cofactor. Mouse brain tissue lysate (Abcam, Cambridge, UK, ab7188) is used as obtained from the supplier. Porcine pancreatin (Sigma Aldrich, St. Louis, MO; P1625-100G) is diluted to 10 mg/mL in 0.1 M Tris buffer, pH 7.4.

To prepare the rat colonic wash, the colon between the ceacum and rectum is resected from a euthanized rat. Five to 10 mL of PBS pH 7.4 buffer (depending on the weight of the rat) is flushed into the lumen of the large intestine and collected into a 250 mL glass beaker at 0°C (ice bath). The colonic wash is transferred into 10 mL conical tubes using a 10 mL syringe fitted with a filter. Samples of 0.5 mL colonic wash are stored at -80°C until the time of use. Colonic wash is used without dilution.

The enzymatic stability assays for a compound in CaCo2 S9, rat liver S9, mouse plasma, mouse brain homogenate, pig pancreatin, and rat colonic wash are performed using the following procedure. Ninety (90) µL of lysate is aliquoted to designated tubes on a cluster plate. The lysate is pre-incubated for 10 min at 37°C. With the exception of the t(0) time point, 10 µL of a 400 µM solution of test compound in 0.1M Tris buffer, pH 7.4 is added to multiple wells, representing different incubation times. The samples are incubated at 37°C. At each time point, the reaction is quenched by adding 300 µL of 100% ethanol. The samples are thoroughly mixed, the tubes transferred to a V-bottom plate, and stored at -20°C. For the t(0) time point, the lysate is quenched with 300 µL of ice cold 100% ethanol, thoroughly mixed, 10 µL of 400 µM test compound is added and mixed, and the sample tube transferred to a V-bottom plate and stored at -20°C. For analysis, 180 µL from each sample is transferred to a 96 well V-bottom plate and sealed. After all time points are collected, the plate is centrifuged for 10 min at 5600 rpm at 4°C. One-hundred fifty (150) µL from each well is then transferred to a 96 well round bottom plate. Samples are analyzed using LC/MS/MS to determine the concentrations of the compound and/or metabolite thereof.

For the pH 8.0 stability studies, 190 µL of 150 mM $NaH_2P0_4$ buffer pH 8.0 is added to each sample tube. Ten (10) µL of 20 mM test compound is added to each tube and mixed. The samples are incubated for 60 min at 37°C. Following incubation, the samples are transferred to room temperature and 800 µL of 50% acetonitrile in water is added to each tube. Samples are analyzed using LC/MS/MS to determine the concentrations of the compound and/or metabolite thereof.

LC/MS/MS analysis for MMF is performed using an API 4000 equipped with an Agilent 1100 HPLC and a Leap Technologies autosampler. An HPLC Phenomenex Onyx Monolithic C18 (CH0-7644) column at a temperature of 35°C, flow rate of 2.0 mL/min, injection volume of 30 µL, and a 3-min run time is used. The

mobile phase A is 0.1% formic acid in water and Mobile phase B is 0.1% formic acid in acetonitrile. The gradient is 98% A /2% B at time 0; 98% A / 2% B at time 0.1 min; 5% A / 95% B at time 1.4 min; 5% A / 95% B at time 2.2 min; 98% A / 2% B at time 2.3 min; and 98% A / 2% B at time 3.0 min. MMF content is determined using negative ion mode (Q1 128.94; Q2 71).

## Example 15

### *In vivo* Pharmacokinetics and Oral Bioavailability

Rats are obtained commercially and are pre-cannulated in the jugular vein. Animals are conscious at the time of the experiment. All animals are fasted overnight and until 4 hours post- dosing of a compound of the disclosure. Blood samples (0.3 mL/sample) are collected from all animals prior to dosing and at different time-points up to 24 h post-dose into tubes containing EDTA. Two aliquots (100 pL each) are quenched with 300 pL methanol and stored at -20°C prior to analysis.

To prepare analysis standards, 90 pL of rat blood is quenched with 300 µ L methanol followed by 10 pL of spiking standard and/or 20 µ L of internal standard. The sample tubes are vortexed for at least 2 min and then centrifuged at 3,400 rpm for 20 min. The supernatant is then transferred to an injection vial or plate for analysis by LC-MS-MS.

To prepare samples for analysis, 20 pL of internal standard is added to each quenched sample tube. The sample tubes are vortexed for at least 2 min and then centrifuged at 3,400 rpm for 20 min. The supernatant is then transferred to an injection vial or plate for analysis by LC/MS/MS.

LC/MS/MS analysis for MMF is performed using an API 4000 equipped with an Agilent 1100 HPLC and a Leap Technologies autosampler. An HPLC Phenomenex Onyx Monolithic C18 (CH0-7644) column at a temperature of 35°C, flow rate of 2.0 mL/min, injection volume of 30 µL, and a 3-min run time is used. The mobile phase A is 0.1% formic acid in water and Mobile phase B is 0.1% formic acid in acetonitrile. The gradient is 98% A /2% B at time 0; 98% A / 2% B at time 0.1 min; 5% A / 95% B at time 1.4 min; 5% A / 95% B at time 2.2 min; 98% A / 2% B at time 2.3 min; and 98% A / 2% B at time 3.0 min. MMF content is determined using negative ion mode (Q1 128.94; Q2 71).

Non-compartmental analysis is performed using WinNonlin software (v.3.1 Professional Version, Pharsight Corporation, Mountain View, California) on individual animal profiles. Summary statistics on major parameter estimates is performed for Cmax (peak observed concentration following dosing), Tmax (time

to maximum concentration is the time at which the peak concentration is observed), AUC(o-t) (area under the plasma concentration-time curve from time zero to last collection time, estimated using the log-linear trapezoidal method), AUC(o-∞), (area under the plasma concentration time curve from time zero to infinity, estimated using the log-linear trapezoidal method to the last collection time with extrapolation to infinity), and ti/2,z (terminal half-life).

A compound of the disclosure is administered by oral gavage to groups of four to six adult male Sprague-Dawley rats (about 250 g). Animals are conscious at the time of the experiment. A compound of the disclosure is orally or colonically administered in 3.4% Phosal at a dose of 70 mg-equivalents MHF per kg body weight.

The percent relative bioavailability (F%) of the administered compound or metabolite thereof is determined by comparing the area under the respective concentration vs time curve (AUC) following oral or colonic administration of a compound of the disclosure with the AUC of the concentration vs time curve following intravenous administration of the compound of the disclosure, respectively, on a dose-normalized basis.

The %F can be reported as the mean %F of all animals dosed orally with the compound of the disclosure at the specified level.

## Example 16

### *EAE model of Multiple Sclerosis*

Female C57BL/6 mice, 8-10 weeks old (Harlan Laboratories, Livermore, CA), are immunized subcutaneously in the flanks and mid-scapular region with 200µg of myelin oligodendrocyte glycoprotein peptide 35-55 ($MOG_{35-55}$) (synthesized by Invitrogen) emulsified (1:1 volume ratio) with complete Freund's adjuvant (CFA) (containing 4 mg/mL *Mycobacterium tuberculosis*). Emulsion is prepared by the syringe-extrusion method with two glass Luer-Lock syringes connected by a 3-way stopcock. Mice are also given an intraperitoneal injection of 200 ng pertussis toxin (List Biological Laboratories, Inc, Campbell, CA) on the day of immunization and on day two post immunization. Mice are weighed and examined daily for clinical signs of experimental autoimmune encephalomyelitis (EAE). Food and water is provided ad libitum and once animals start to show disease, food is provided on the cage bottom. All experiments are approved by the Institutional Animal Care and Use Committee.

Clinical Evaluation

Mice are scored daily beginning on day 7 post immunization. The clinical scoring scale is as follows: 0 = normal; I = limp tail or hind limb weakness (defined by foot slips between bars of cage top while walking); 2 = limp tail and hind limb weakness; 3 = partial hind limb paralysis (defined as no weight bearing on hind limbs but can still move one or both hind limbs to some extent); 4 = complete hind limb paralysis; 5 = moribund state (includes forelimb paralysis) or death.

Treatment

Compound(s) of the disclosure (i.e., "test compound(s)") are dissolved in 0.5% methocellulose/0.1% Tween80 in distilled water and administered by oral gavage twice daily starting from day 3 post-immunization until termination. Dexamethasone is dissolved in 1x PBS buffer and administered subcutaneously once daily. Treatment groups are, for example, as follows: vehicle alone, 15 mg/kg dimethylfumarate, 20 mg/kg compound of the disclosure, and 1 mg/kg dexamethasone.

Alternate Animal Models of Multiple Sclerosis

The following experiment confirmed that MMF is the active moiety of MMF prodrugs, DMF and the compounds of the disclosure and examined the relationship between MMF exposure and effect in animal models of multiple sclerosis (MS). Efficacy of representative compound of the disclosure and dimethylfumarate is compared in the $MOG_{35-55}$ mouse EAE model of multiple sclerosis. C57BF/6 mice (6 females) are injected subcutaneously with $MOG_{35-55}$ peptide in CFA with *Mycobacterium tuberculosis*. Pertussis toxin (200 mg) is injected IV on Day 0 and Day 2 post-immunization. Animals received oral test compound or DMF (90 mg-eq MMF/kg twice daily) or vehicle on Days 3 to 29. Daily EAE clinical disease scores (5 point scale) are recorded. End of study MMF blood levels are determined by LC/MS/MS.

## Example 17

### *Animal Model for Assessing Therapeutic Efficacy in Escalating Dose of MMF Prodrugs for Multiple Sclerosis*

Experiments are conducted on female mice aged 4-6 weeks belong to the C57BL/6 strain weighing 17-20 g. Experimental autoimmune encephalomyelitis (EAE) is actively induced using >95% pure synthetic $MOG_{35-55}$. Each mouse is anesthetized and receives 200 pg of $MOG_{35-55}$ peptide and 15 µg of Saponin extract from Quilija bark emulsified in 100 µL of phosphate-buffered saline. A 25 µL volume is injected

subcutaneously over four flank areas. Mice are also intraperitoneally injected with 200 ng of pertussis toxin in 200 µL of PBS. A second, identical injection of pertussis toxin is given after 48 h.

A compound of the disclosure is administered at varying doses. Control animals receive 25 µL of DMSO. Daily treatment extends from day 26 to day 36 post-immunization. Clinical scores are obtained daily from day 0 post-immunization until day 60. Clinical signs are scored using the following protocol: 0, no detectable signs; 0.5, distal tail limpness, hunched appearance and quiet demeanor; I, completely limp tail; 1.5, limp tail and hindlimb weakness (unsteady gait and poor grip with hind limbs); 2, unilateral partial hind limb paralysis; 2.5, bilateral hind limb paralysis; 3, complete bilateral hindlimb paralysis; 3.5, complete hindlimb paralysis and unilateral forelimb paralysis; 4, total paralysis of hind limbs and forelimbs.

Inflammation and demyelination are assessed by histology on sections from the CNS of EAE mice. Mice are sacrificed after 30 or 60 days and whole spinal cords are removed and placed in 0.32 M sucrose solution at 4°C overnight. Tissues are prepared and sectioned. Luxol fast blue stain is used to observe areas of demyelination. Haematoxylin and eosin staining is used to highlight areas of inflammation by darkly staining the nuclei of mononuclear cells. Immune cells stained with H&E are counted in a blinded manner under a light microscope. Sections are separated into gray and white matter and each sector is counted manually before being combined to give a total for the section. T cells are immunolabeled with anti-CD3+ monoclonal antibody. After washing, sections are incubated with goat anti-rat HRP secondary antibody. Sections are then washed and counterstained with methyl green. Splenocytes isolated from mice at 30 and 60 days post-immunization are treated with lysis buffer to remove red blood cells. Cells are then resuspended in PBS and counted. Cells at a density of about $3x10^6$ cells/mL are incubated overnight with 20 µg/mL of MOG peptide. Supernatants from stimulated cells are assayed for IFN-γ protein levels using an appropriate mouse IFN-γ immunoassay system.

## Example 18

### *Animal Models of Parkinson's Disease*

MPTP Induced Neurotoxicity

MPTP, or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, is a neurotoxin that produces a Parkinsonian syndrome in both humans and experimental animals. Studies of the mechanism of MPTP neurotoxicity show that it involves the generation of a major metabolite, $MPP^+$, formed by the activity of monoamine

oxidase on MPTP. Inhibitors of monoamine oxidase block the neurotoxicity of MPTP in both mice and primates. The specificity of the neurotoxic effects of $MPP^+$ for dopaminergic neurons appears to be due to the uptake of $MPP^+$ by the synaptic dopamine transporter. Blockers of this transporter prevent $MPP^+$ neurotoxicity. $MPP^+$ has been shown to be a relatively specific inhibitor of mitochondrial complex I activity, binding to complex I at the rotenone binding site and impairing oxidative phosphorylation. *In vivo* studies have shown that MPTP can deplete striatal ATP concentrations in mice. It has been demonstrated that $MPP^+$ administered intrastriatally to rats produces significant depletion of ATP as well as increased lactate concentration confined to the striatum at the site of the injections. Compounds that enhance ATP production can protect against MPTP toxicity in mice.

A MMF prodrug is administered to animals such as mice or rats for three weeks before treatment with MPTP. MPTP is administered at an appropriate dose, dosing interval, and mode of administration for 1 week before sacrifice. Control groups receive either normal saline or MPTP hydrochloride alone. Following sacrifice the two striate are rapidly dissected and placed in chilled 0.1 M perchloric acid. Tissue is subsequently sonicated and aliquots analyzed for protein content using a fluorometer assay. Dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) are also quantified. Concentrations of dopamine and metabolites are expressed as nmol/mg protein. MMF prodrugs that protect against DOPAC depletion induced by MPTP, HVA, and/or dopamine depletion are neuroprotective and therefore can be useful for the treatment of Parkinson's disease.

Haloperidol-Induced Hypolocomotion

The ability of a compound to reverse the behavioral depressant effects of dopamine antagonists, such as haloperidol, in rodents is considered a valid method for screening drugs with potential anti-Parkinsonian effects. Hence, the ability of compounds of Formulas 1-3 to block haloperidol-induced deficits in locomotor activity in mice can be used to assess potential anti-Parkinsonian efficacy.

Mice used in the experiments are housed in a controlled environment and allowed to acclimatize before experimental use. One and one-half (1.5) hours before testing, mice are administered 0.2 mg/kg haloperidol, a dose that reduces baseline locomotor activity by at least 50%. A test compound is administered 5-60 min prior to testing. The animals are then placed individually into clean, clear polycarbonate cages with a flat perforated lid. Horizontal locomotor activity is determined by placing the cages within a frame containing a 3x6 array of photocells interfaced to a computer to tabulate beam interrupts. Mice are left undisturbed to explore for 1 h, and the number of beam interruptions made

during this period serves as an indicator of locomotor activity, which is compared with data for control animals for statistically significant differences.

6-Hydroxydopamine Animal Model

The neurochemical deficits seen in Parkinson's disease can be reproduced by local injection of the dopaminergic neurotoxin, 6-hydroxydopamine (6-OHDA) into brain regions containing either the cell bodies or axonal fibers of the nigrostriatal neurons. By unilaterally lesioning the nigrostriatal pathway on only one-side of the brain, a behavioral asymmetry in movement inhibition is observed. Although unilaterally-lesioned animals are still mobile and capable of self-maintenance, the remaining dopamine-sensitive neurons on the lesioned side become supersensitive to stimulation. This is demonstrated by the observation that following systemic administration of dopamine agonists, such as apomorphine, animals show a pronounced rotation in a direction contralateral to the side of lesioning. The ability of compounds to induce contralateral rotations in 6-OHDA lesioned rats has been shown to be a sensitive model to predict drug efficacy in the treatment of Parkinson's disease.

Male Sprague-Dawley rats are housed in a controlled environment and allowed to acclimatize before experimental use. Fifteen minutes prior to surgery, animals are given an intraperitoneal injection of the noradrenergic uptake inhibitor desipramine (25 mg/kg) to prevent damage to nondopamine neurons. Animals are then placed in an anesthetic chamber and anesthetized using a mixture of oxygen and isoflurane. Once unconscious, the animals are transferred to a stereotaxic frame, where anesthesia is maintained through a mask. The top of the head is shaved and sterilized using an iodine solution. Once dry, a 2 cm long incision is made along the midline of the scalp and the skin retracted and clipped back to expose the skull. A small hole is then drilled through the skull above the injection site. In order to lesion the nigrostriatal pathway, the injection cannula is slowly lowered to position above the right medial forebrain bundle at -3.2 mm anterior posterior, -1.5 mm medial lateral from the bregma, and to a depth of 7.2 mm below the dura mater. Two minutes after lowering the cannula, 6-OHDA is infused at a rate of 0.5 µL/min over 4 min, to provide a final dose of 8 µg. The cannula is left in place for an additional 5 min to facilitate diffusion before being slowly withdrawn. The skin is then sutured shut, the animal removed from the sterereotaxic frame, and returned to its housing. The rats are allowed to recover from surgery for two weeks before behavioral testing.

Rotational behavior is measured using a rotameter system having stainless steel bowls (45 cm diameter x 15 cm height) enclosed in a transparent Plexiglas cover around the edge of the bowl and extending to a

height of 29 cm. To assess rotation, rats are placed in a cloth jacket attached to a spring tether connected to an optical rotameter positioned above the bowl, which assesses movement to the left or right either as partial (45°) or full (360°) rotations.

To reduce stress during administration of a test compound, rats are initially habituated to the apparatus for 15 min on four consecutive days. On the test day, rats are given a test compound, e.g., a compound of Formula 1-3. Immediately prior to testing, animals are given a subcutaneous injection of a sub-threshold dose of apomorphine, and then placed in the harness and the number of rotations recorded for one hour. The total number of full contralateral rotations during the hour test period serves as an index of anti-Parkinsonian drug efficacy.

**Example 19**

***Animal Model for Assessing Therapeutic Efficacy of MMF prodrugs for Treating Alzheimer's Disease***

Heterozygous transgenic mice expressing the Swedish AD mutant gene, hAPPK670N, are used as an animal model of Alzheimer's disease. Animals are housed under standard conditions with a 12:12 light/dark cycle and food and water available ad libitum. Beginning at 9 months of age, mice are divided into three groups. The first two groups of animals receive increasing doses of a compound of Formula 1-3 over six weeks. The remaining control group receives daily saline injections for six weeks.

Behavioral testing is performed at each drug dose using the same sequence over two weeks in all experimental groups: (I) spatial reversal learning, (2) locomotion, (3) fear conditioning, and (4) shock sensitivity.

Acquisition of the spatial learning paradigm and reversal learning are tested during the first five days of test compound administration using a water T-maze. Mice are habituated to the water T-maze during days 1-3, and task acquisition begins on day 4. On day 4, mice are trained to find the escape platform in one choice arm of the maze until 6 to 8 correct choices are made on consecutive trails. The reversal learning phase is then conducted on day 5. During the reversal learning phase, mice are trained to find the escape platform in the choice arm opposite from the location of the escape platform on day 4. The same performance criteria and inter-trial interval are used as during task acquisition.

Large ambulatory movements are assessed to determine that the results of the spatial reversal learning paradigm are not influenced by the capacity for ambulation. After a rest period of two days, horizontal

ambulatory movements, excluding vertical and fine motor movements, are assessed in a chamber equipped with a grid of motion-sensitive detectors on day 8. The number of movements accompanied by simultaneous blocking and unblocking of a detector in the horizontal dimension are measured during a one-hour period.

The capacity of an animal for contextual and cued memory is tested using a fear conditioning paradigm beginning on day 9. Testing takes place in a chamber that contains a piece of absorbent cotton soaked in an odor-emitting solution such as mint extract placed below the grid floor. A 5-min, 3 trial 80 db, 2800 Hz tone-foot shock sequence is administered to train the animals on day 9. On day 10, memory for context is tested by returning each mouse to the chamber without exposure to the tone and foot shock, and recording the presence or absence of freezing behavior every 10 seconds for 8 minutes. Freezing is defined as no movement, such as ambulation, sniffing or stereotypy, other than respiration.

On day 11, the response of the animal to an alternate context and to the auditory cue is tested. Coconut extract is placed in a cup and the 80 dB tone is presented, but no foot shock is delivered. The presence or absence of freezing in response to the alternate context is then determined during the first 2 minutes of the trial. The tone is then presented continuously for the remaining 8 minutes of the trial, and the presence or absence of freezing in response to the tone is determined.

On day 12, the animals are tested to assess their sensitivity to the conditioning stimulus, *i.e*., foot shock. Following the last day of behavioral testing, animals are anesthetized and the brains removed, post-fixed overnight, and sections cut through the hippocampus. The sections are stained to image β-amyloid plaques. Data is analyzed using appropriate statistical methods.

## Example 20

***Animal Model for Assessing Therapeutic Efficacy of MMF Prodrugs for Treating Huntington's Disease***

Neuroprotective Effects in a Transgenic Mouse Model of Huntington's Disease

Transgenic HD mice of the N171-82Q strain and non-transgenic littermates are treated with a compound of Formula 1-3 or a vehicle from 10 weeks of age. The mice are placed on a rotating rod ("rotarod"). The length of time at which a mouse falls from the rotarod is recorded as a measure of motor coordination. The total distance traveled by a mouse is also recorded as a measure of overall locomotion. Mice

administered MMF prodrugs that are neuroprotective in the N171-82Q transgenic HD mouse model remain on the rotarod for a longer period of time and travel farther than mice administered vehicle.

Malonate Model of Huntington's Disease

A series of reversible and irreversible inhibitors of enzymes involved in energy generating pathways has been used to generate animal models for neurodegenerative diseases such as Parkinson's and Huntington's diseases. In particular, inhibitors of succinate dehydrogenase, an enzyme that impacts cellular energy homeostasis, has been used to generate a model for Huntington's disease.

To evaluate the effect of compounds of Formula 1-3 in this malonate model for Huntington's disease, a compound of Formula 1-3 is administered at an appropriate dose, dosing interval, and route, to male Sprague-Dawley rats. A compound of Formula 1-3 is administered for two weeks prior to the administration of malonate and then for an additional week prior to sacrifice. Malonate is dissolved in distilled deionized water and the pH adjusted to 7.4 with 0.1 M HCl. Intrastriatal injections of 1.5 µL of 3 µmol malonate are made into the left striatum at the level of the Bregma, 2.4 mm lateral to the midline and 4.5 mm ventral to the dura. Animals are sacrificed at 7 days by decapitation and the brains quickly removed and placed in ice cold 0.9% saline solution. Brains are sectioned at 2 mm intervals in a brain mold. Slices are then placed posterior side down in 2% 2,3,5-triphenyltetrazolium chloride. Slices are stained in the dark at room temperature for 30 min and then removed and placed in 4% paraformaldehyde pH 7.3. Lesions, noted by pale staining, are evaluated on the posterior surface of each section. The measurements are validated by comparison with measurements obtained on adjacent Nissl stain sections. Compounds exhibiting a neuroprotective effect and therefore potentially useful in treating Huntington's disease show a reduction in malonate-induced lesions.

## Example 21

### *Animal Model for Assessing Therapeutic Efficacy of MMF Prodrugs for Treating Amyotrophic Lateral Sclerosis*

A murine model of SOD1 mutation-associated ALS has been developed in which mice express the human superoxide dismutase (SOD) mutation glycine—>alanine at residue 93 (SOD1). These SOD1 mice exhibit a dominant gain of the adverse property of SOD, and develop motor neuron degeneration and dysfunction similar to that of human ALS. The SOD1 transgenic mice show signs of posterior limb weakness at about 3 months of age and die at 4 months. Features common to human ALS include astrocytosis, microgliosis,

oxidative stress, increased levels of cyclooxygenase/prostaglandin, and, as the disease progresses, profound motor neuron loss.

Studies are performed on transgenic mice overexpressing human Cu/Zn-SOD G93A mutations (B6SJL-TgN (SOD1-G93A) I Gur) and non-transgenic B6/SJL mice and their wild litter mates. Mice are housed on a 12-hr day/light cycle and (beginning at 45 d of age) allowed ad libitum access to either test compound-supplemented chow, or, as a control, regular formula cold press chow processed into identical pellets. Genotyping can be conducted at 21 days of age. The SODl mice are separated into groups and treated with a test compound, e.g., compound of Formula 1-3, or serve as controls.

The mice are observed daily and weighed weekly. To assess health status mice are weighed weekly and examined for changes in lacrimation/salivation, palpebral closure, ear twitch and pupillary responses, whisker orienting, postural and righting reflexes and overall body condition score. A general pathological examination is conducted at the time of sacrifice.

Motor coordination performance of the animals can be assessed by one or more methods known to those skilled in the art. For example, motor coordination can be assessed using a neurological scoring method. In neurological scoring, the neurological score of each limb is monitored and recorded according to a defined 4-point scale: O - normal reflex on the hind limbs (animal will splay its hind limbs when lifted by its tail); I - abnormal reflex of hind limbs (lack of splaying of hind limbs when animal is lifted by the tail); 2 - abnormal reflex of limbs and evidence of paralysis; 3 - lack of reflex and complete paralysis; and 4 - inability to right when placed on the side in 30 seconds or found dead. The primary end point is survival with secondary end points of neurological score and body weight. Neurological score observations and body weight are made and recorded five days per week. Data analysis is performed using appropriate statistical methods.

The rotarod test evaluates the ability of an animal to stay on a rotating dowel allowing evaluation of motor coordination and proprioceptive sensitivity. The apparatus is a 3 cm diameter automated rod turning at, for example, 12 rounds per min. The rotarod test measures how long the mouse can maintain itself on the rod without falling. The test can be stopped after an arbitrary limit, for example at 120 sec. If the animal falls down before 120 sec, the performance is recorded and two additional trials are performed. The mean time of 3 trials is calculated. A motor deficit is indicated by a decrease of walking time.

In the grid test, mice are placed on a grid (length: 37 cm, width: 10.5 cm, mesh size: 1x1 cm) situated above a plane support. The number of times the mice put their paws through the grid is counted and serves as a measure for motor coordination.

The hanging test evaluates the ability of an animal to hang on a wire. The apparatus is a wire stretched horizontally 40 cm above a table. The animal is attached to the wire by its forepaws. The time needed by the animal to catch the string with its hind paws is recorded (60 sec max) during three consecutive trials.

Electrophysiological measurements (EMG) can also be used to assess motor activity condition. Electromyographic recordings are performed using an electromyography apparatus.

During EMG monitoring mice are anesthetized. The measured parameters are the amplitude and the latency of the compound muscle action potential (CMAP). CMAP is measured in gastrocnemius muscle after stimulation of the sciatic nerve. A reference electrode is inserted near the Achilles tendon and an active needle placed at the base of the tail. A ground needle is inserted on the lower back of the mice. The sciatic nerve is stimulated with a single 0.2 msec pulse at supramaximal intensity (12.9 mA). The amplitude (mV) and the latency of the response (ms) are measured. The amplitude is indicative of the number of active motor units, while distal latency reflects motor nerve conduction velocity.

The efficacy of test compounds can also be evaluated using biomarker analysis. To assess the regulation of protein biomarkers in SODl mice during the onset of motor impairment, samples of lumbar spinal cord (protein extracts) are applied to ProteinChip Arrays with varying surface chemical/biochemical properties and analyzed, for example, by surface enhanced laser desorption ionization time of flight mass spectrometry. Then, using integrated protein mass profile analysis methods data is used to compare protein expression profiles of the various treatment groups. Analysis can be performed using appropriate statistical methods.

## **Example 22**

### ***Animal Model for Assessing GI Irritation of MMF Prodrugs***

At least one MMF prodrug, e.g., dimethyl fumarate, is known to cause gastrointestinal irritation. The Annamalai-Ma gastrointestinal irritation rat model is predictive of gastrointestinal irritation of MMF prodrugs in humans. This animal model has several common features of other published GI irritation animal models including the Whiteley-Dalrymple model.

In order to assess gastrointestinal irritation using this model, rats are treated orally with either vehicle or the MMF prodrug of the present disclosure (n = 10 per group) at 180 mg-equivalents MMF/kg of animal body weight, dosed once per day for 4 days, followed by necropsy and gastrointestinal evaluation at 24 hours after the final dose. Evans Blue dye is injected IV (tail vein) to visually emphasize any lesions in the gastrointestinal tissue.

Accordingly, rats are dosed once per day for 4 consecutive days with 180 mg-equivalents MMF/kg body weight per day. The animals are fasted overnight prior to necropsy. On Day 5, to help visualize lesions, I mL of 1% Evan's blue in saline is injected into the tail vein 30 minutes prior to euthanasia. The animals are euthanized by inhalation of carbon dioxide. A partial necropsy, limited to the abdominal cavities, is then performed. The stomach and small intestine are removed. Residual material is washed away, using an irrigation syringe filled with saline. The stomach is cut along the larger curvature and washed gently with normal saline, and is examined for any lesions. The stomachs are scored in accordance with the scoring system outlined below in **Table 5**.

**Table 5**

***Scoring System for Stomach Lesions in the Rat***

| Score | Characteristics |
|---|---|
| 0 | Normal mucosa. |
| 1 | Non-erosive mucosal changes. Swelling and reddening without any apparent mucosal defect. |
| 2 | Apparent mucosal erosions. |
| 3 | Mild ulceration 1-5 small lesions (l-2mm). |
| 4 | Moderate ulceration: More than 5 small lesions or I intermediate lesion (34mm). |
| 5 | Severe ulceration: two or more intermediate lesions or gross lesions (longer than 4 mm). |

While certain embodiments have been described, these embodiments have been presented by way of example only and are not intended to limit the scope of the present disclosures. Indeed, the novel methods, apparatuses and systems described herein can be embodied in a variety of other forms; furthermore, various omissions, substitutions, and changes in the form of the methods, apparatuses and

systems described herein can be made without departing from the spirit of the present disclosures. The accompanying claims and their equivalents are intended to cover such forms or modifications as would fall within the scope and spirit of the present disclosure.

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**FOREIGN PATENT DOCUMENTS**

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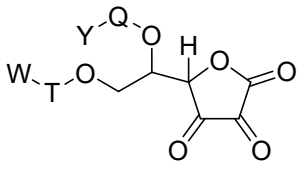
Japan Pat. No. 5,781,983

## CLAIMS

What is claimed is:

1. A compound of Formula 1:



1 ;

wherein:

Q is a single bond or C(O),

T is a single bond or C(O),

W is selected from the group consisting of hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, and $C(H)=C(H)CO_2R^{50}$,

Y is hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, or $C(H)=C(H)CO_2R^{50}$,

when Y is $C(H)=C(H)CO_2R^{50}$, then Q is C(O),

when Y or W is hydrogen, then Q or T is a single bond,

when W is $C(H)=C(H)CO_2R^{50}$, then T is C(O),

$R^{50}$ is $C_1$-$C_6$ alkyl, and,

at least one of W or Y is $C(H)=C(H)CO_2R^{50}$ with (E) regiochemistry;

or a pharmaceutically acceptable salt thereof.

2. A compound of Claim 1, wherein T is C(O) and Q is a single bond.
3. A compound of Claim 1, wherein Q is C(O) and S is a single bond.
4. A compound of Claim 1, wherein T is C(O) and Q is C(O).
5. The compound of Claim 1, wherein the compound is selected from the group consisting of:

2-hydroxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;

2-methoxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;

2-acetoxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;

2-(benzoyloxy)-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;

methyl (2-(2-phenylacetoxy)-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl) fumarate;

methyl (2-(pivaloyloxy)-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl) fumarate;

2-hydroxy-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;

2-acetoxy-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;

methyl (2-(pivaloyloxy)-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl) fumarate;

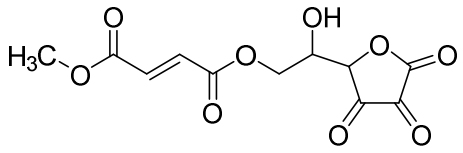
2-(benzoyloxy)-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;

methyl (2-(2-phenylacetoxy)-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl) fumarate;

2-(tert-butoxy)-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate; and,

dimethyl O,O'-((1S)-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethane-1,2-diyl) difumarate.

6. The compound of Claim 1, wherein the compound is:



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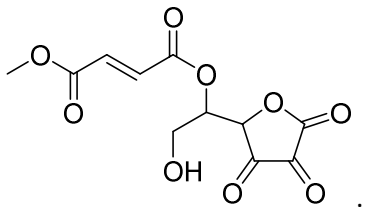
7. The compound of Claim 1, wherein the compound is:



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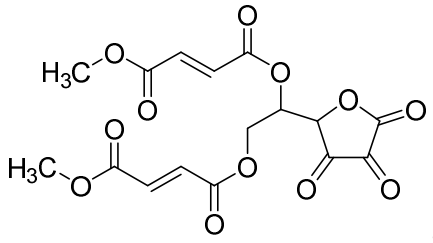
8. The compound of Claim 1, wherein the compound is:



.

9. A composition comprising:

at least one compound of any one of Claims 1-8; and

at least one pharmaceutically acceptable excipient.

10. A composition comprising:

at least one compound of any one of Claims 1-8; and

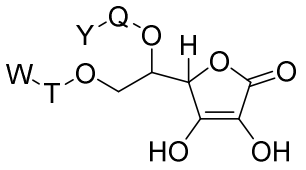
another compound selected from the group consisting of monomethyl fumarate and dimethylfumarate, clofibrate, bezofibrate, gemfibrizil, and fenofibrate and meclofenamic acid.

11. A method of treating and/or preventing a human disease by administering a compound of any one of Claims 1-8, or a composition of claim 9, to a human being.
12. The method of claim 11, wherein the disease a mitochondrial disease or a disease associated with lowered mitochondrial activity.
13. The method of Claim 12, wherein the disease is a primary mitochondrial disease.
14. The method of Claim 13, wherein the disease is selected from Leigh's syndrome, Leber hereditary optic neuropathy, Kearns-Sayre syndrome, Alpers-Huttenlocher syndrome, ataxia neuropathy syndrome, Mitochondrial Encephalomyopathy / Lactic Acid / Stroke (MELAS) syndrome, and Myoclonic Epilepsy/Red Ragged Fiber (MERRF) syndrome.
15. The method of Claim 12, wherein the disease is a secondary mitochondrial disorder.
16. The method of Claim 15, wherein the secondary mitochondrial disorder is selected from the group consisting of spinal muscular atrophy, Friedreich's ataxia, Charcot-Marie-Tooth syndrome, hereditary spastic paraplegia, and Wilson's disease.
17. The method of Claim 11, wherein the disease is a neurological disease.
18. The method of Claim 17, wherein the disease is a neurological disease selected from the group consisting of: Alzheimer's Disease, Frontotemporal Dementia, Amyotrophic Lateral Sclerosis, Lewy Body Dementia, Vascular dementia, Parkinson's Disease, traumatic brain injury, stroke, multiple sclerosis, Balo Concentric Sclerosis, and Huntington's Disease.
19. The method of Claim 17, wherein the neurological disease is selected from the group consisting of: schizophrenia, obsessive compulsive disorder, bipolar disorder, depressive disorder, drug addiction, psychotic disorders, anxiety disorder, personality disorder, mood disorder, major depressive disorder, post-traumatic stress disorder, and panic disorder.
20. The method of Claim 17, wherein the neurological disease is selected from the group consisting of: autism, Asperger's syndrome, Rett syndrome, childhood disintegrative disorder, Kanner's syndrome, pervasive developmental disorder, high functioning autism, low functioning autism, and classical autism.
21. The method of Claim 11, wherein the disease is selected from the group consisting of: Down Syndrome, retinitis pigmentosa, adrenal leukodystrophy, Alexanders Disease, Alper's Disease, Canavan Disease, Childhood Ataxia with Central Nervous System Hypomyelination, Globoid Cell Leukodystrophy, and Aicardi-Goutieres syndrome.

22. The method of Claim 11, wherein the disease is a proliferative disease.

23. The method of Claim 22, wherein the proliferative disease is selected from the group consisting of: brain cancer, glioblastoma multiforme, neuroblastoma, leukemia, lymphoma, sarcoma, and psoriasis.

24. A compound of Formula 2:




2 ;

wherein:

Q is a single bond or C(O),

T is a single bond or C(O),

W is selected from the group consisting of hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, and $C(H)=C(H)CO_2R^{50}$,

Y is selected from the group consisting of hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, and $C(H)=C(H)CO_2R^{50}$,

when Y is $C(H)=C(H)CO_2R^{50}$, then Q is C(O),

when Y or W is hydrogen, then Q or T is a single bond,

when W is $C(H)=C(H)CO_2R^{50}$, then S is C(O),

$R^{50}$ is $C_1$-$C_6$ alkyl,

at least one of Y or W is $C(H)=C(H)CO_2R^{50}$ with (E) regiochemistry, and,

if T is C(O) and W is $C(H)=C(H)CO_2R^{50}$ then Y is not hydrogen;

or a pharmaceutically acceptable salt thereof.

25. The compound of Claim 22, wherein the compound is selected from the group consisting of:

O,O'-(1-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)ethane-1,2-diyl) dimethyl difumarate;

2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-methoxyethyl methyl fumarate;

2-acetoxy-2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)ethyl methyl fumarate;

2-(benzoyloxy)-2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)ethyl methyl fumarate;

2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-(2-phenylacetoxy)ethyl methyl fumarate;

2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-(pivaloyloxy)ethyl methyl fumarate;

1-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-hydroxyethyl methyl fumarate;

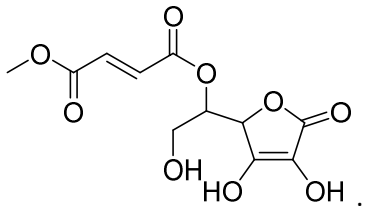
1-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-(pivaloyloxy)ethyl methyl fumarate;

2-(benzoyloxy)-1-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)ethyl methyl fumarate;

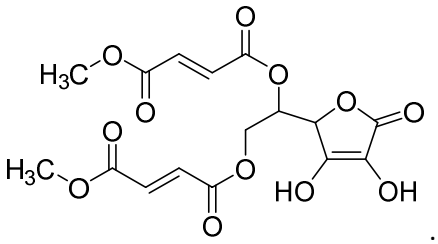
1-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-(2-phenylacetoxy)ethyl methyl fumarate; and,

2-(tert-butoxy)-1-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)ethyl methyl fumarate.

26. The compound of Claim 18 wherein the compound is:



27. The compound of Claim 18 wherein the compound is:



28. A composition comprising:

    at least one compound of any one of Claims 23-27; and

    at least one pharmaceutically acceptable excipient.

29. A method of treating and/or preventing a human disease by administering a compound of any one of Claims 24-27, or a composition of claim 28 to a human being.

30. The method of claim 29, wherein the disease a mitochondrial disease or a disease associated with lowered mitochondrial activity.

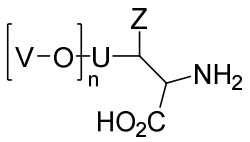
31. The method of Claim 30, wherein the disease is a primary mitochondrial disease.

32. The method of Claim 31, wherein the disease is selected from Leigh's syndrome, Leber hereditary optic neuropathy, Kearns-Sayre syndrome, Alpers-Huttenlocher syndrome, ataxia neuropathy syndrome, Mitochondrial Encephalomyopathy / Lactic Acid / Stroke (MELAS) syndrome, and Myoclonic Epilepsy/Red Ragged Fiber (MERRF) syndrome.

33. The method of Claim 30, wherein the disease is a secondary mitochondrial disorder.

34. The method of Claim 33, wherein the secondary mitochondrial disorder is selected from the group consisting of spinal muscular atrophy, Friedreich's ataxia, Charcot-Marie-Tooth syndrome, hereditary spastic paraplegia, and Wilson's disease.
35. The method of Claim 29, wherein the disease is a neurological disease.
36. The method of Claim 35, wherein the neurological disease is selected from the group consisting of Alzheimer's Disease, Frontotemporal Dementia, Amyotrophic Lateral Sclerosis, Lewy Body Dementia, Vascular dementia, Parkinson's Disease, traumatic brain injury, stroke, multiple sclerosis, Balo Concentric Sclerosis, and Huntington's Disease.
37. The method of Claim 35, wherein the neurological disease is selected from the group consisting of schizophrenia, obsessive compulsive disorder, bipolar disorder, depressive disorder, drug addiction, psychotic disorders, anxiety disorder, personality disorder, mood disorder, major depressive disorder, post-traumatic stress disorder, and panic disorder.
38. The method of Claim 35, wherein the neurological disease is selected from the group consisting of autism, Asperger's syndrome, Rett syndrome, childhood disintegrative disorder, Kanner's syndrome, pervasive developmental disorder, high functioning autism, low functioning autism, and classical autism.
39. The method of Claim 29, wherein the disease is selected from the group consisting of Down Syndrome, retinitis pigmentosa, adrenal leukodystrophy, Alexanders Disease, Alper's Disease, Canavan Disease, Childhood Ataxia with Central Nervous System Hypomyelination, Globoid Cell Leukodystrophy, and Aicardi-Goutieres syndrome.
40. The method of Claim 29, wherein the disease is a proliferative disease.
41. The method of Claim 40, wherein the proliferative disease is selected from the group consisting of brain cancer, glioblastoma multiforme, neuroblastoma, leukemia, lymphoma, sarcoma, and psoriasis.

42. A compound of Formula 3:




3 ;

wherein

n is 1 or 2,

U is a single bond or an aryl ring comprised of phenyl or pyridyl,

V is $C(O)C(H)=C(H)CO_2R^{50}$ with (E) regiochemistry,

Z is selected from the group consisting of hydrogen, methyl, trifluoromethyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, and benzyl,

if U is a single bond, Z is hydrogen,

if U is phenyl or pyridyl, Z is selected from the group consisting of hydrogen, methyl and trifluoromethyl, and,

$R^{50}$ is $C_1$-$C_6$ alkyl;

or a pharmaceutically acceptable salt thereof.

43. The compound of Claim 42, wherein U is a single bond and Z is selected from the group consisting of methyl, trifluoromethyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, and benzyl.

44. The compound of Claim 42, wherein:

U is a phenyl ring; and

Z is selected from the group consisting of hydrogen, methyl, trifluoromethyl, and ethyl.

45. The compound of Claim 42, wherein the compound is selected from the group consisting of:

(E)-2-amino-3-((4-methoxy-4-oxobut-2-enoyl)oxy)butanoic acid;

(E)-2-amino-3-((4-methoxy-4-oxobut-2-enoyl)oxy)-4,4-dimethylpentanoic acid;

(E)-2-amino-4,4,4-trifluoro-3-((4-methoxy-4-oxobut-2-enoyl)oxy)butanoic acid;

(E)-2-amino-3-((4-methoxy-4-oxobut-2-enoyl)oxy)-3-phenylpropanoic acid;

(E)-2-amino-3-((4-methoxy-4-oxobut-2-enoyl)oxy)-4-phenylbutanoic acid;

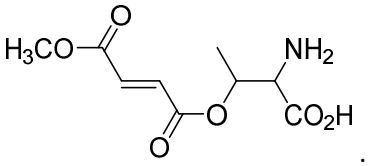
(E)-2-amino-3-(4-((4-methoxy-4-oxobut-2-enoyl)oxy)phenyl)propanoic acid;

(E)-2-amino-3-(3-((4-methoxy-4-oxobut-2-enoyl)oxy)phenyl)propanoic acid;

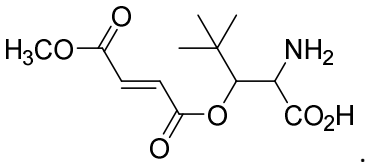
(E)-2-amino-3-(2-((4-methoxy-4-oxobut-2-enoyl)oxy)phenyl)propanoic acid; and,

2-amino-3-(3,5-bis(((E)-4-methoxy-4-oxobut-2-enoyl)oxy)phenyl)propanoic acid.

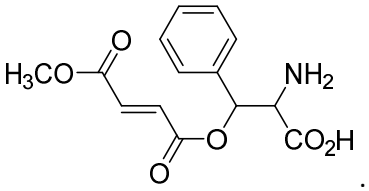
46. The compound of Claim 42, wherein the compound is:



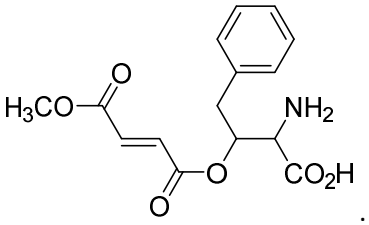
47. The compound of Claim 42, wherein the compound is:



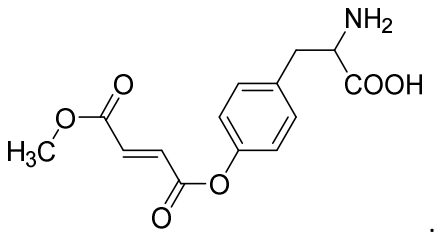
48. The compound of Claim 42, wherein the compound is:



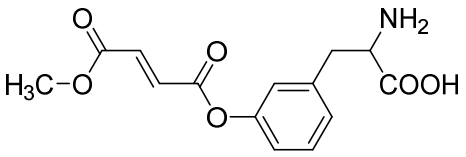
49. The compound of Claim 42, wherein the compound is:



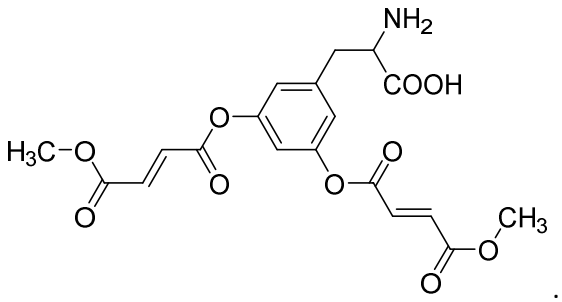
50. The compound of Claim 42, wherein the compound is:



51. The compound of Claim 42, wherein the compound is:



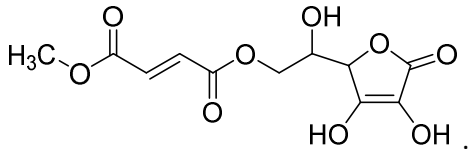
52. The compound of Claim 42, wherein the compound is:



53. A composition comprising:
    at least one compound of any one of Claims 42-52; and
    at least one pharmaceutically acceptable excipient.
54. A method of treating and/or preventing a human disease by administering a compound of any one of Claims 42-52, or a composition of claim 53, to a human being.
55. The method of claim 54, wherein the disease a mitochondrial disease or a disease associated with lowered mitochondrial activity.
56. The method of Claim 55, wherein the disease is a primary mitochondrial disease.
57. The method of Claim 56, wherein the disease is selected from Leigh's syndrome, Leber hereditary optic neuropathy, Kearns-Sayre syndrome, Alpers-Huttenlocher syndrome, ataxia neuropathy syndrome, Mitochondrial Encephalomyopathy / Lactic Acid / Stroke (MELAS) syndrome, and Myoclonic Epilepsy/Red Ragged Fiber (MERRF) syndrome.
58. The method of Claim 55, wherein the disease is a secondary mitochondrial disorder.
59. The method of Claim 58, wherein the secondary mitochondrial disorder is selected from the group consisting of spinal muscular atrophy, Friedreich's ataxia, Charcot-Marie-Tooth syndrome, hereditary spastic paraplegia, and Wilson's disease.
60. The method of Claim 54, wherein the disease is a neurological disease.
61. The method of Claim 60, wherein the neurological disease is selected from the group consisting of Alzheimer's Disease, Frontotemporal Dementia, Amyotrophic Lateral Sclerosis, Lewy Body Dementia, Vascular dementia, Parkinson's Disease, traumatic brain injury, stroke, multiple sclerosis, Balo Concentric Sclerosis, and Huntington's Disease.
62. The method of Claim 60, wherein the neurological disease is selected from the group consisting of schizophrenia, obsessive compulsive disorder, bipolar disorder, depressive disorder, drug addiction,

psychotic disorders, anxiety disorder, personality disorder, mood disorder, major depressive disorder, post-traumatic stress disorder, and panic disorder.

63. The method of Claim 60, wherein the neurological disease is selected from the group consisting of autism, Asperger's syndrome, Rett syndrome, childhood disintegrative disorder, Kanner's syndrome, pervasive developmental disorder, high functioning autism, low functioning autism, and classical autism.
64. The method of Claim 54, wherein the disease is selected from the group consisting of Down Syndrome, retinitis pigmentosa, adrenal leukodystrophy, Alexanders Disease, Alper's Disease, Canavan Disease, Childhood Ataxia with Central Nervous System Hypomyelination, Globoid Cell Leukodystrophy, and Aicardi-Goutieres syndrome.
65. The method of Claim 54, wherein the disease is a proliferative disease.
66. The method of Claim 65, wherein the proliferative disease is selected from the group consisting of brain cancer, glioblastoma multiforme, neuroblastoma, leukemia, lymphoma, sarcoma, and psoriasis.
67. A compound of the structure:



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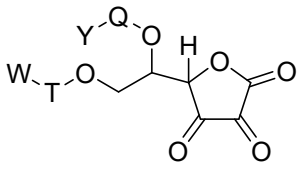
68. A compound of Formula 4:



1 ;

wherein:

Q is a single bond or C(O),

T is a single bond or C(O),

W is selected from the group consisting of hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, and $C(H)=C(H)CO_2R^{50}$,

Y is hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, or $C(H)=C(H)CO_2R^{50}$,

when Y is $C(H)=C(H)CO_2R^{50}$, then Q is C(O),

when W is $C(H)=C(H)CO_2R^{50}$, then T is C(O),

when Y or W is hydrogen, then Q or T is a single bond,

when W is H, then Y is methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, or $C(H)=C(H)CO_2R^{50}$;

$R^{50}$ is $C_1$-$C_6$ alkyl, and,

when W is methyl, i-propyl, or phenyl, and T is C(O), then i) Q may not be a single bond, and ii) Y may not be hydrogen;

or a pharmaceutically acceptable salt thereof.

69. The compound of Claim 68, wherein the compound is selected from the group consisting of:

5-(1-hydroxy-2-methoxyethyl)furan-2,3,4(5H)-trione;

5-(2-ethoxy-1-hydroxyethyl)furan-2,3,4(5H)-trione;

5-(2-butoxy-1-hydroxyethyl)furan-2,3,4(5H)-trione;

5-(1-hydroxy-2-isopropoxyethyl)furan-2,3,4(5H)-trione;

5-(2-butoxy-1-hydroxyethyl)furan-2,3,4(5H)-trione;

5-(1-hydroxy-2-isobutoxyethyl)furan-2,3,4(5H)-trione;

5-(2-(tert-butoxy)-1-hydroxyethyl)furan-2,3,4(5H)-trione;

5-(2-(benzyloxy)-1-hydroxyethyl)furan-2,3,4(5H)-trione;

2-hydroxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;

2-hydroxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl propionate;

2-hydroxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl butyrate;

2-hydroxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl pentanoate;

2-hydroxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl 3-methylbutanoate;

2-hydroxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl pivalate;

2-hydroxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl 2-phenylacetate; and

2-hydroxy-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate.

70. A compound of the structure:

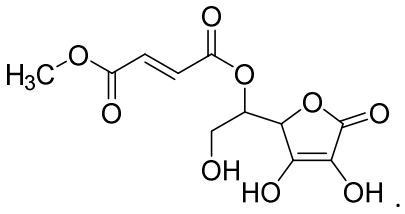



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Figure 1

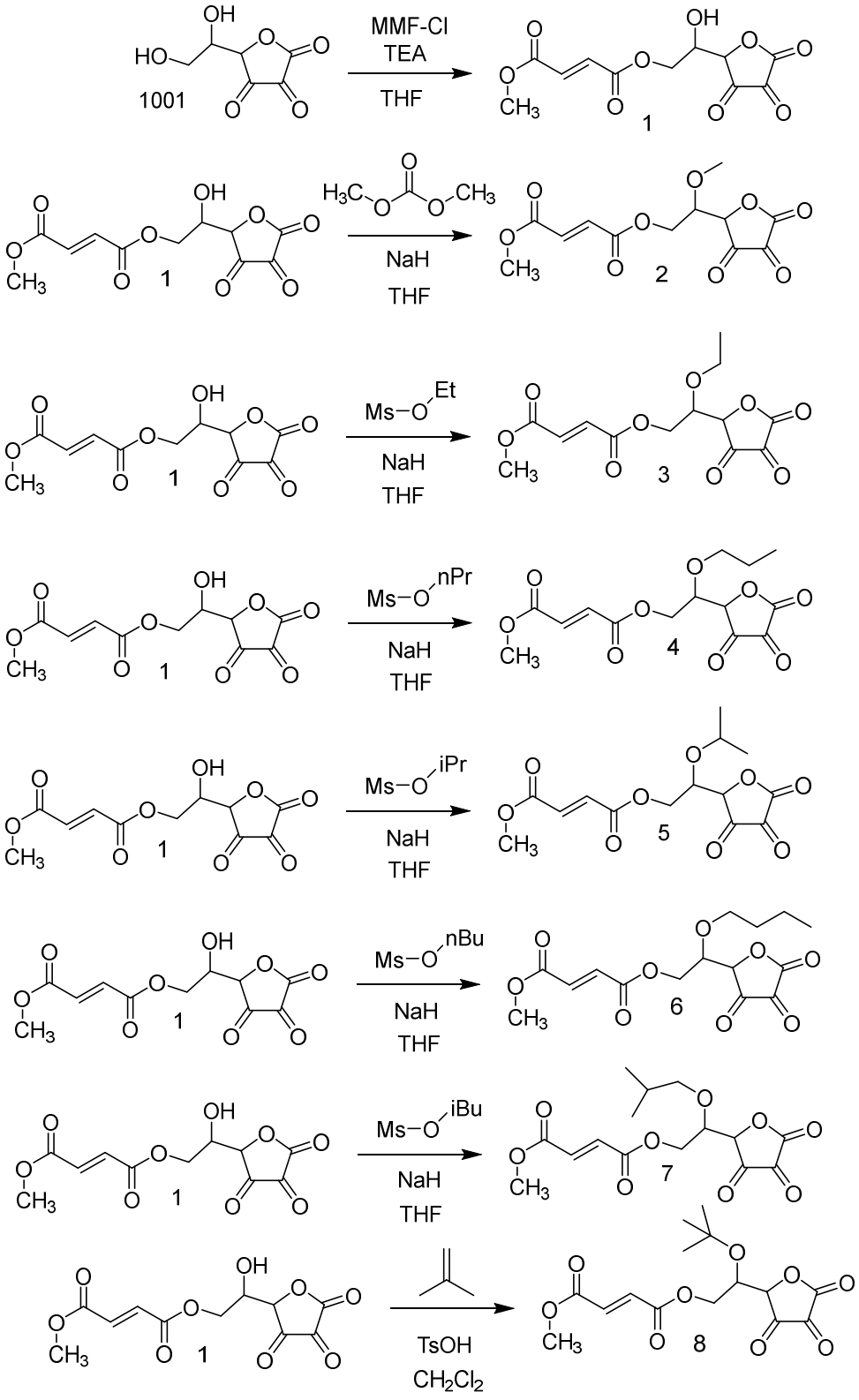

1001
MMF-Cl
TEA
THF
1
NaH
THF
2
Et
3
nPr
4
iPr
5
nBu
6
iBu
7
TsOH
CH2Cl2
8

Figure 2

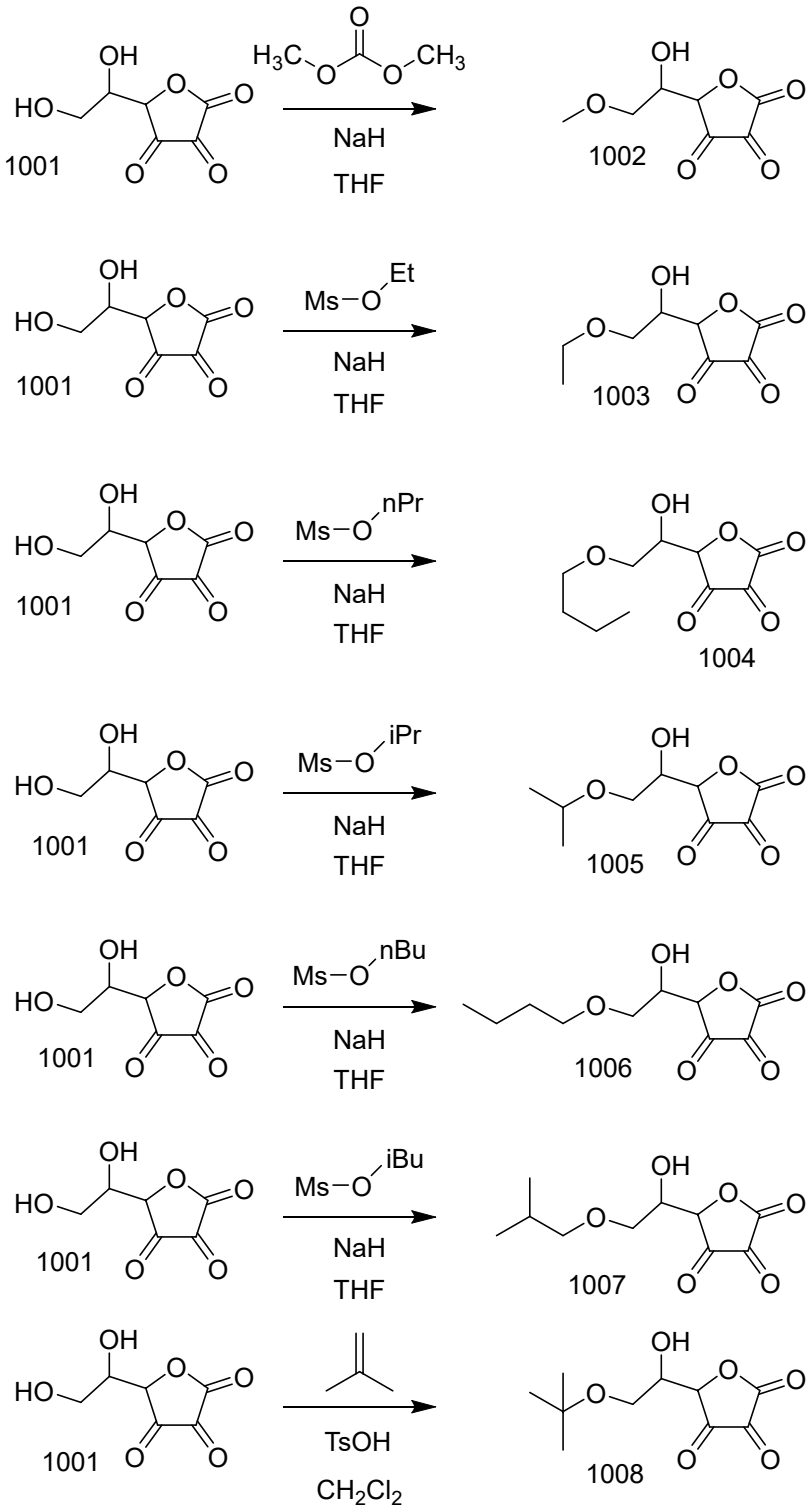

1001
NaH
THF
1002
Ms—O Et
1003
Ms—O nPr
1004
Ms—O iPr
1005
Ms—O nBu
1006
Ms—O iBu
1007
TsOH
$CH_2Cl_2$
1008

Figure 3

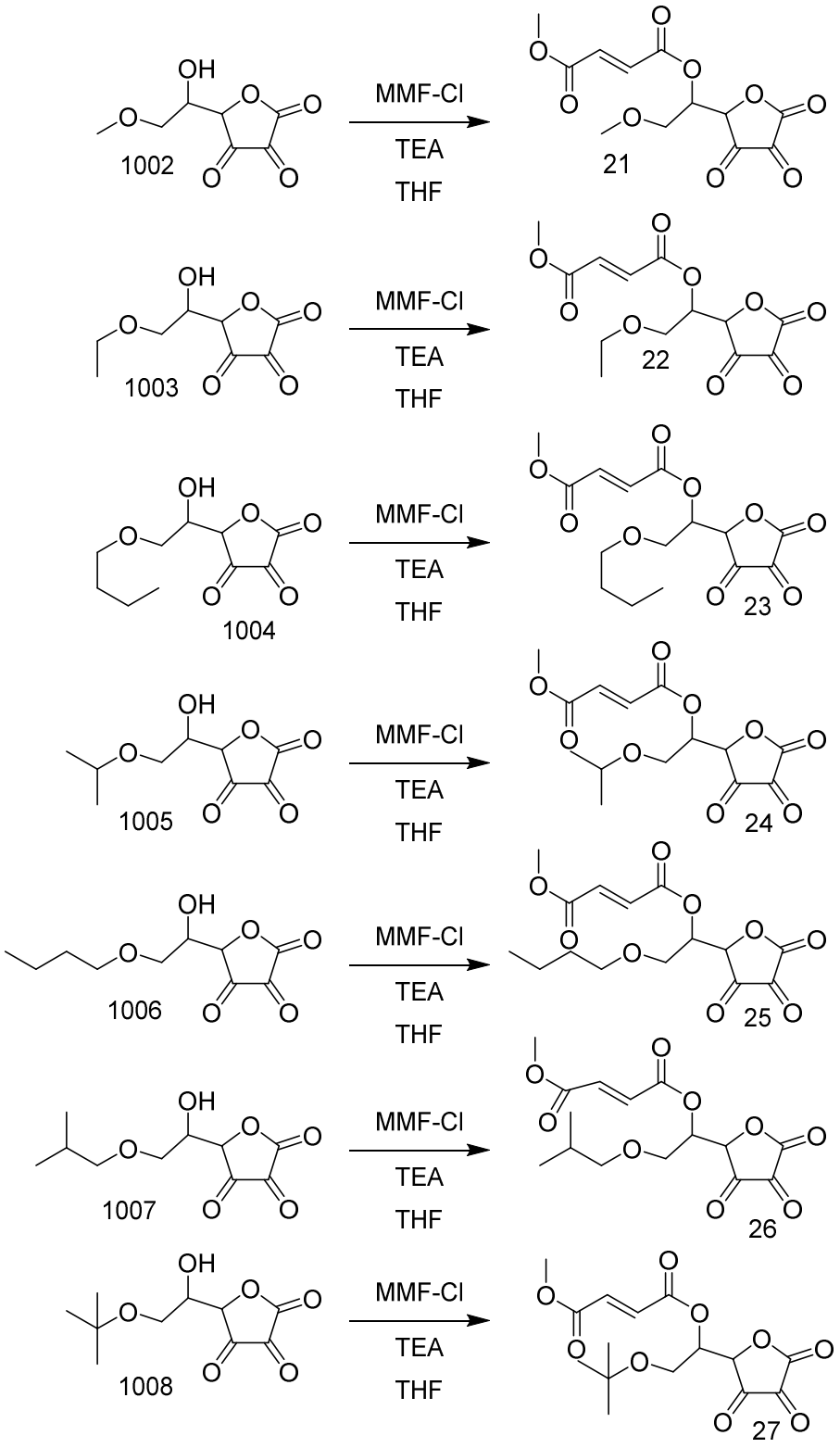

OH
O
O
O
O
O
1002
MMF-Cl
TEA
THF
21
1003
22
1004
23
1005
24
1006
25
1007
26
1008
27

Figure 4

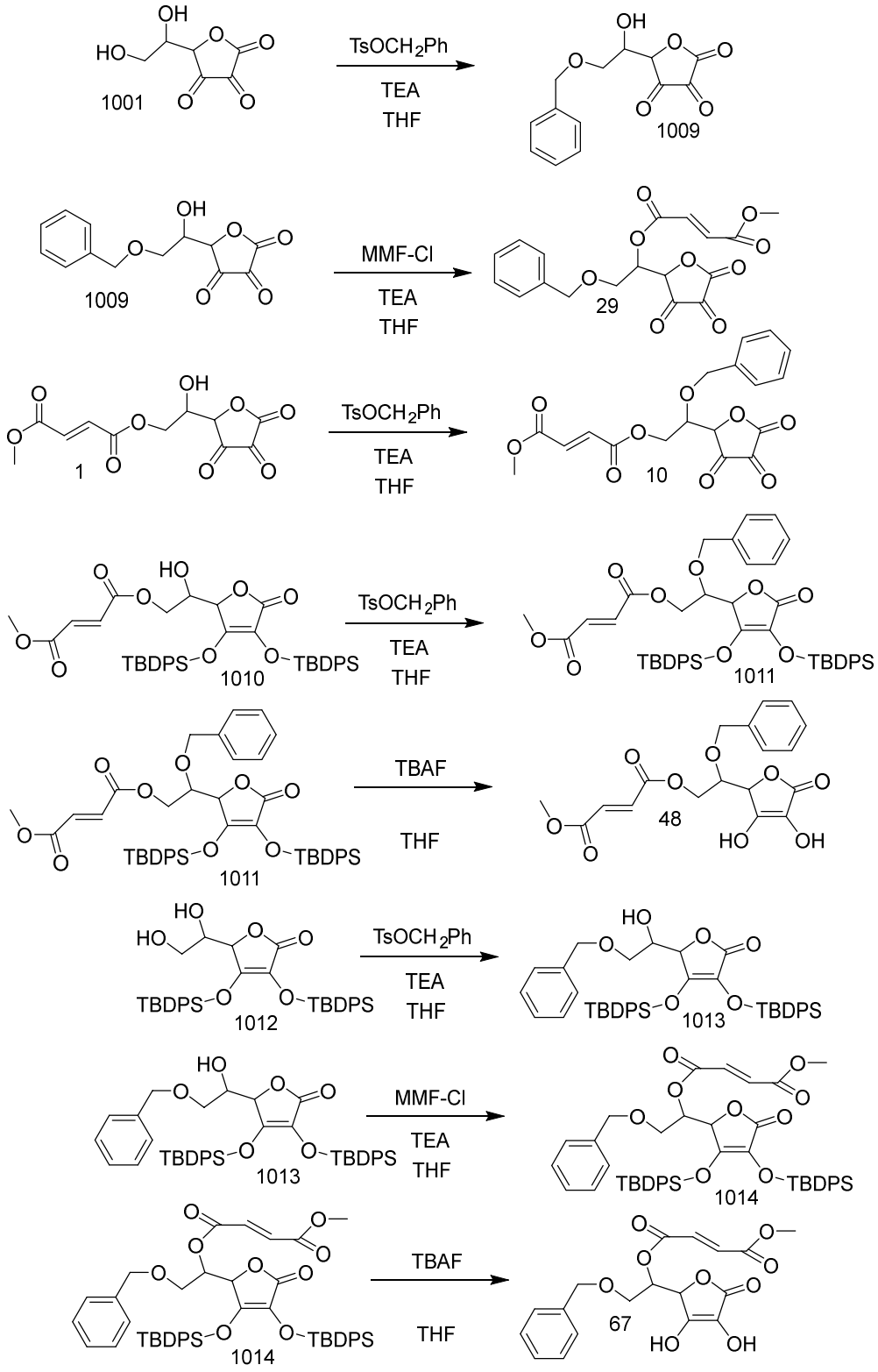

1001
TsOCH2Ph
TEA
THF
1009
1009
MMF-Cl
TEA
THF
29
1
TsOCH2Ph
TEA
THF
10
1010
TsOCH2Ph
TEA
THF
1011
1011
TBAF
THF
48
1012
TsOCH2Ph
TEA
THF
1013
1013
MMF-Cl
TEA
THF
1014
1014
TBAF
THF
67

Figure 5

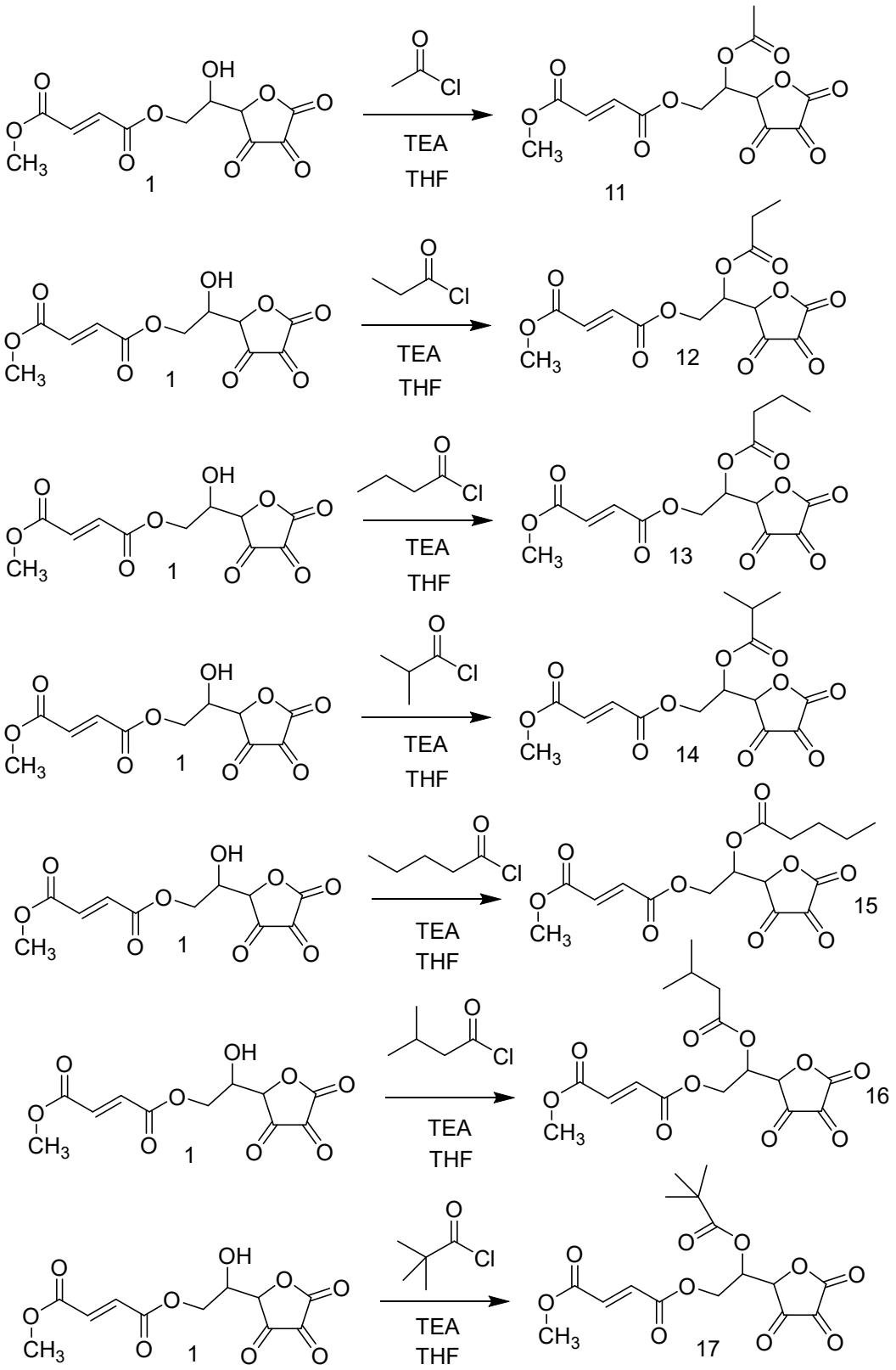


Figure 6

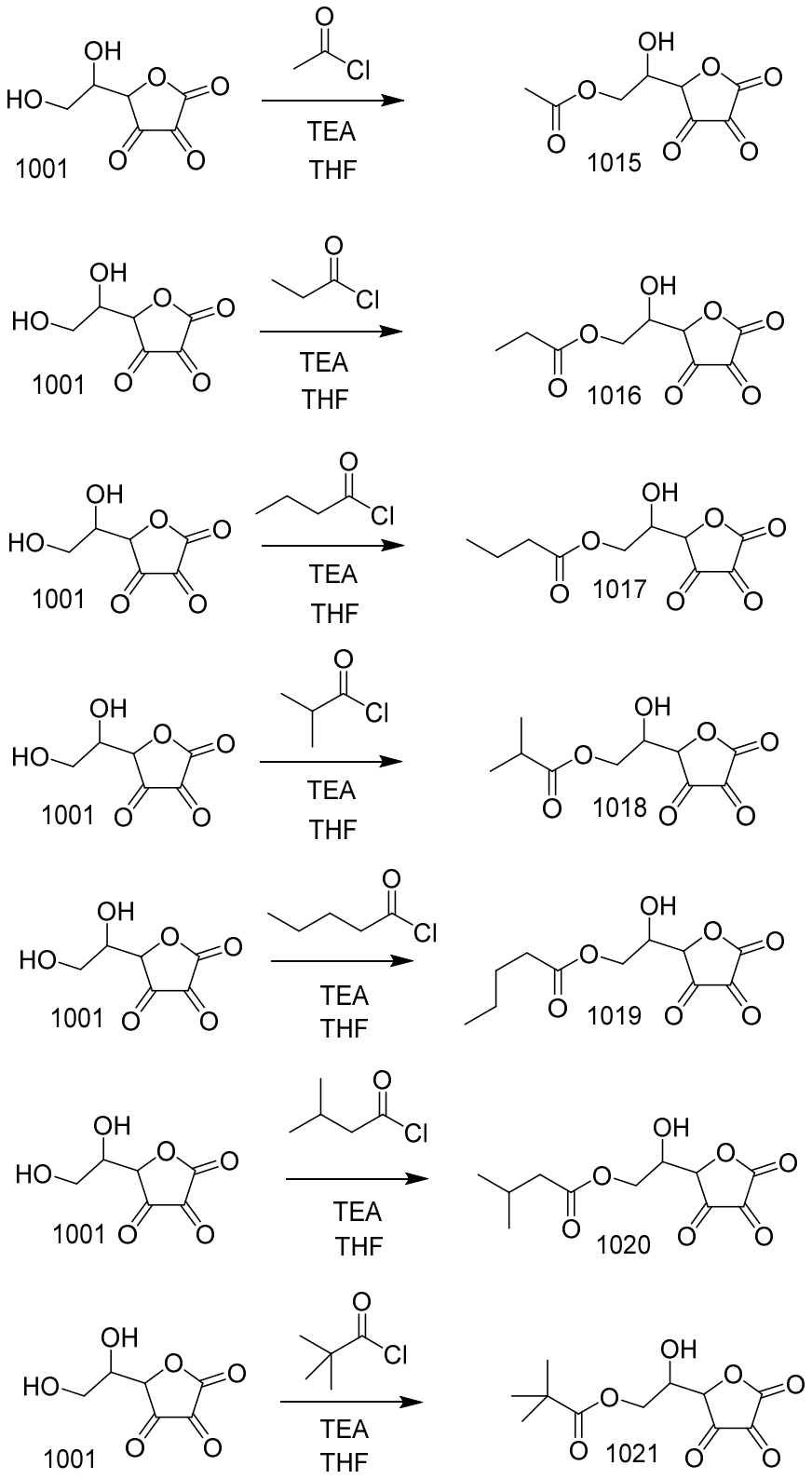


Figure 7

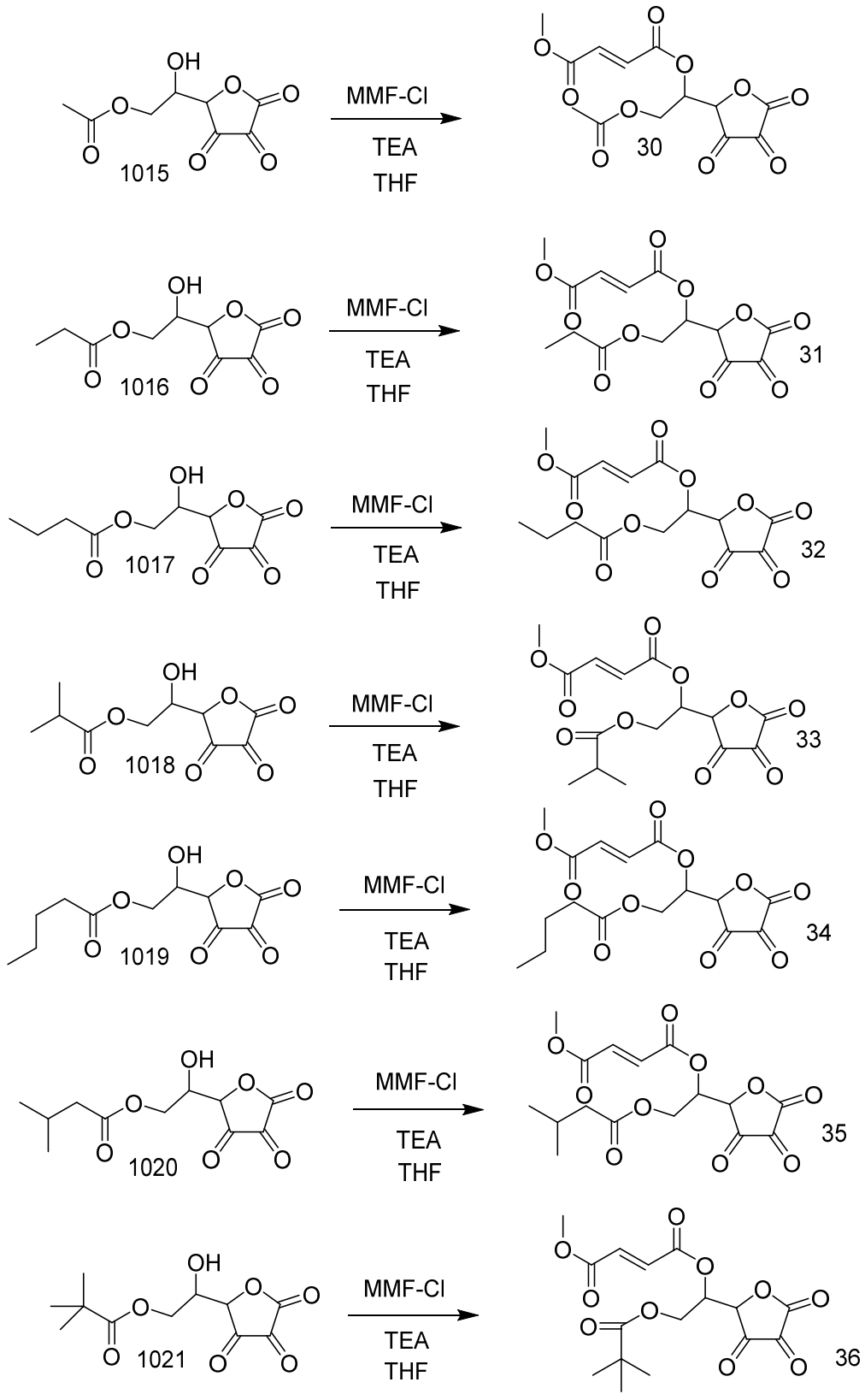

OH
O
O
O
O
O
O
1015
MMF-Cl
TEA
THF
30
1016
31
1017
32
1018
33
1019
34
1020
35
1021
36

Figure 8

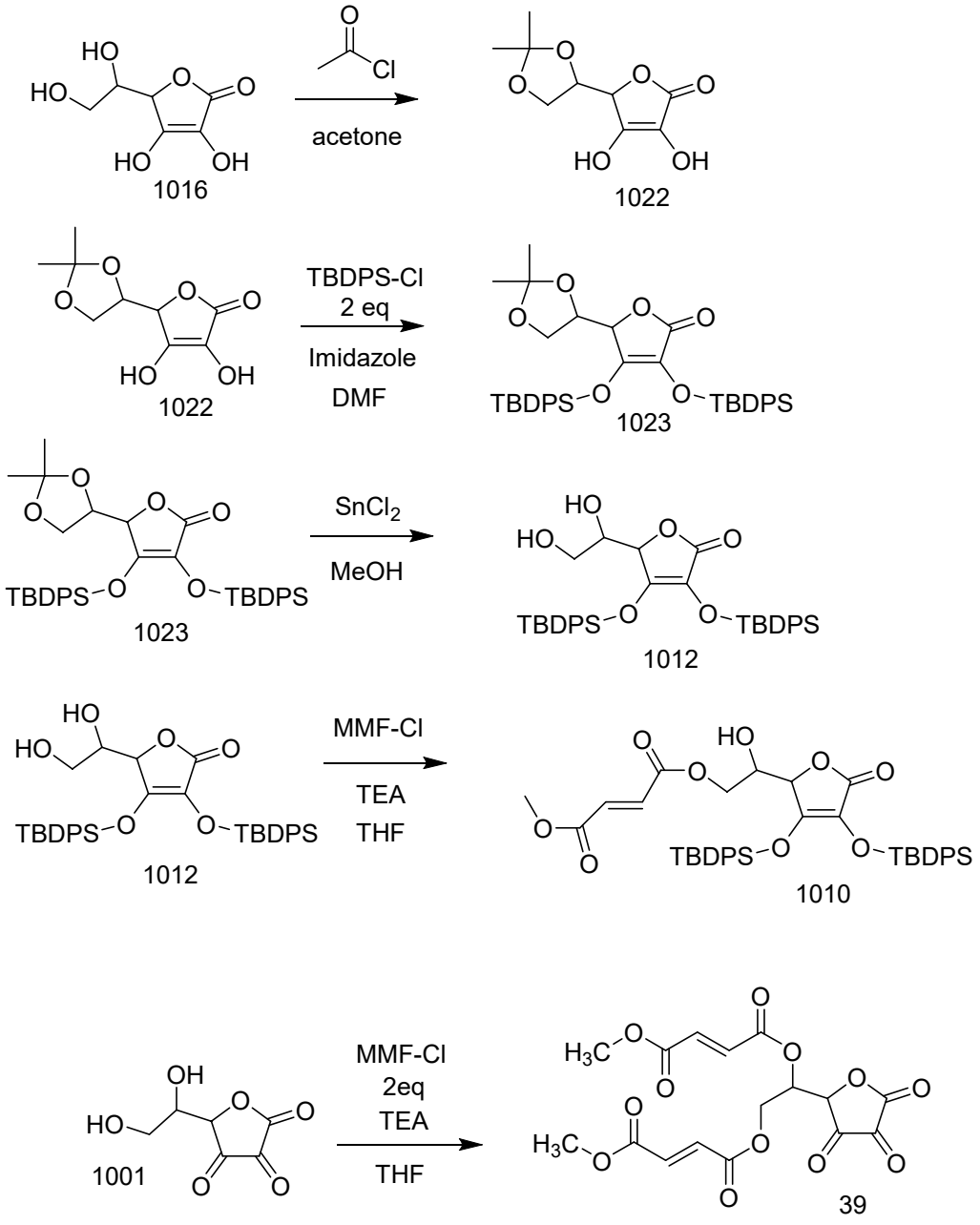

1016
acetone
1022
TBDPS-Cl
2 eq
Imidazole
DMF
1023
SnCl2
MeOH
1012
MMF-Cl
TEA
THF
1010
1001
MMF-Cl
2eq
TEA
THF
39

Figure 9

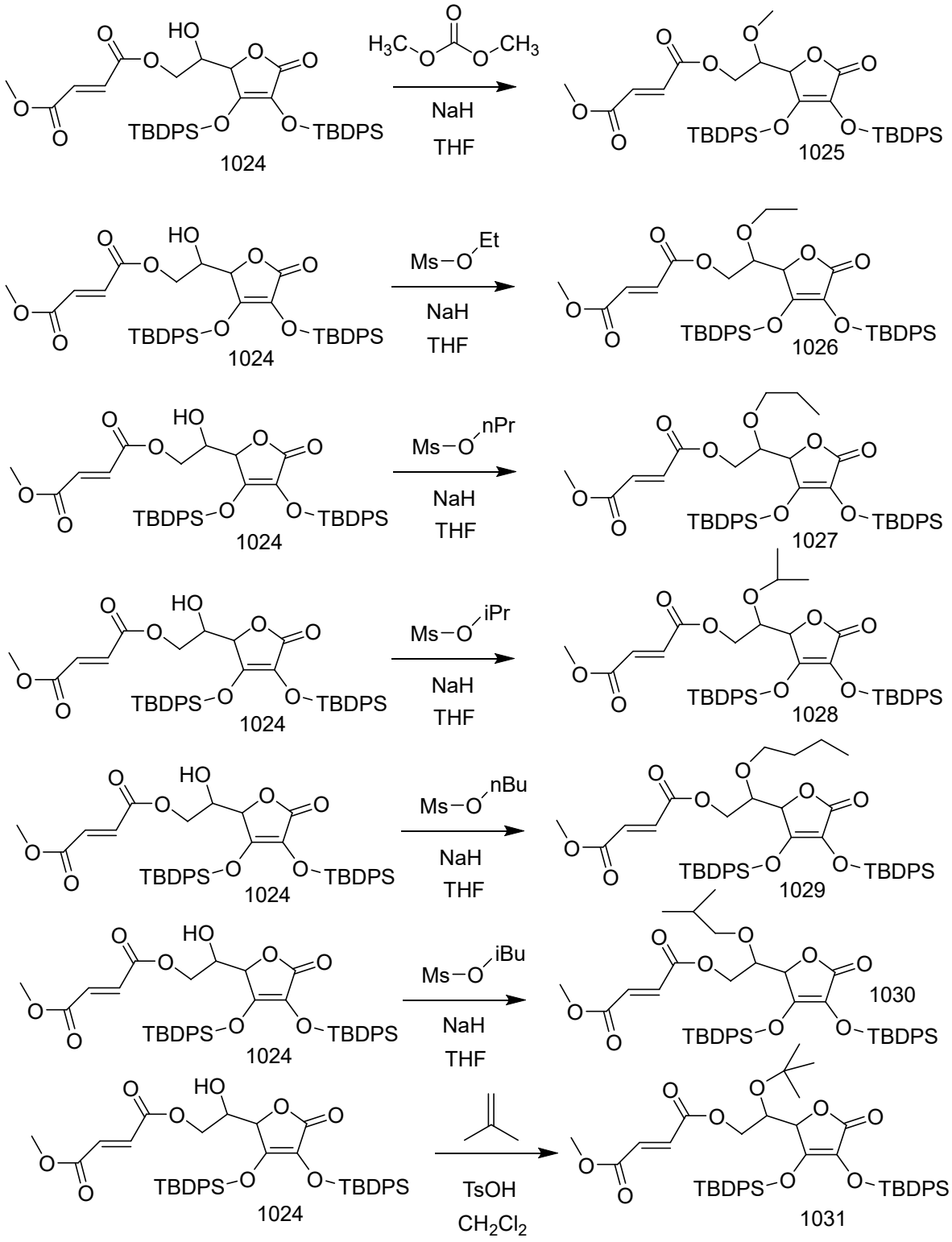

HO
TBDPS
TBDPS
1024
H3C
CH3
NaH
THF
1025
Ms
Et
1026
nPr
1027
iPr
1028
nBu
1029
iBu
1030
TsOH
CH2Cl2
1031

Figure 10

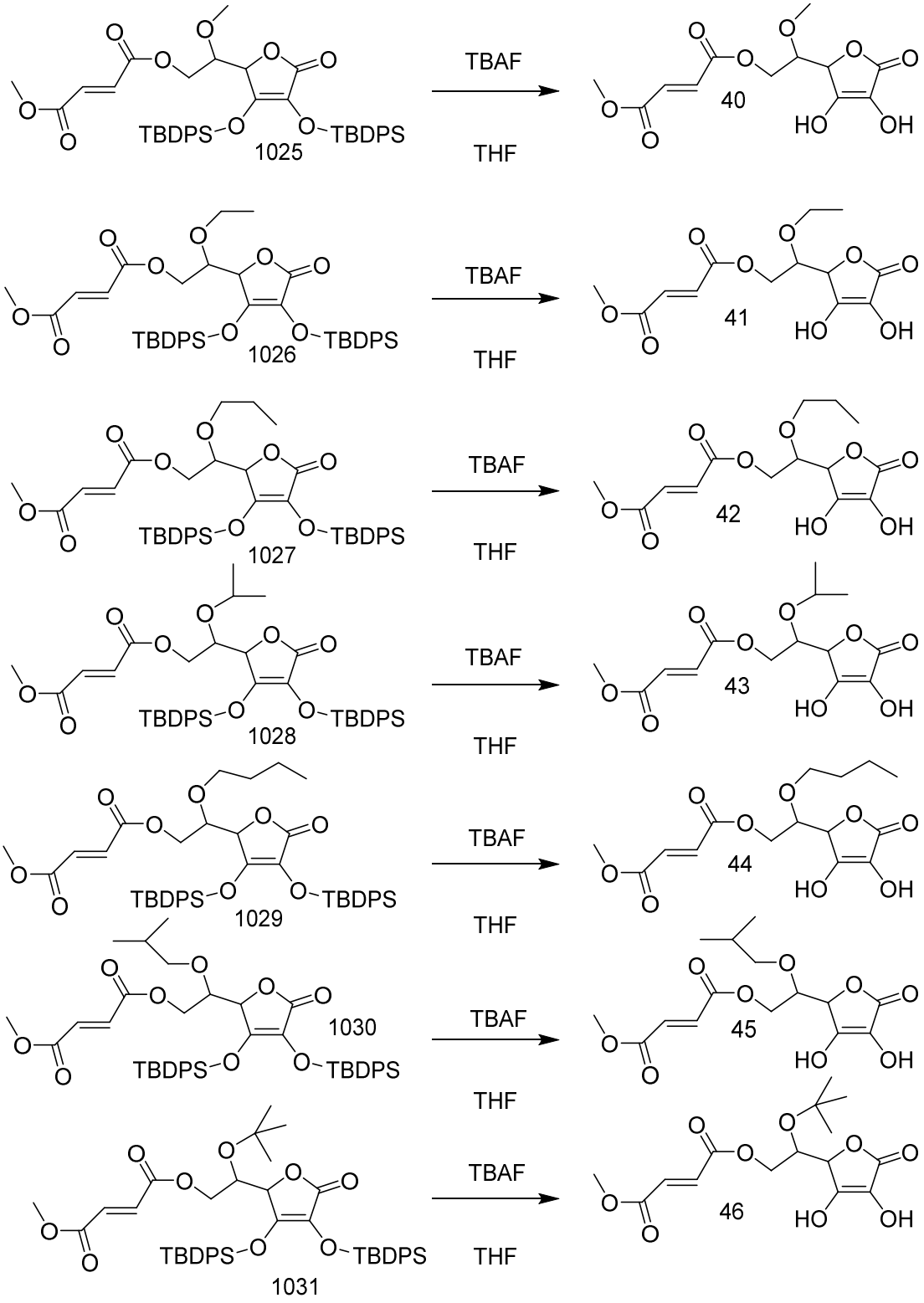

TBAF
THF
TBDPS
TBDPS
1025
40
1026
41
1027
42
1028
43
1029
44
1030
45
1031
46
HO
OH

Figure 11

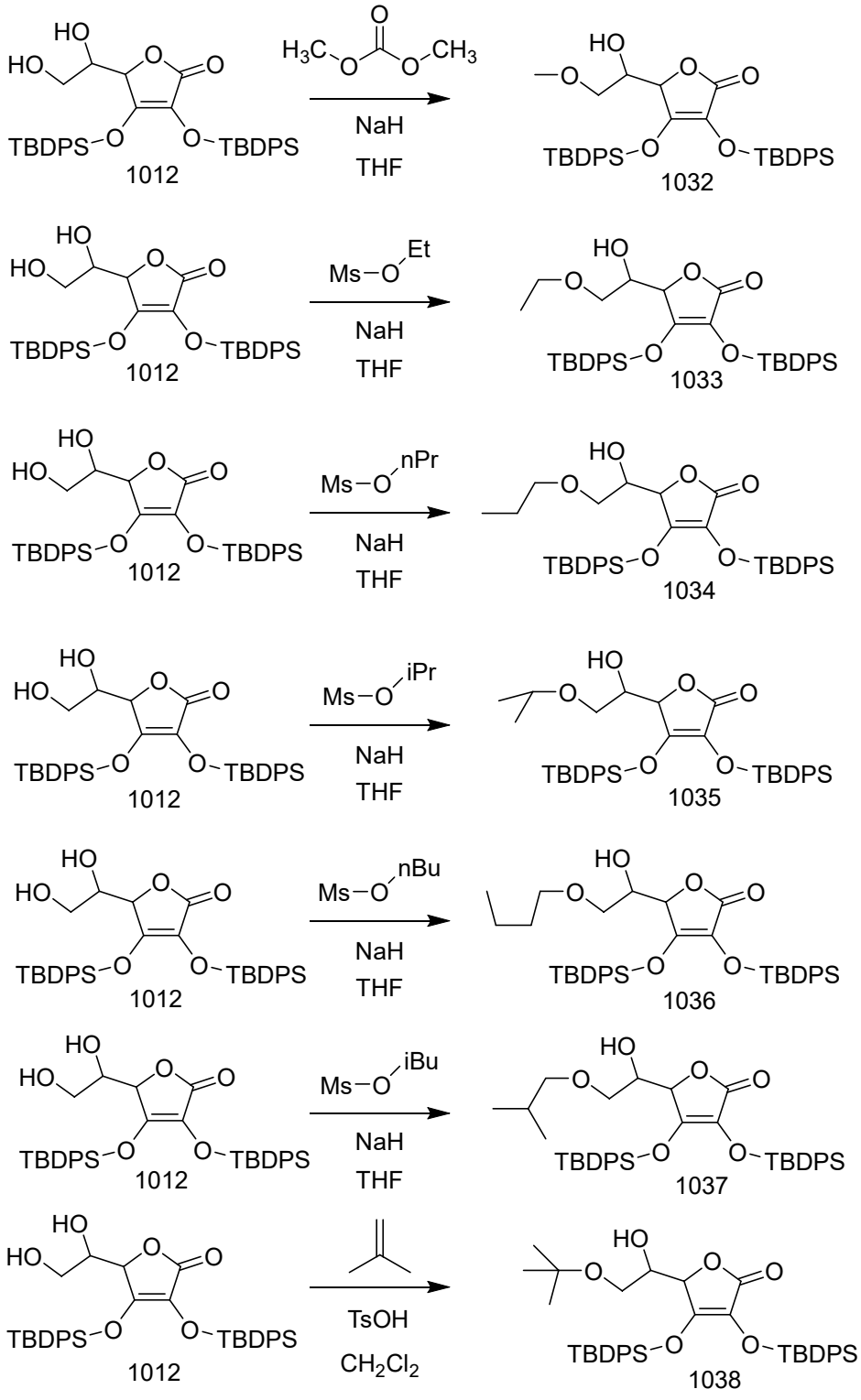

HO
HO
O
O
TBDPS
O
O
TBDPS
1012
H3C
O
O
CH3
NaH
THF
1032
Ms
O
Et
1033
nPr
1034
iPr
1035
nBu
1036
iBu
1037
TsOH
CH2Cl2
1038

Figure 12

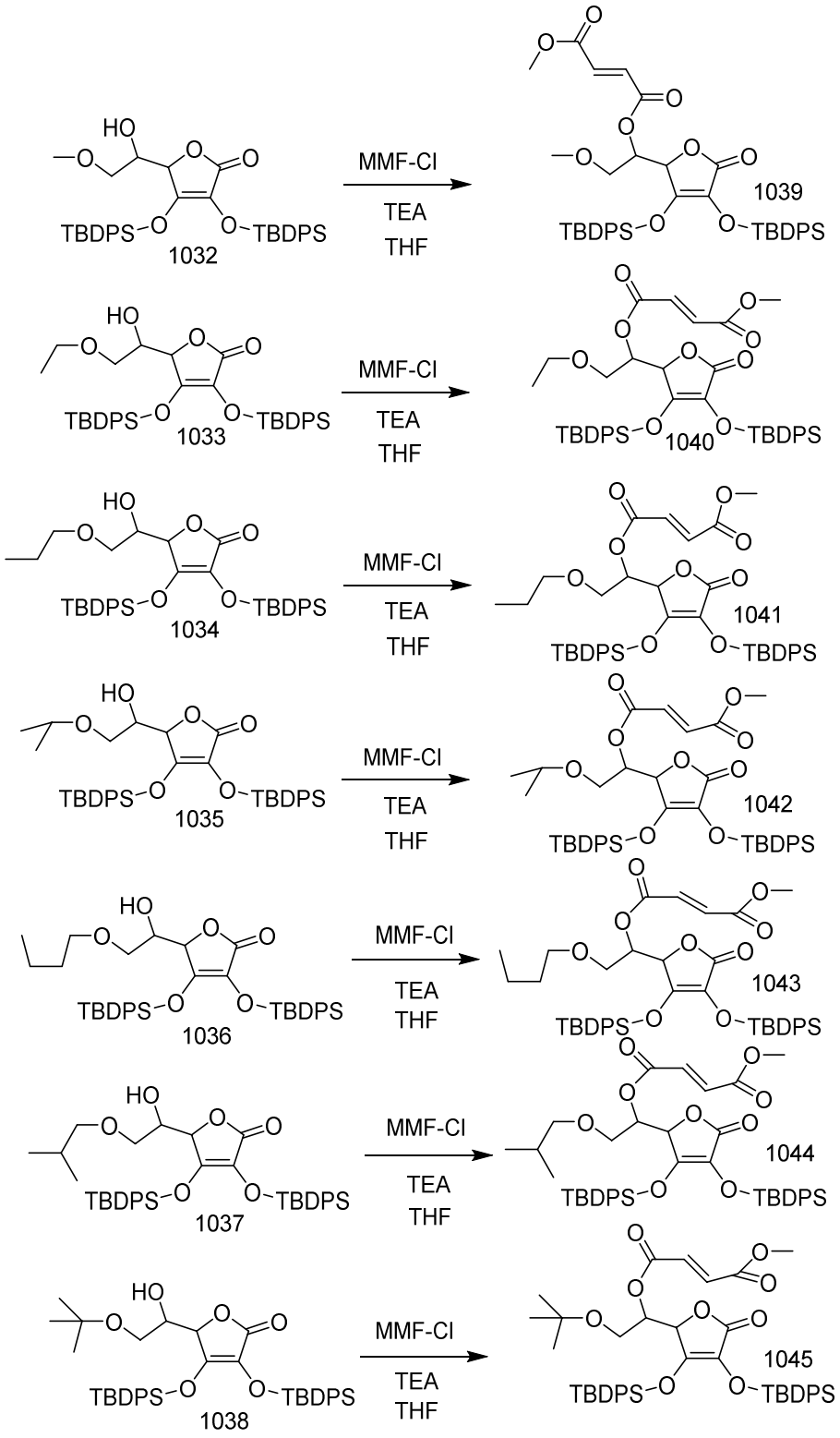

HO
TBDPS
TBDPS
1032
MMF-CI
TEA
THF
1039
1033
1040
1034
1041
1035
1042
1036
1043
1037
1044
1038
1045

Figure 13

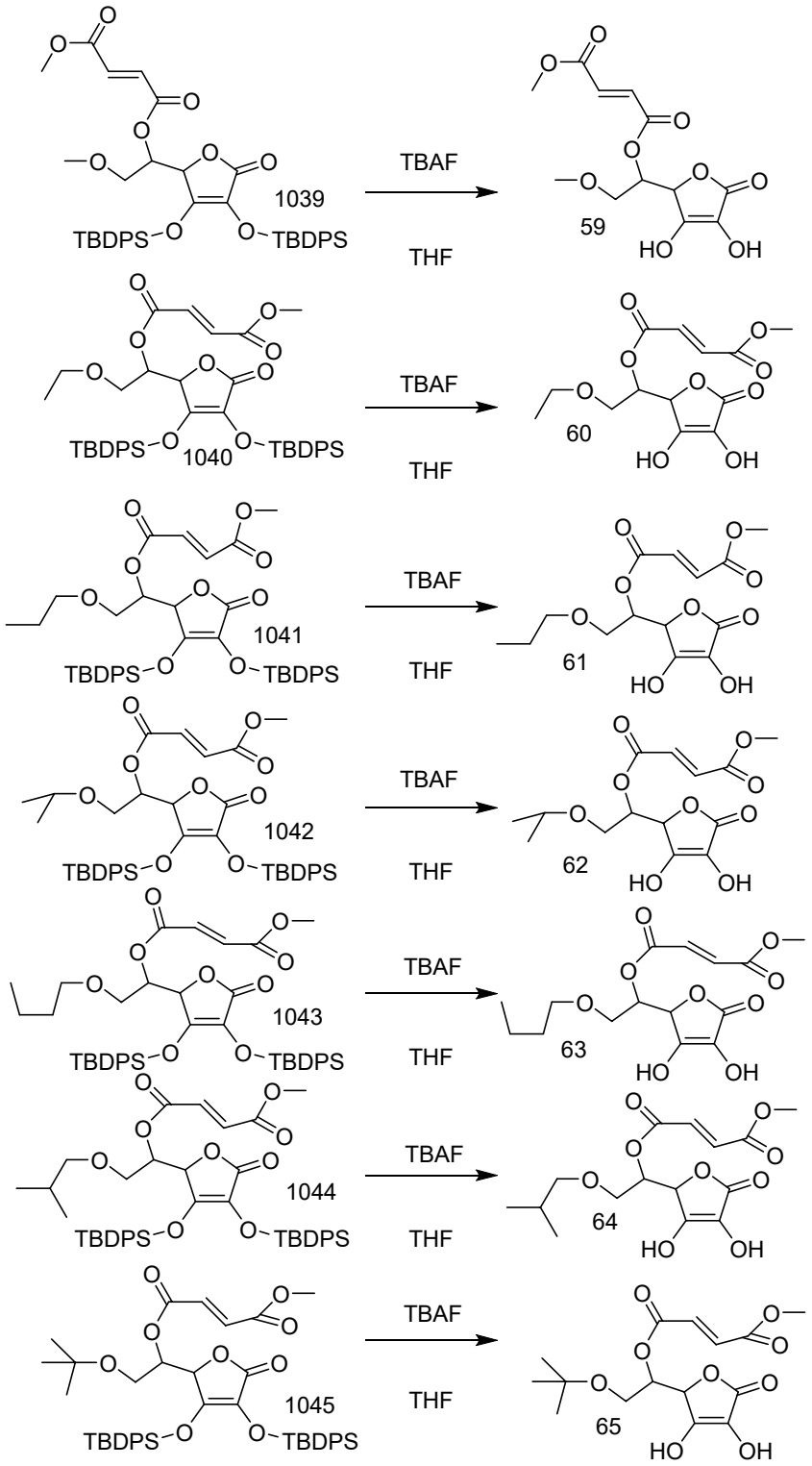

1039
TBDPS
TBDPS
TBAF
THF
59
HO
OH
1040
60
1041
61
1042
62
1043
63
1044
64
1045
65

Figure 14

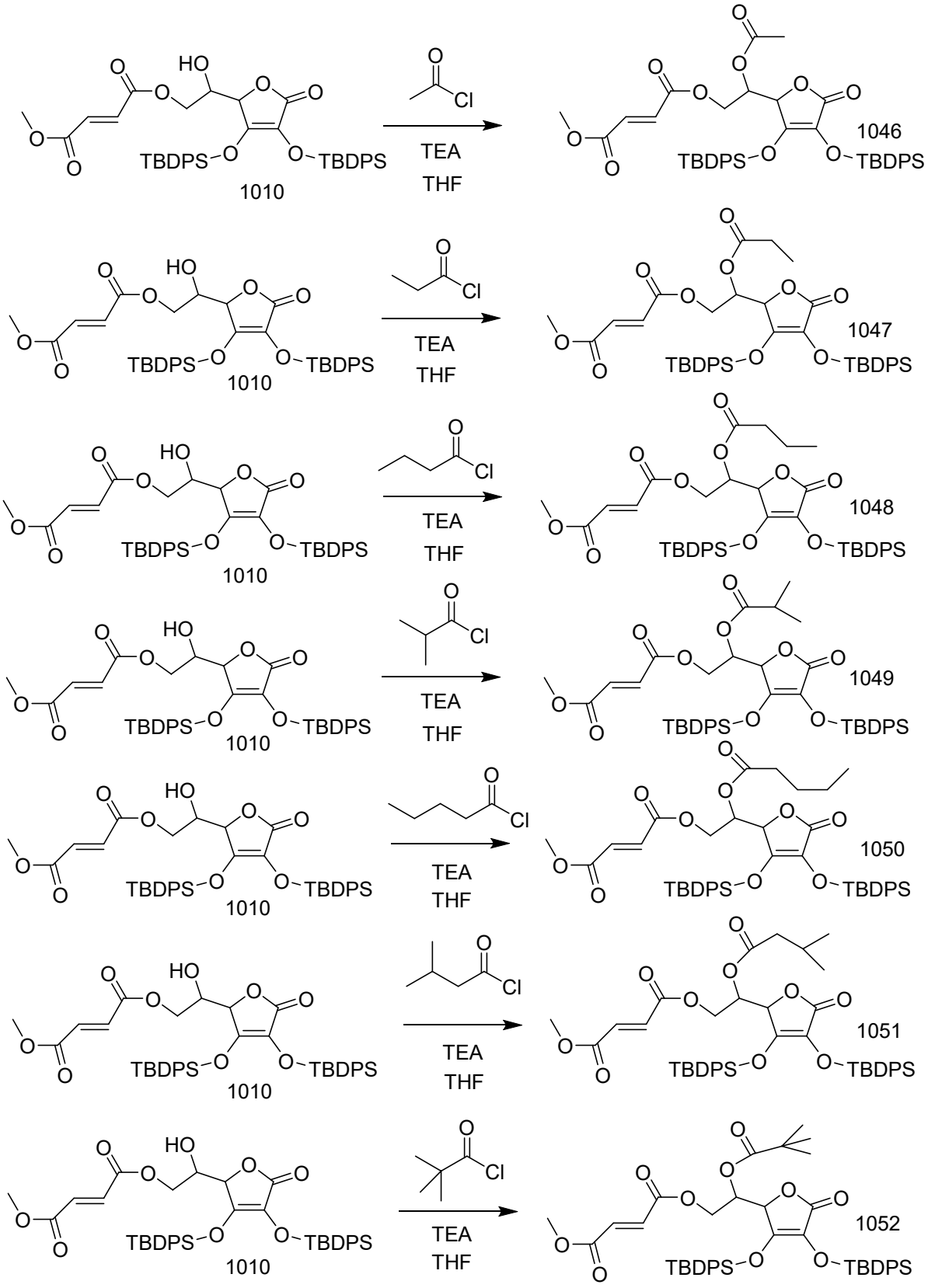

HO
TBDPS
TBDPS
1010
TEA
THF
1046
1047
1048
1049
1050
1051
1052

Figure 15

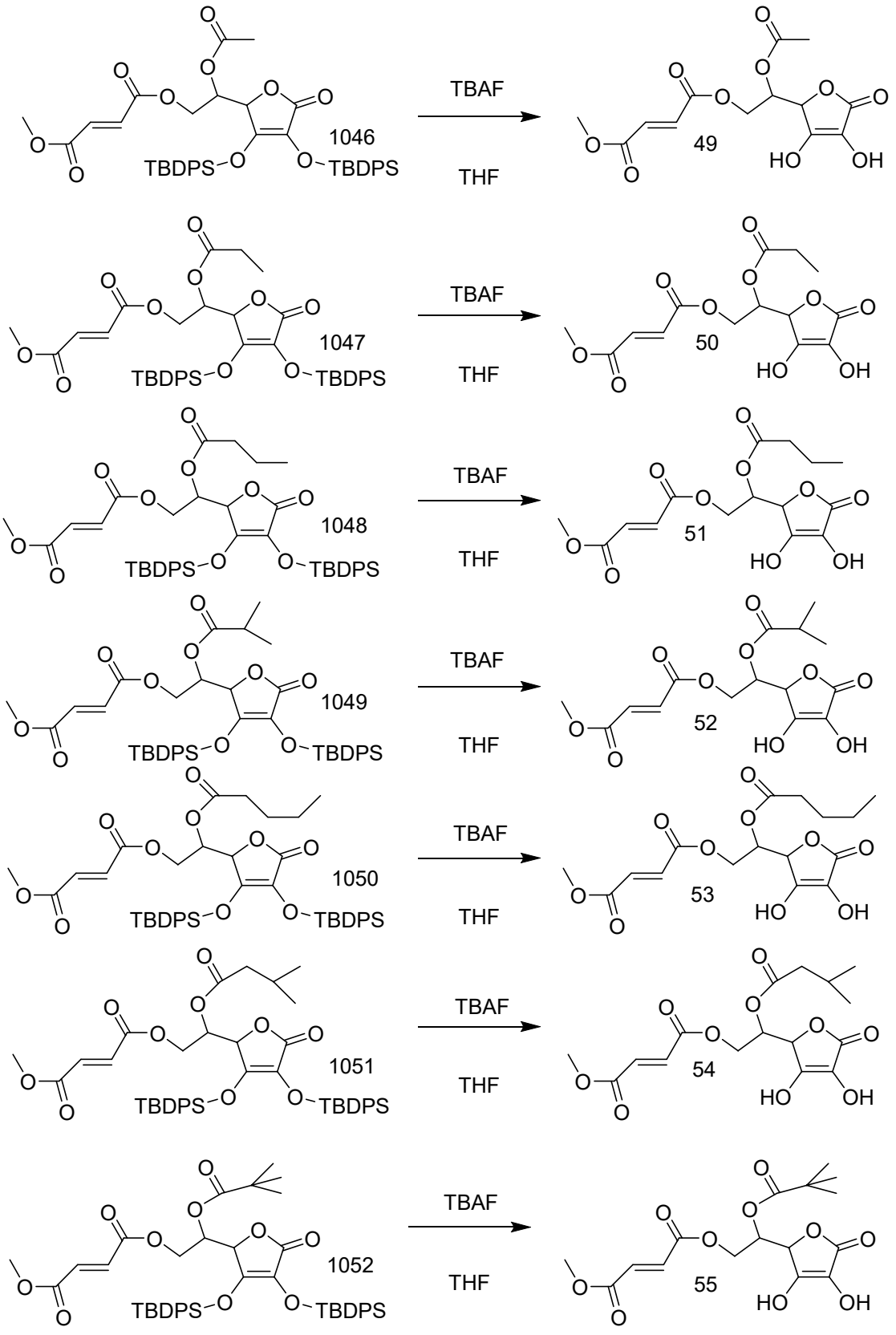

1046
TBDPS
TBDPS
TBAF
THF
49
HO
OH
1047
TBDPS
TBDPS
TBAF
THF
50
HO
OH
1048
TBDPS
TBDPS
TBAF
THF
51
HO
OH
1049
TBDPS
TBDPS
TBAF
THF
52
HO
OH
1050
TBDPS
TBDPS
TBAF
THF
53
HO
OH
1051
TBDPS
TBDPS
TBAF
THF
54
HO
OH
1052
TBDPS
TBDPS
TBAF
THF
55
HO
OH

Figure 16

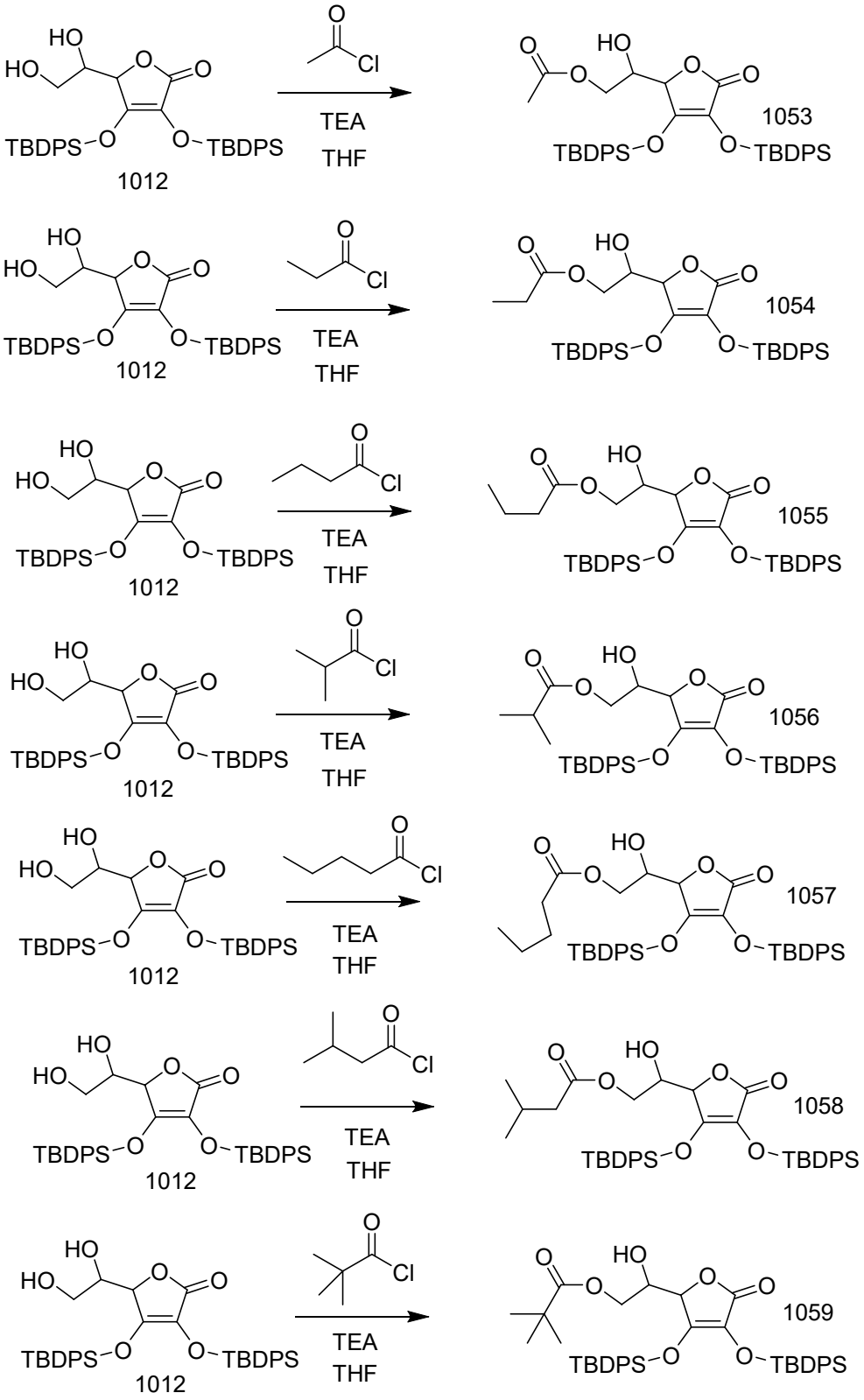

HO
HO
O
O
TBDPS
O
O
TBDPS
1012
O
Cl
TEA
THF
1053
1054
1055
1056
1057
1058
1059

Figure 17

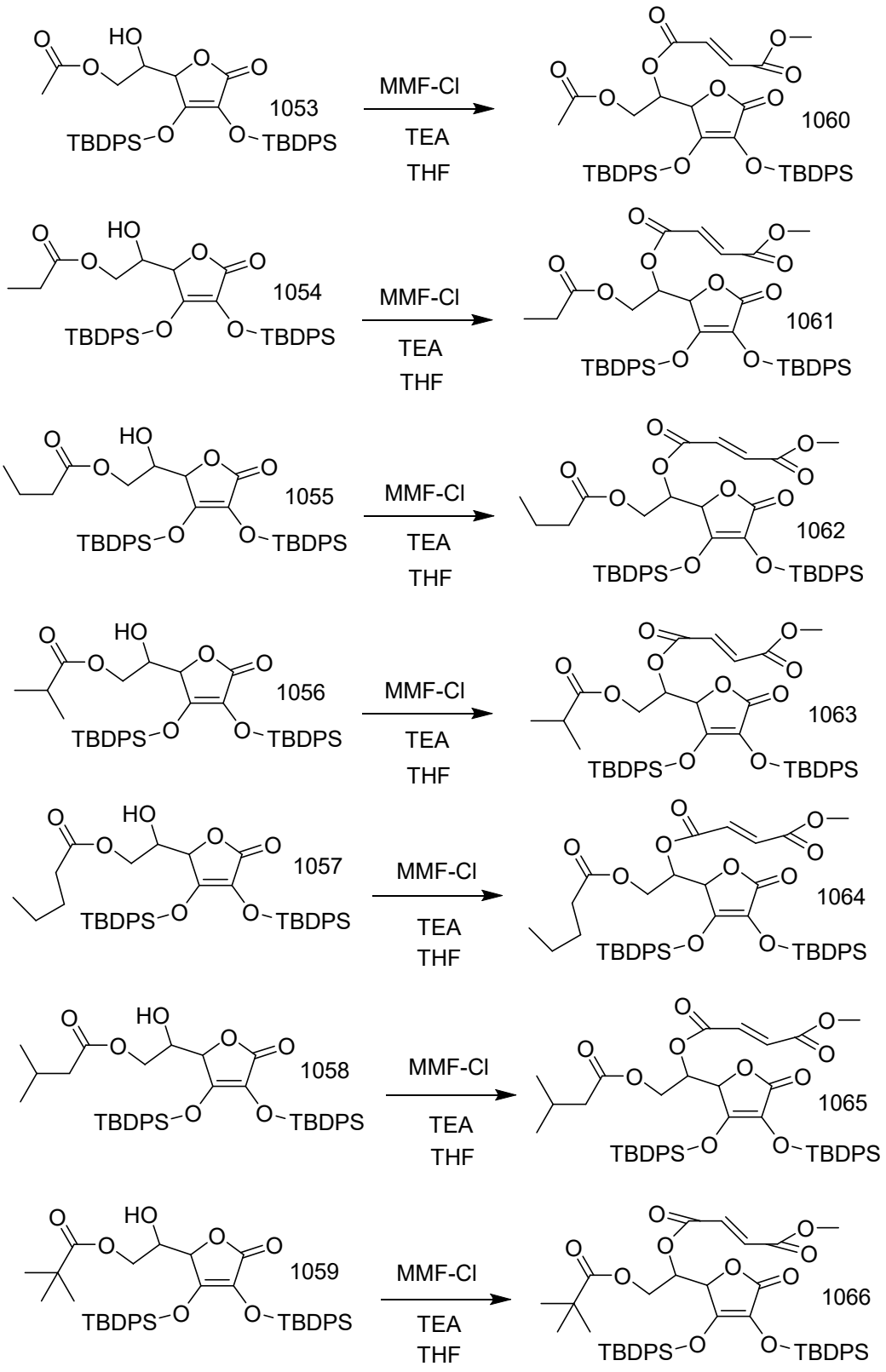

1053
MMF-Cl
TEA
THF
1060
1054
MMF-Cl
TEA
THF
1061
1055
MMF-Cl
TEA
THF
1062
1056
MMF-Cl
TEA
THF
1063
1057
MMF-Cl
TEA
THF
1064
1058
MMF-Cl
TEA
THF
1065
1059
MMF-Cl
TEA
THF
1066

Figure 18

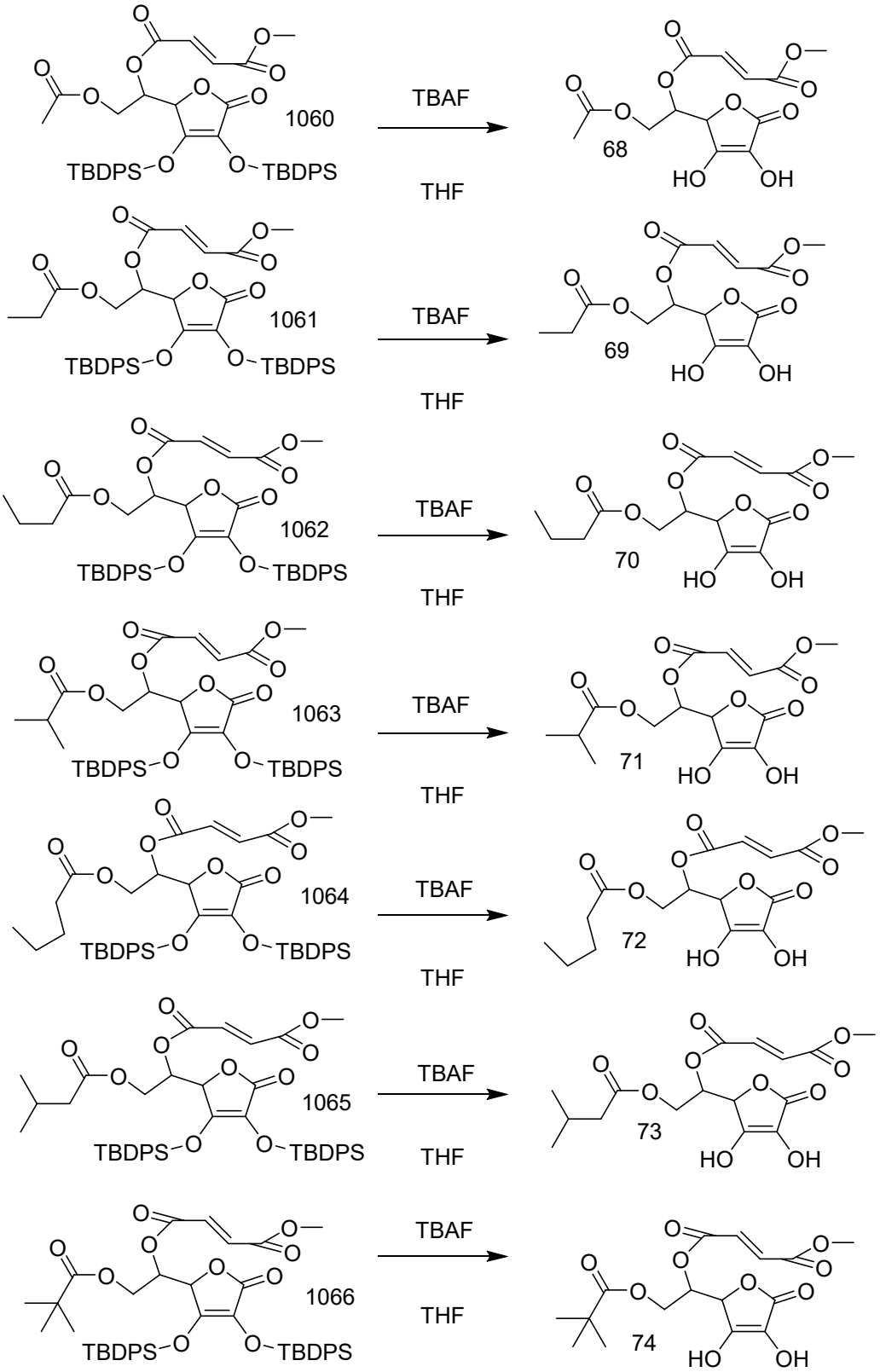

1060
TBAF
THF
68
1061
TBAF
THF
69
1062
TBAF
THF
70
1063
TBAF
THF
71
1064
TBAF
THF
72
1065
TBAF
THF
73
1066
TBAF
THF
74
TBDPS
TBDPS
HO
OH

Figure 19

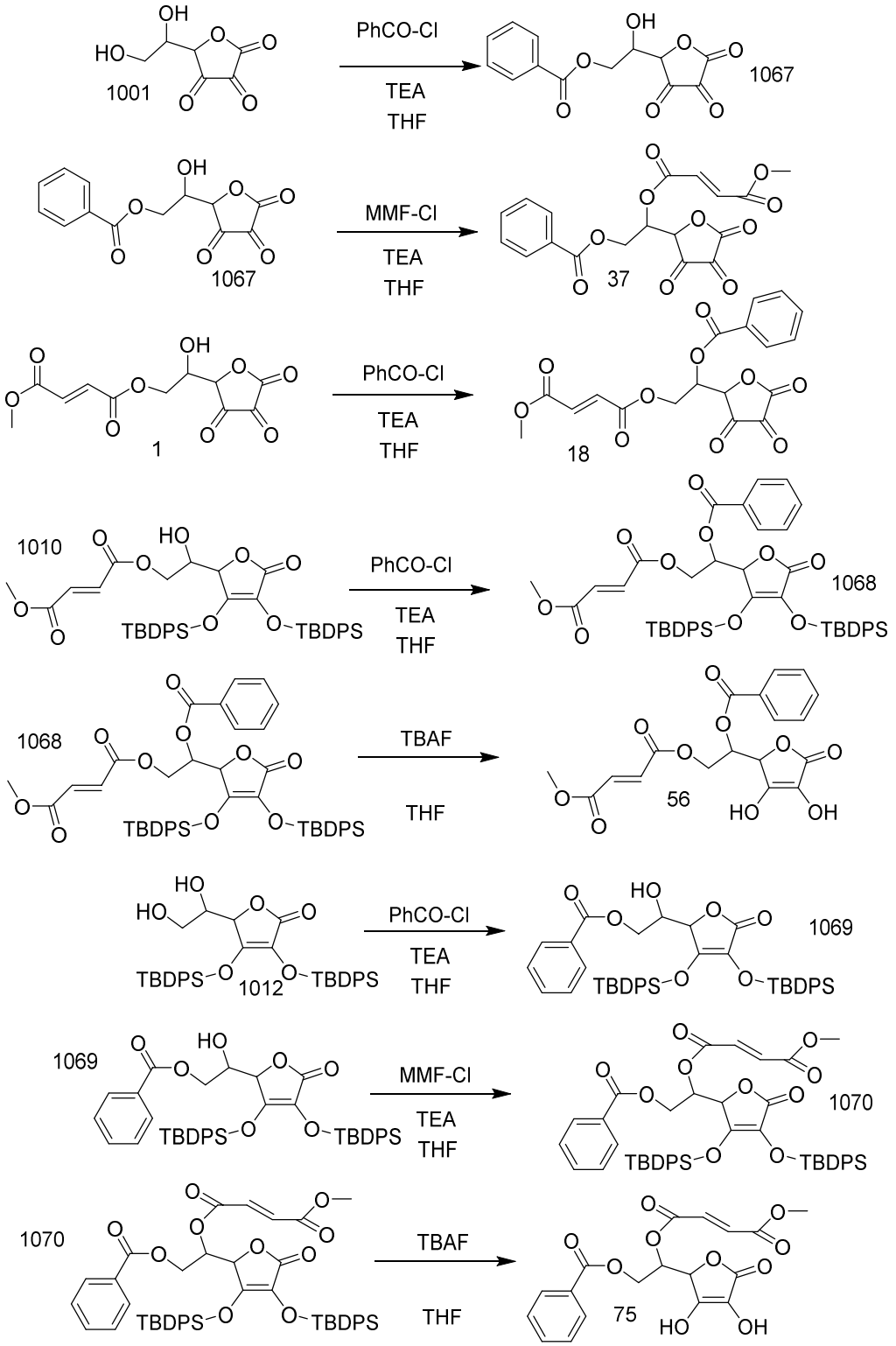

1001
PhCO-Cl
TEA
THF
1067
MMF-Cl
TEA
THF
37
1
PhCO-Cl
TEA
THF
18
1010
PhCO-Cl
TEA
THF
1068
TBAF
THF
56
1012
PhCO-Cl
TEA
THF
1069
1069
MMF-Cl
TEA
THF
1070
1070
TBAF
THF
75
TBDPS
HO
OH

Figure 20

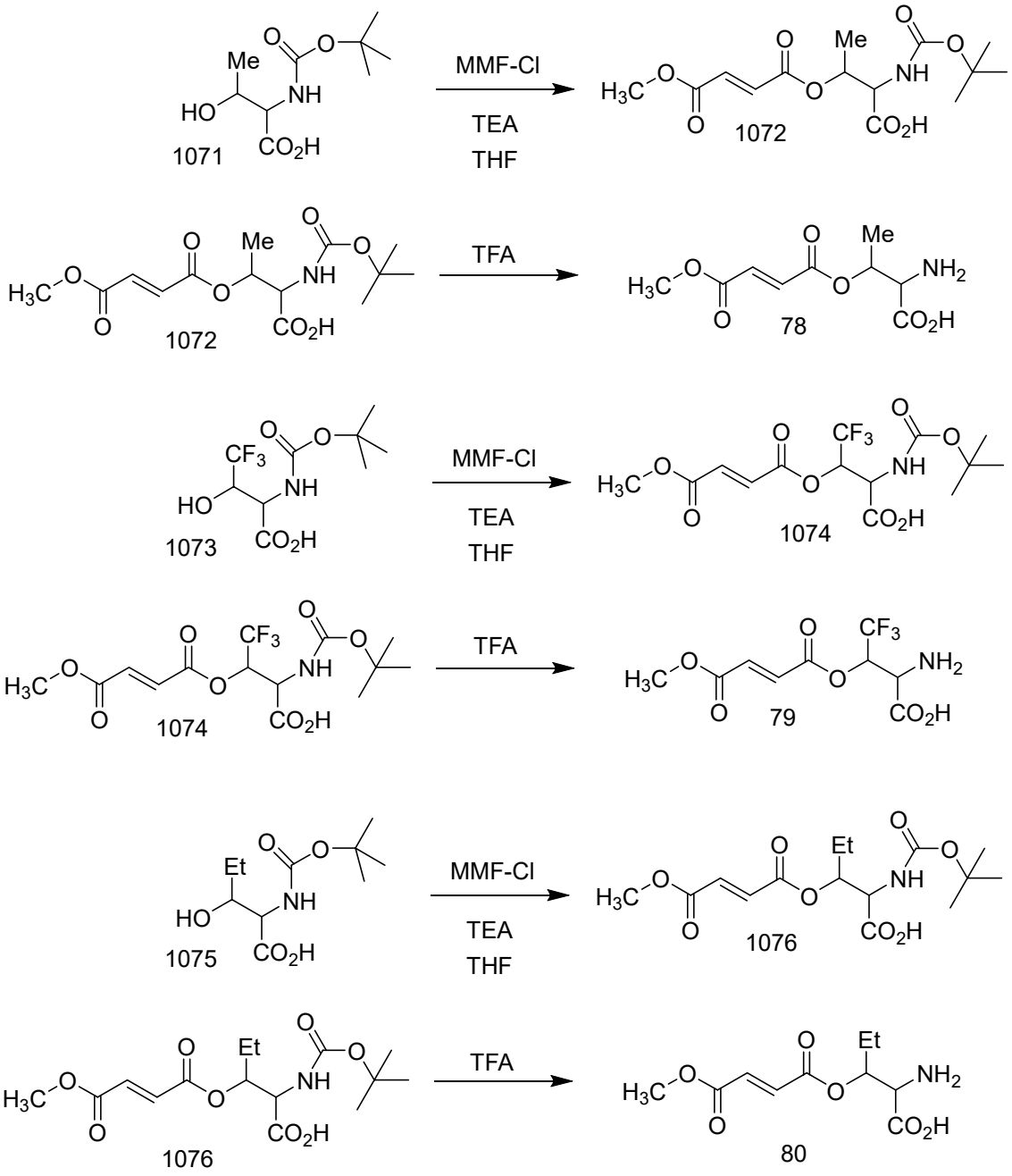


Figure 21

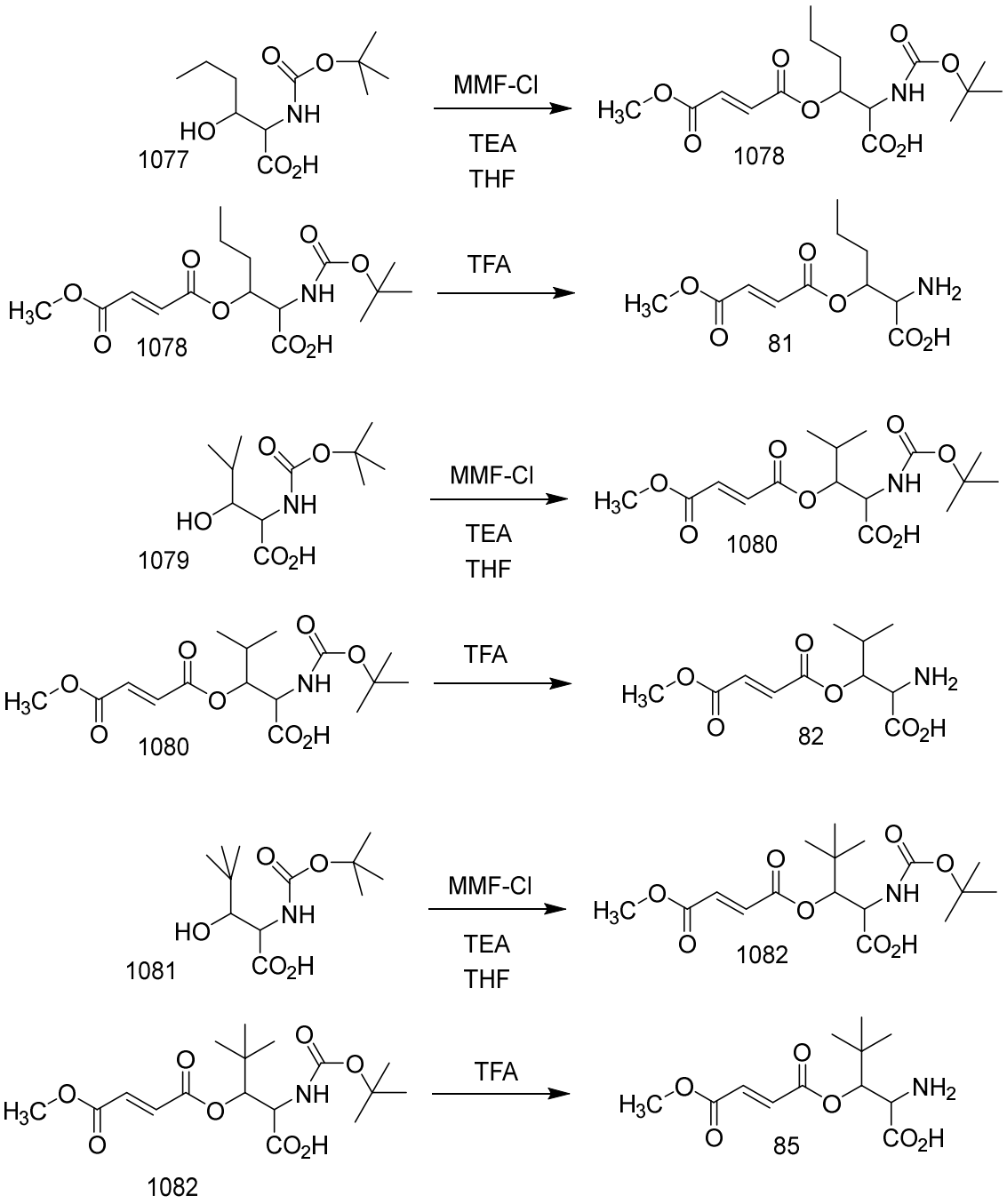


Figure 22

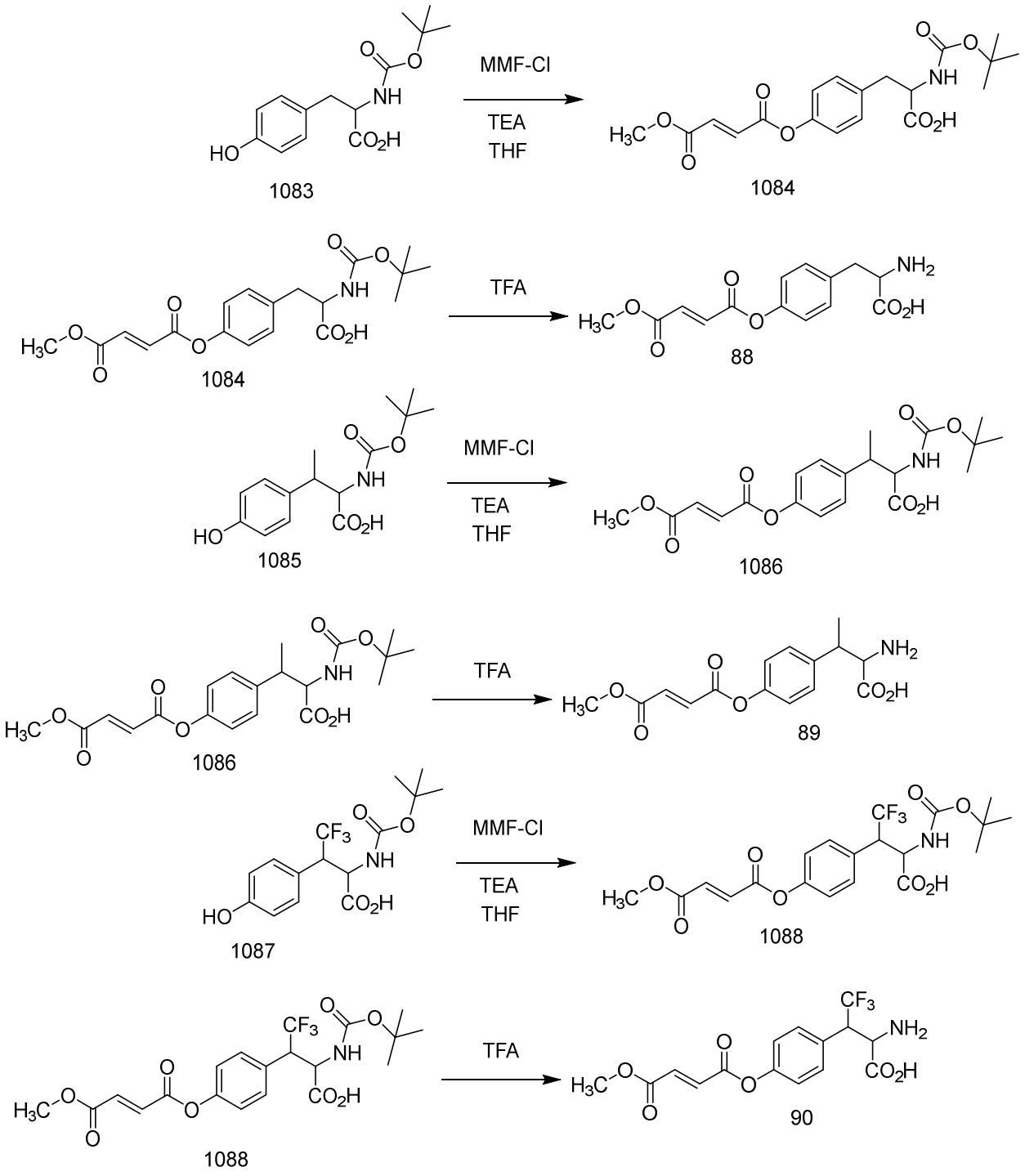


Figure 23

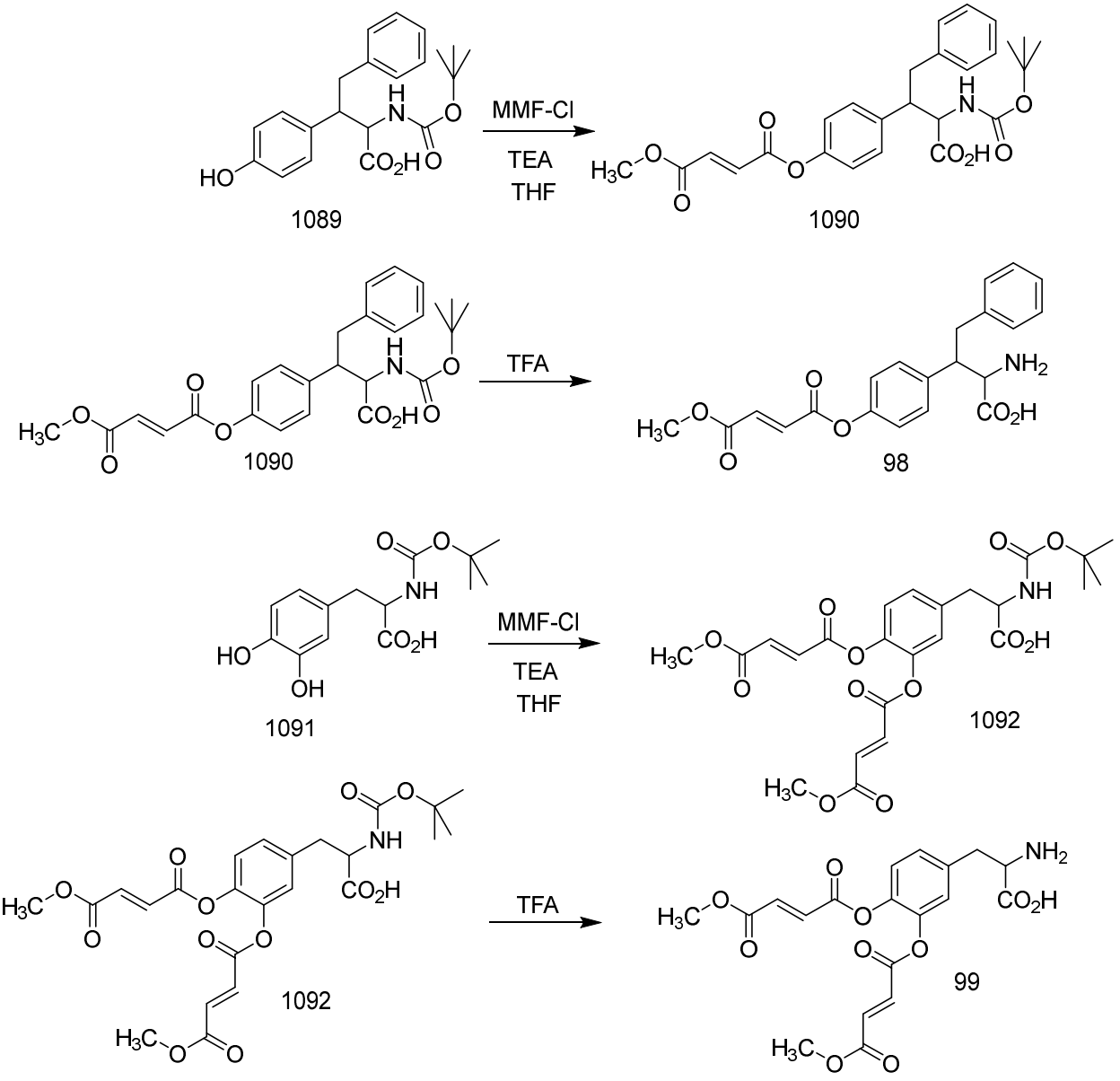
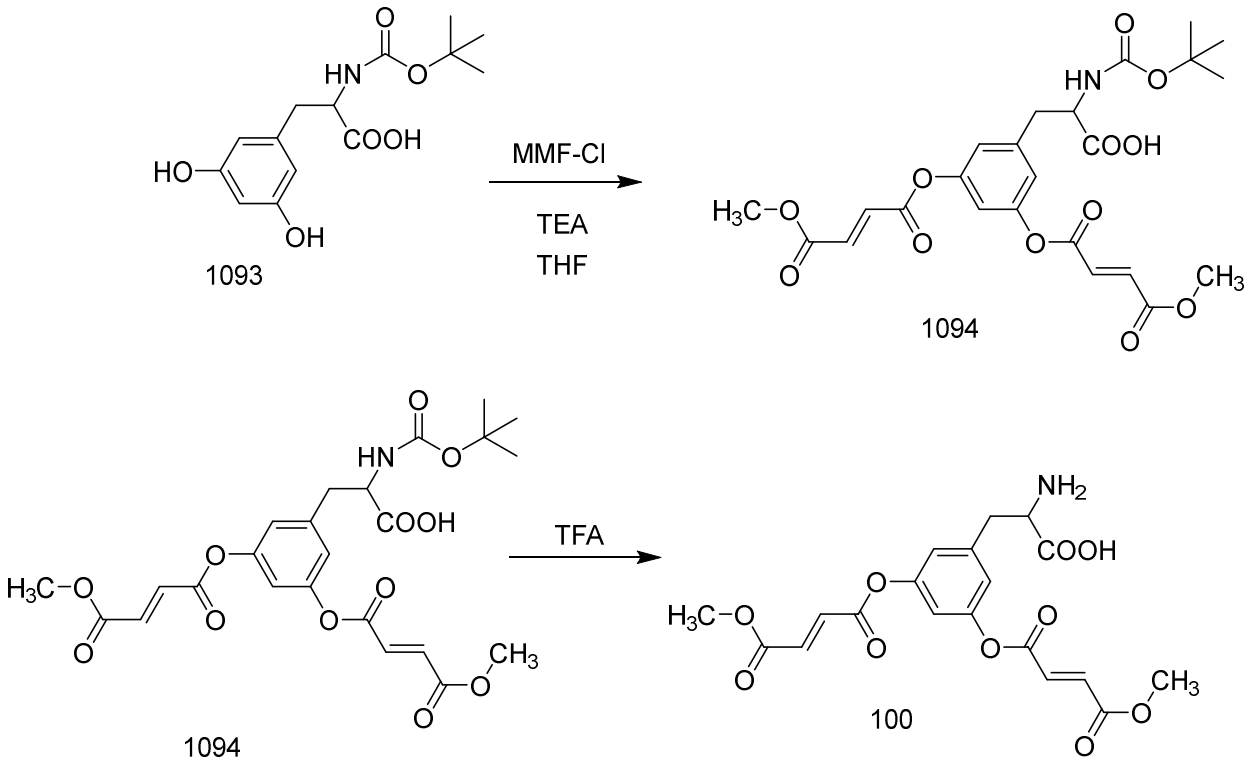


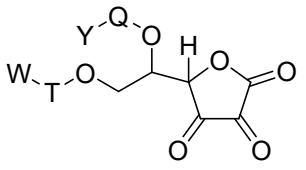
Figure 24



## CLAIMS

What is claimed is:

1. A compound of Formula 1:



1 ;

wherein:

Q is a single bond or C(O),

T is a single bond or C(O),

W is selected from the group consisting of hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, and $C(H)=C(H)CO_2R^{50}$,

Y is hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, or $C(H)=C(H)CO_2R^{50}$,

when Y is $C(H)=C(H)CO_2R^{50}$, then Q is C(O),

when Y or W is hydrogen, then Q or T is a single bond,

when W is $C(H)=C(H)CO_2R^{50}$, then T is C(O),

$R^{50}$ is $C_1$-$C_6$ alkyl, and,

at least one of W or Y is $C(H)=C(H)CO_2R^{50}$ with (E) regiochemistry;

or a pharmaceutically acceptable salt thereof.

2. A compound of Claim 1, wherein T is C(O) and Q is a single bond.
3. A compound of Claim 1, wherein Q is C(O) and S is a single bond.
4. A compound of Claim 1, wherein T is C(O) and Q is C(O).
5. The compound of Claim 1, wherein the compound is selected from the group consisting of:

2-hydroxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;

2-methoxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;

2-acetoxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;

2-(benzoyloxy)-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;

methyl (2-(2-phenylacetoxy)-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl) fumarate;

methyl (2-(pivaloyloxy)-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl) fumarate;

2-hydroxy-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;

2-acetoxy-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;

methyl (2-(pivaloyloxy)-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl) fumarate;

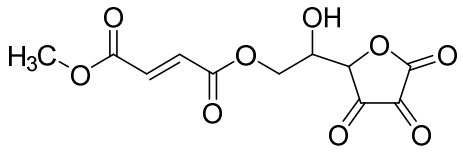
2-(benzoyloxy)-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;

methyl (2-(2-phenylacetoxy)-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl) fumarate;

2-(tert-butoxy)-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate; and,

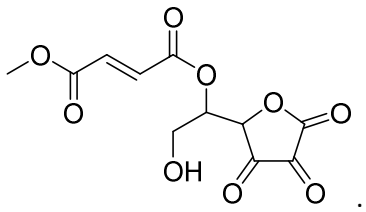
dimethyl O,O'-((1S)-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethane-1,2-diyl) difumarate.

6. The compound of Claim 1, wherein the compound is:



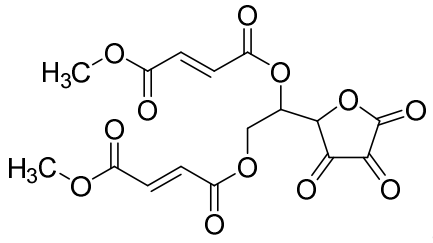
.

7. The compound of Claim 1, wherein the compound is:



.

8. The compound of Claim 1, wherein the compound is:



.

9. A composition comprising:

at least one compound of any one of Claims 1-8; and

at least one pharmaceutically acceptable excipient.

10. A composition comprising:

at least one compound of any one of Claims 1-8; and

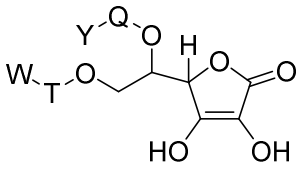
another compound selected from the group consisting of monomethyl fumarate and dimethylfumarate, clofibrate, bezofibrate, gemfibrizil, and fenofibrate and meclofenamic acid.

11. A method of treating and/or preventing a human disease by administering a compound of any one of Claims 1-8, or a composition of claim 9, to a human being.
12. The method of claim 11, wherein the disease a mitochondrial disease or a disease associated with lowered mitochondrial activity.
13. The method of Claim 12, wherein the disease is a primary mitochondrial disease.
14. The method of Claim 13, wherein the disease is selected from Leigh's syndrome, Leber hereditary optic neuropathy, Kearns-Sayre syndrome, Alpers-Huttenlocher syndrome, ataxia neuropathy syndrome, Mitochondrial Encephalomyopathy / Lactic Acid / Stroke (MELAS) syndrome, and Myoclonic Epilepsy/Red Ragged Fiber (MERRF) syndrome.
15. The method of Claim 12, wherein the disease is a secondary mitochondrial disorder.
16. The method of Claim 15, wherein the secondary mitochondrial disorder is selected from the group consisting of spinal muscular atrophy, Friedreich's ataxia, Charcot-Marie-Tooth syndrome, hereditary spastic paraplegia, and Wilson's disease.
17. The method of Claim 11, wherein the disease is a neurological disease.
18. The method of Claim 17, wherein the disease is a neurological disease selected from the group consisting of: Alzheimer's Disease, Frontotemporal Dementia, Amyotrophic Lateral Sclerosis, Lewy Body Dementia, Vascular dementia, Parkinson's Disease, traumatic brain injury, stroke, multiple sclerosis, Balo Concentric Sclerosis, and Huntington's Disease.
19. The method of Claim 17, wherein the neurological disease is selected from the group consisting of: schizophrenia, obsessive compulsive disorder, bipolar disorder, depressive disorder, drug addiction, psychotic disorders, anxiety disorder, personality disorder, mood disorder, major depressive disorder, post-traumatic stress disorder, and panic disorder.
20. The method of Claim 17, wherein the neurological disease is selected from the group consisting of: autism, Asperger's syndrome, Rett syndrome, childhood disintegrative disorder, Kanner's syndrome, pervasive developmental disorder, high functioning autism, low functioning autism, and classical autism.
21. The method of Claim 11, wherein the disease is selected from the group consisting of: Down Syndrome, retinitis pigmentosa, adrenal leukodystrophy, Alexanders Disease, Alper's Disease, Canavan Disease, Childhood Ataxia with Central Nervous System Hypomyelination, Globoid Cell Leukodystrophy, and Aicardi-Goutieres syndrome.

22. The method of Claim 11, wherein the disease is a proliferative disease.

23. The method of Claim 22, wherein the proliferative disease is selected from the group consisting of: brain cancer, glioblastoma multiforme, neuroblastoma, leukemia, lymphoma, sarcoma, and psoriasis.

24. A compound of Formula 2:




2 ;

wherein:

Q is a single bond or C(O),

T is a single bond or C(O),

W is selected from the group consisting of hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, and $C(H)=C(H)CO_2R^{50}$,

Y is selected from the group consisting of hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, and $C(H)=C(H)CO_2R^{50}$,

when Y is $C(H)=C(H)CO_2R^{50}$, then Q is C(O),

when Y or W is hydrogen, then Q or T is a single bond,

when W is $C(H)=C(H)CO_2R^{50}$, then S is C(O),

$R^{50}$ is $C_1$-$C_6$ alkyl,

at least one of Y or W is $C(H)=C(H)CO_2R^{50}$ with (E) regiochemistry, and,

if T is C(O) and W is $C(H)=C(H)CO_2R^{50}$ then Y is not hydrogen;

or a pharmaceutically acceptable salt thereof.

25. The compound of Claim 22, wherein the compound is selected from the group consisting of:

O,O'-(1-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)ethane-1,2-diyl) dimethyl difumarate;

2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-methoxyethyl methyl fumarate;

2-acetoxy-2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)ethyl methyl fumarate;

2-(benzoyloxy)-2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)ethyl methyl fumarate;

2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-(2-phenylacetoxy)ethyl methyl fumarate;

2-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-(pivaloyloxy)ethyl methyl fumarate;

1-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-hydroxyethyl methyl fumarate;

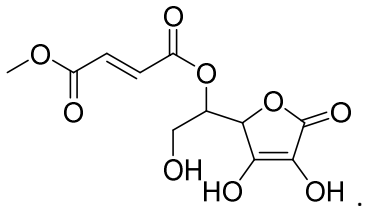
1-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-(pivaloyloxy)ethyl methyl fumarate;

2-(benzoyloxy)-1-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)ethyl methyl fumarate;

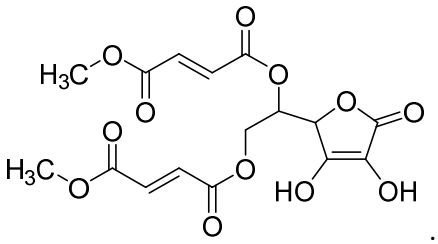
1-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-(2-phenylacetoxy)ethyl methyl fumarate; and,

2-(tert-butoxy)-1-(3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)ethyl methyl fumarate.

26. The compound of Claim 18 wherein the compound is:



27. The compound of Claim 18 wherein the compound is:



28. A composition comprising:

    at least one compound of any one of Claims 23-27; and

    at least one pharmaceutically acceptable excipient.

29. A method of treating and/or preventing a human disease by administering a compound of any one of Claims 24-27, or a composition of claim 28 to a human being.

30. The method of claim 29, wherein the disease a mitochondrial disease or a disease associated with lowered mitochondrial activity.

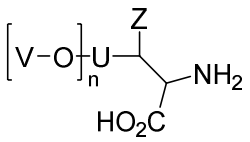
31. The method of Claim 30, wherein the disease is a primary mitochondrial disease.

32. The method of Claim 31, wherein the disease is selected from Leigh's syndrome, Leber hereditary optic neuropathy, Kearns-Sayre syndrome, Alpers-Huttenlocher syndrome, ataxia neuropathy syndrome, Mitochondrial Encephalomyopathy / Lactic Acid / Stroke (MELAS) syndrome, and Myoclonic Epilepsy/Red Ragged Fiber (MERRF) syndrome.

33. The method of Claim 30, wherein the disease is a secondary mitochondrial disorder.

34. The method of Claim 33, wherein the secondary mitochondrial disorder is selected from the group consisting of spinal muscular atrophy, Friedreich's ataxia, Charcot-Marie-Tooth syndrome, hereditary spastic paraplegia, and Wilson's disease.
35. The method of Claim 29, wherein the disease is a neurological disease.
36. The method of Claim 35, wherein the neurological disease is selected from the group consisting of Alzheimer's Disease, Frontotemporal Dementia, Amyotrophic Lateral Sclerosis, Lewy Body Dementia, Vascular dementia, Parkinson's Disease, traumatic brain injury, stroke, multiple sclerosis, Balo Concentric Sclerosis, and Huntington's Disease.
37. The method of Claim 35, wherein the neurological disease is selected from the group consisting of schizophrenia, obsessive compulsive disorder, bipolar disorder, depressive disorder, drug addiction, psychotic disorders, anxiety disorder, personality disorder, mood disorder, major depressive disorder, post-traumatic stress disorder, and panic disorder.
38. The method of Claim 35, wherein the neurological disease is selected from the group consisting of autism, Asperger's syndrome, Rett syndrome, childhood disintegrative disorder, Kanner's syndrome, pervasive developmental disorder, high functioning autism, low functioning autism, and classical autism.
39. The method of Claim 29, wherein the disease is selected from the group consisting of Down Syndrome, retinitis pigmentosa, adrenal leukodystrophy, Alexanders Disease, Alper's Disease, Canavan Disease, Childhood Ataxia with Central Nervous System Hypomyelination, Globoid Cell Leukodystrophy, and Aicardi-Goutieres syndrome.
40. The method of Claim 29, wherein the disease is a proliferative disease.
41. The method of Claim 40, wherein the proliferative disease is selected from the group consisting of brain cancer, glioblastoma multiforme, neuroblastoma, leukemia, lymphoma, sarcoma, and psoriasis.

42. A compound of Formula 3:




3 ;

wherein

n is 1 or 2,

U is a single bond or an aryl ring comprised of phenyl or pyridyl,

V is $C(O)C(H)=C(H)CO_2R^{50}$ with (E) regiochemistry,

Z is selected from the group consisting of hydrogen, methyl, trifluoromethyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, and benzyl,

if U is a single bond, Z is hydrogen,

if U is phenyl or pyridyl, Z is selected from the group consisting of hydrogen, methyl and trifluoromethyl, and,

$R^{50}$ is $C_1$-$C_6$ alkyl;

or a pharmaceutically acceptable salt thereof.

43. The compound of Claim 42, wherein U is a single bond and Z is selected from the group consisting of methyl, trifluoromethyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, and benzyl.

44. The compound of Claim 42, wherein:

U is a phenyl ring; and

Z is selected from the group consisting of hydrogen, methyl, trifluoromethyl, and ethyl.

45. The compound of Claim 42, wherein the compound is selected from the group consisting of:

(E)-2-amino-3-((4-methoxy-4-oxobut-2-enoyl)oxy)butanoic acid;

(E)-2-amino-3-((4-methoxy-4-oxobut-2-enoyl)oxy)-4,4-dimethylpentanoic acid;

(E)-2-amino-4,4,4-trifluoro-3-((4-methoxy-4-oxobut-2-enoyl)oxy)butanoic acid;

(E)-2-amino-3-((4-methoxy-4-oxobut-2-enoyl)oxy)-3-phenylpropanoic acid;

(E)-2-amino-3-((4-methoxy-4-oxobut-2-enoyl)oxy)-4-phenylbutanoic acid;

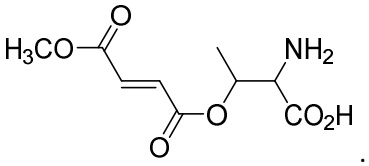
(E)-2-amino-3-(4-((4-methoxy-4-oxobut-2-enoyl)oxy)phenyl)propanoic acid;

(E)-2-amino-3-(3-((4-methoxy-4-oxobut-2-enoyl)oxy)phenyl)propanoic acid;

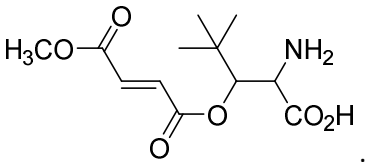
(E)-2-amino-3-(2-((4-methoxy-4-oxobut-2-enoyl)oxy)phenyl)propanoic acid; and,

2-amino-3-(3,5-bis(((E)-4-methoxy-4-oxobut-2-enoyl)oxy)phenyl)propanoic acid.

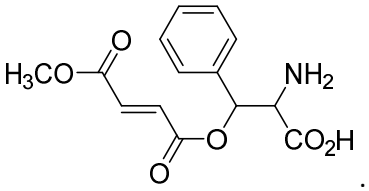
46. The compound of Claim 42, wherein the compound is:



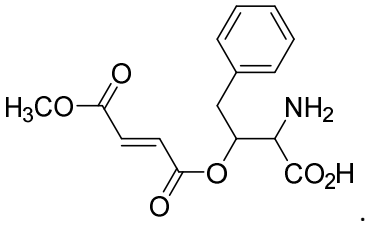
47. The compound of Claim 42, wherein the compound is:



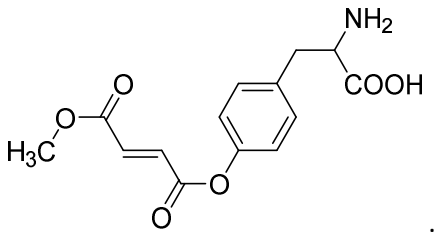
48. The compound of Claim 42, wherein the compound is:



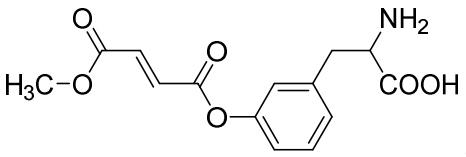
49. The compound of Claim 42, wherein the compound is:



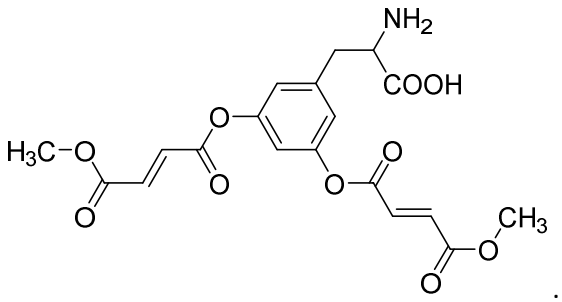
50. The compound of Claim 42, wherein the compound is:



51. The compound of Claim 42, wherein the compound is:



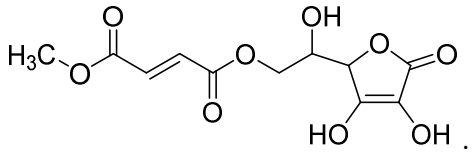
52. The compound of Claim 42, wherein the compound is:



53. A composition comprising:
    at least one compound of any one of Claims 42-52; and
    at least one pharmaceutically acceptable excipient.
54. A method of treating and/or preventing a human disease by administering a compound of any one of Claims 42-52, or a composition of claim 53, to a human being.
55. The method of claim 54, wherein the disease a mitochondrial disease or a disease associated with lowered mitochondrial activity.
56. The method of Claim 55, wherein the disease is a primary mitochondrial disease.
57. The method of Claim 56, wherein the disease is selected from Leigh's syndrome, Leber hereditary optic neuropathy, Kearns-Sayre syndrome, Alpers-Huttenlocher syndrome, ataxia neuropathy syndrome, Mitochondrial Encephalomyopathy / Lactic Acid / Stroke (MELAS) syndrome, and Myoclonic Epilepsy/Red Ragged Fiber (MERRF) syndrome.
58. The method of Claim 55, wherein the disease is a secondary mitochondrial disorder.
59. The method of Claim 58, wherein the secondary mitochondrial disorder is selected from the group consisting of spinal muscular atrophy, Friedreich's ataxia, Charcot-Marie-Tooth syndrome, hereditary spastic paraplegia, and Wilson's disease.
60. The method of Claim 54, wherein the disease is a neurological disease.
61. The method of Claim 60, wherein the neurological disease is selected from the group consisting of Alzheimer's Disease, Frontotemporal Dementia, Amyotrophic Lateral Sclerosis, Lewy Body Dementia, Vascular dementia, Parkinson's Disease, traumatic brain injury, stroke, multiple sclerosis, Balo Concentric Sclerosis, and Huntington's Disease.
62. The method of Claim 60, wherein the neurological disease is selected from the group consisting of schizophrenia, obsessive compulsive disorder, bipolar disorder, depressive disorder, drug addiction,

psychotic disorders, anxiety disorder, personality disorder, mood disorder, major depressive disorder, post-traumatic stress disorder, and panic disorder.

63. The method of Claim 60, wherein the neurological disease is selected from the group consisting of autism, Asperger's syndrome, Rett syndrome, childhood disintegrative disorder, Kanner's syndrome, pervasive developmental disorder, high functioning autism, low functioning autism, and classical autism.
64. The method of Claim 54, wherein the disease is selected from the group consisting of Down Syndrome, retinitis pigmentosa, adrenal leukodystrophy, Alexanders Disease, Alper's Disease, Canavan Disease, Childhood Ataxia with Central Nervous System Hypomyelination, Globoid Cell Leukodystrophy, and Aicardi-Goutieres syndrome.
65. The method of Claim 54, wherein the disease is a proliferative disease.
66. The method of Claim 65, wherein the proliferative disease is selected from the group consisting of brain cancer, glioblastoma multiforme, neuroblastoma, leukemia, lymphoma, sarcoma, and psoriasis.
67. A compound of the structure:



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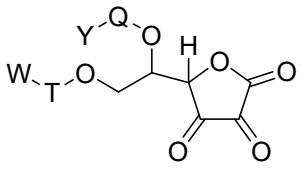
68. A compound of Formula 4:



1 ;

wherein:

Q is a single bond or C(O),

T is a single bond or C(O),

W is selected from the group consisting of hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, and $C(H)=C(H)CO_2R^{50}$,

Y is hydrogen, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, or $C(H)=C(H)CO_2R^{50}$,

when Y is $C(H)=C(H)CO_2R^{50}$, then Q is C(O),

when W is $C(H)=C(H)CO_2R^{50}$, then T is C(O),

when Y or W is hydrogen, then Q or T is a single bond,

when W is H, then Y is methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, phenyl, benzyl, or $C(H)=C(H)CO_2R^{50}$;

$R^{50}$ is $C_1$-$C_6$ alkyl, and,

when W is methyl, i-propyl, or phenyl, and T is C(O), then i) Q may not be a single bond, and ii) Y may not be hydrogen;

or a pharmaceutically acceptable salt thereof.

69. The compound of Claim 68, wherein the compound is selected from the group consisting of:

5-(1-hydroxy-2-methoxyethyl)furan-2,3,4(5H)-trione;

5-(2-ethoxy-1-hydroxyethyl)furan-2,3,4(5H)-trione;

5-(2-butoxy-1-hydroxyethyl)furan-2,3,4(5H)-trione;

5-(1-hydroxy-2-isopropoxyethyl)furan-2,3,4(5H)-trione;

5-(2-butoxy-1-hydroxyethyl)furan-2,3,4(5H)-trione;

5-(1-hydroxy-2-isobutoxyethyl)furan-2,3,4(5H)-trione;

5-(2-(tert-butoxy)-1-hydroxyethyl)furan-2,3,4(5H)-trione;

5-(2-(benzyloxy)-1-hydroxyethyl)furan-2,3,4(5H)-trione;

2-hydroxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate;

2-hydroxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl propionate;

2-hydroxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl butyrate;

2-hydroxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl pentanoate;

2-hydroxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl 3-methylbutanoate;

2-hydroxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl pivalate;

2-hydroxy-2-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl 2-phenylacetate; and

2-hydroxy-1-(3,4,5-trioxotetrahydrofuran-2-yl)ethyl methyl fumarate.

70. A compound of the structure:

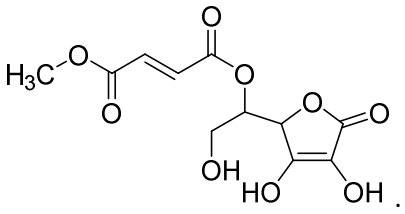



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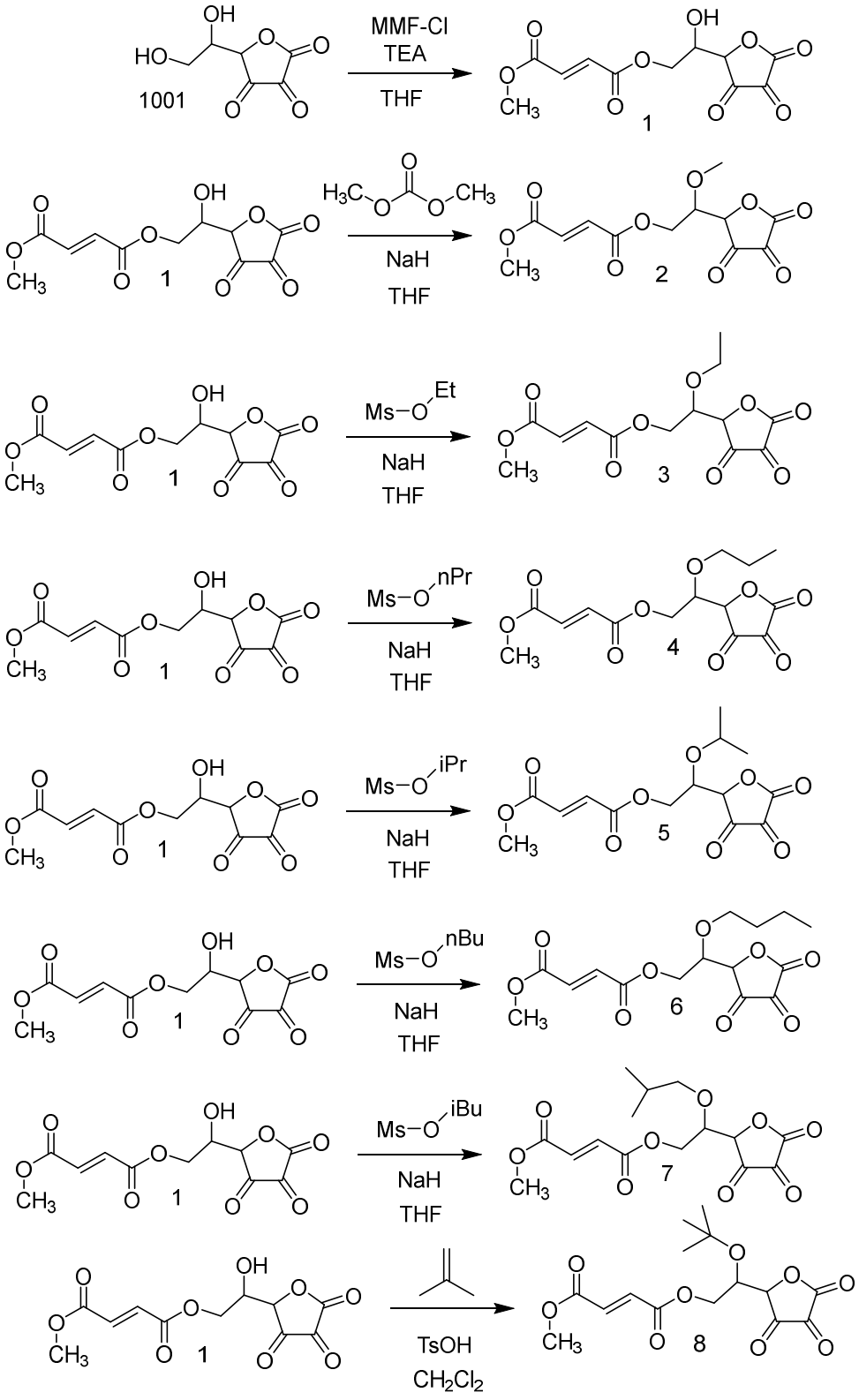
Figure 1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MMF-Cl
TEA
THF
1
NaH
THF
2
Et
3
nPr
4
iPr
5
nBu
6
iBu
7
TsOH
CH2Cl2
8

Figure 2

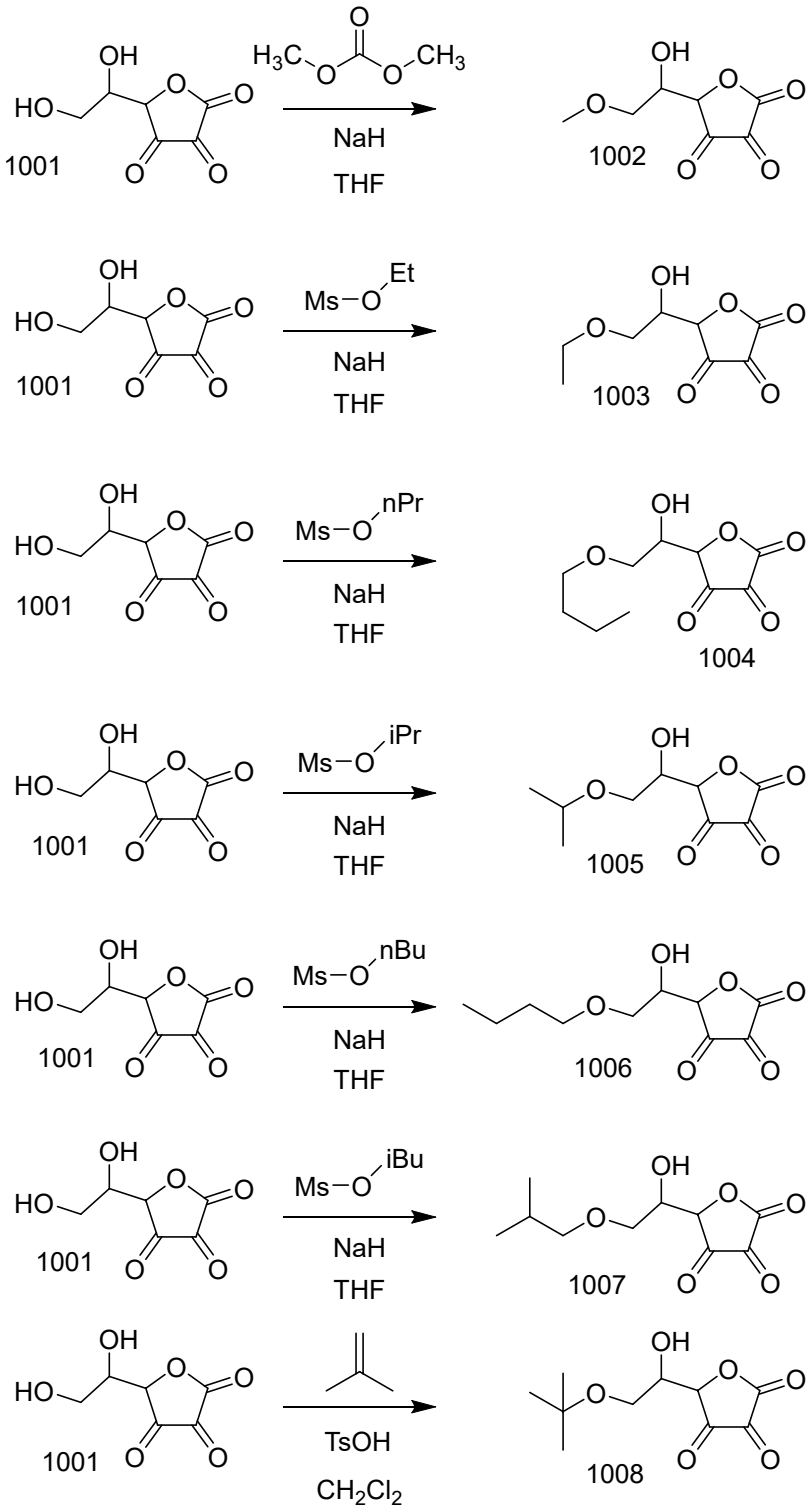

1001
NaH
THF
1002
Ms—O Et
1003
Ms—O nPr
1004
Ms—O iPr
1005
Ms—O nBu
1006
Ms—O iBu
1007
TsOH
$CH_2Cl_2$
1008

Figure 3

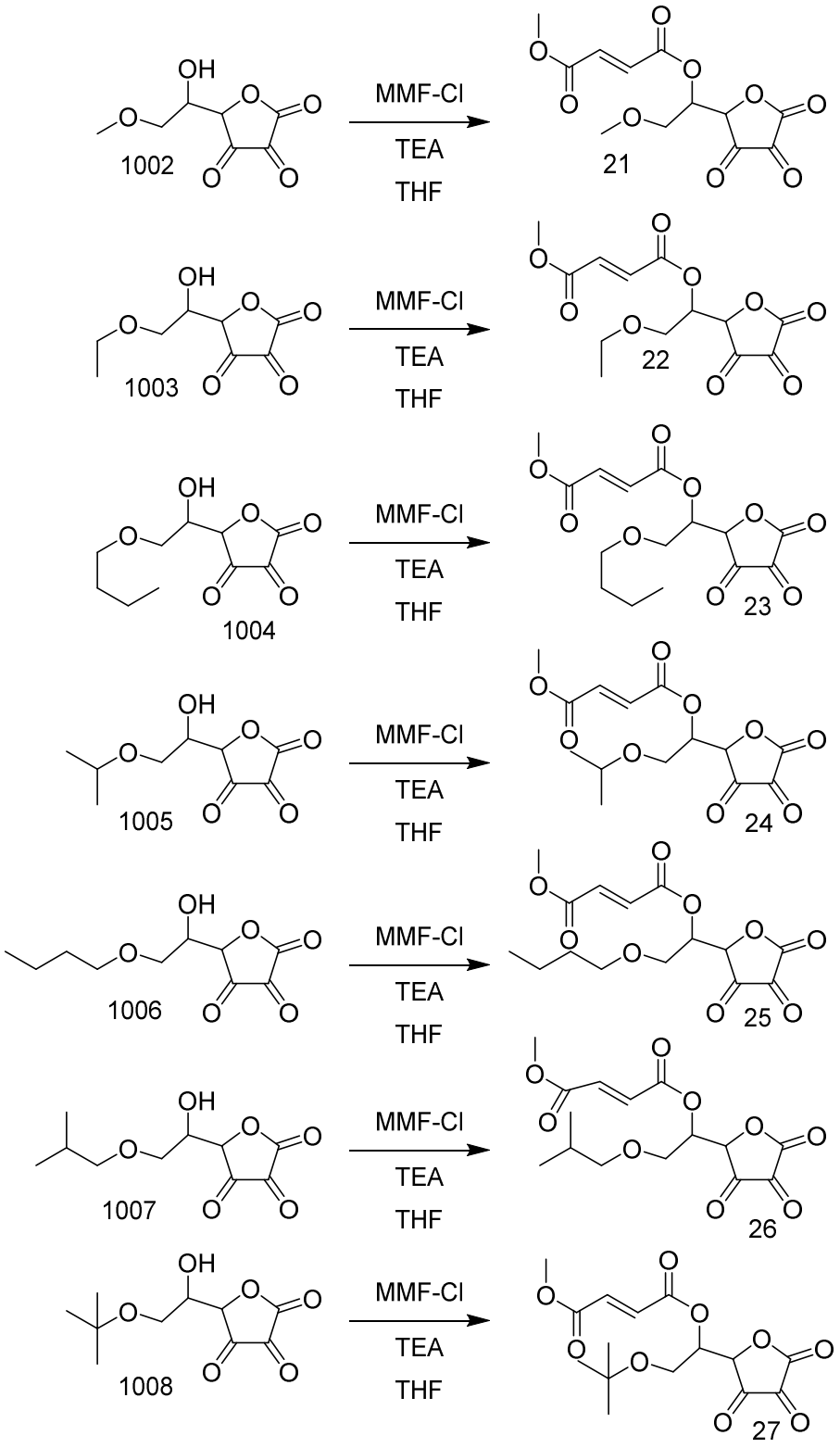

OH
O
O
O
O
O
1002
MMF-Cl
TEA
THF
21
1003
22
1004
23
1005
24
1006
25
1007
26
1008
27

Figure 4

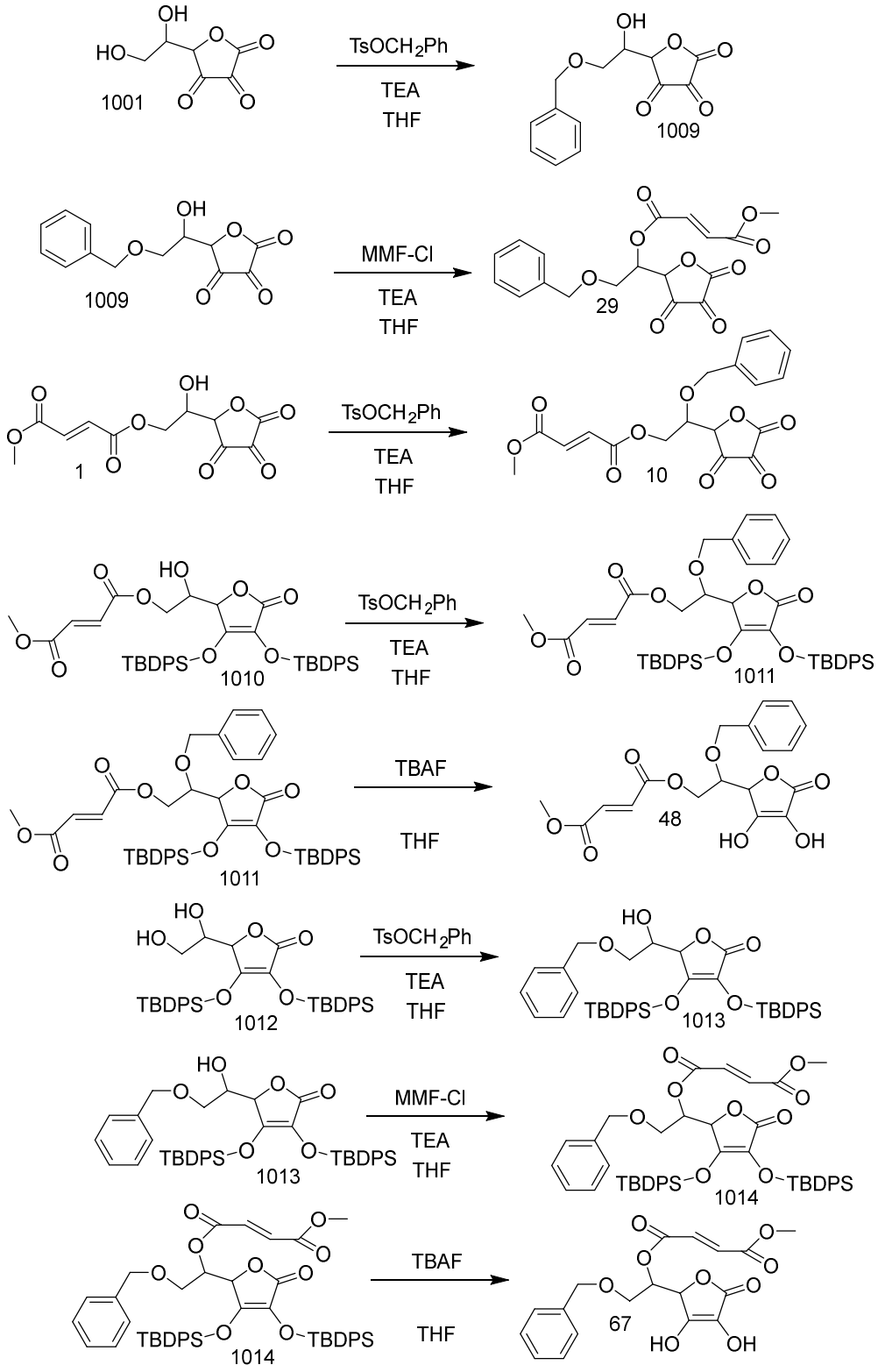

1001
TsOCH2Ph
TEA
THF
1009
1009
MMF-Cl
TEA
THF
29
1
TsOCH2Ph
TEA
THF
10
1010
TsOCH2Ph
TEA
THF
1011
1011
TBAF
THF
48
1012
TsOCH2Ph
TEA
THF
1013
1013
MMF-Cl
TEA
THF
1014
1014
TBAF
THF
67

Figure 5

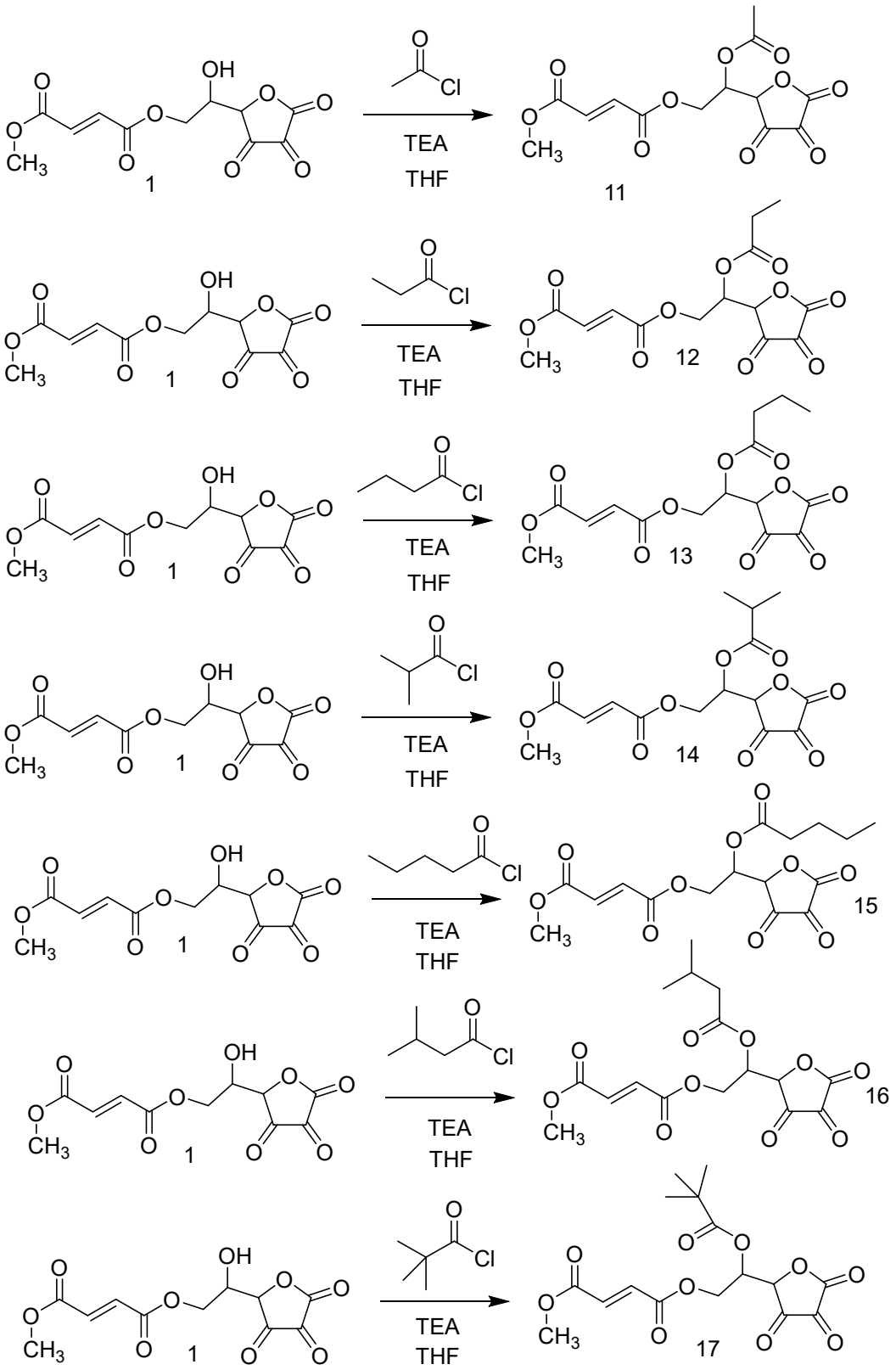


Figure 6

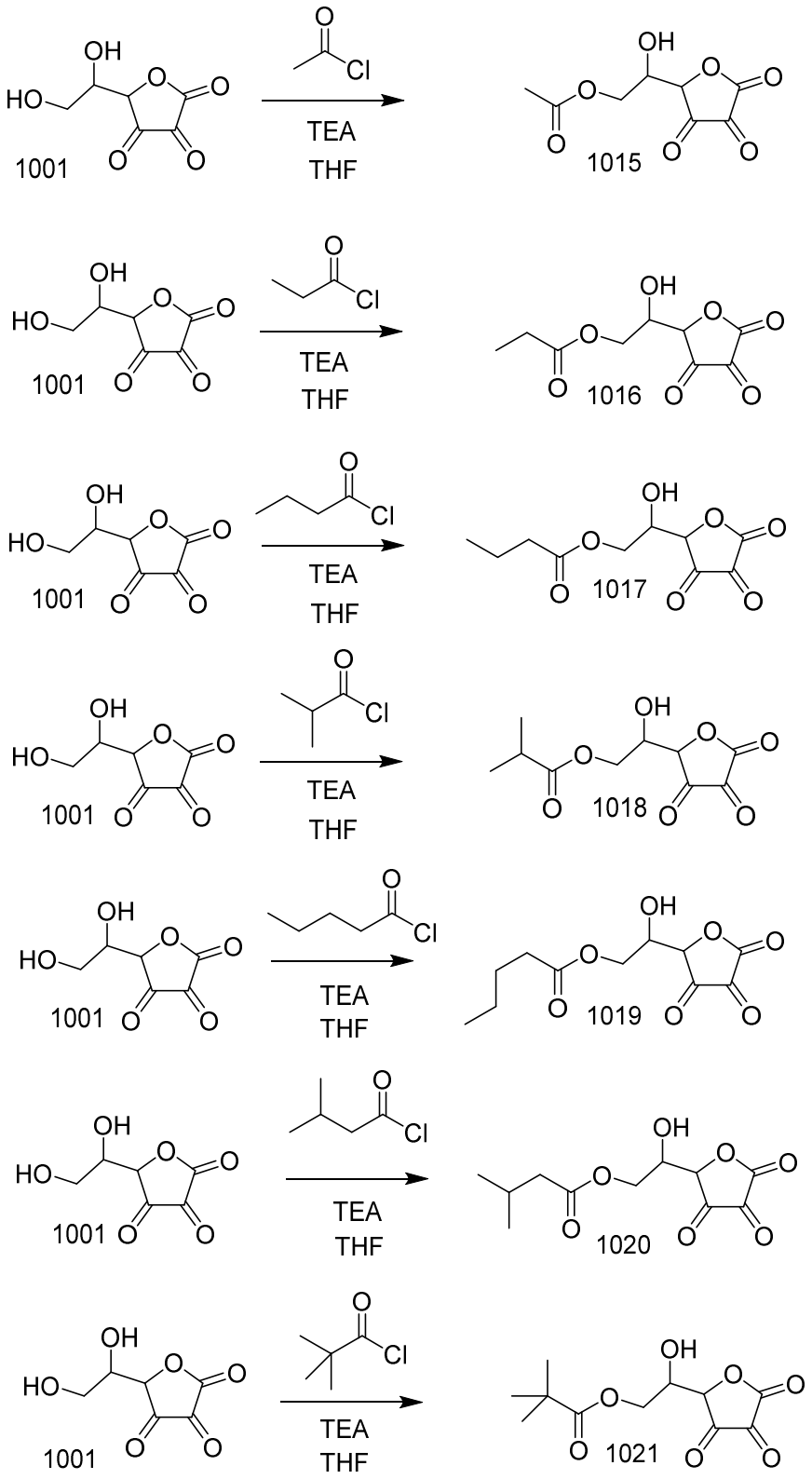


Figure 7

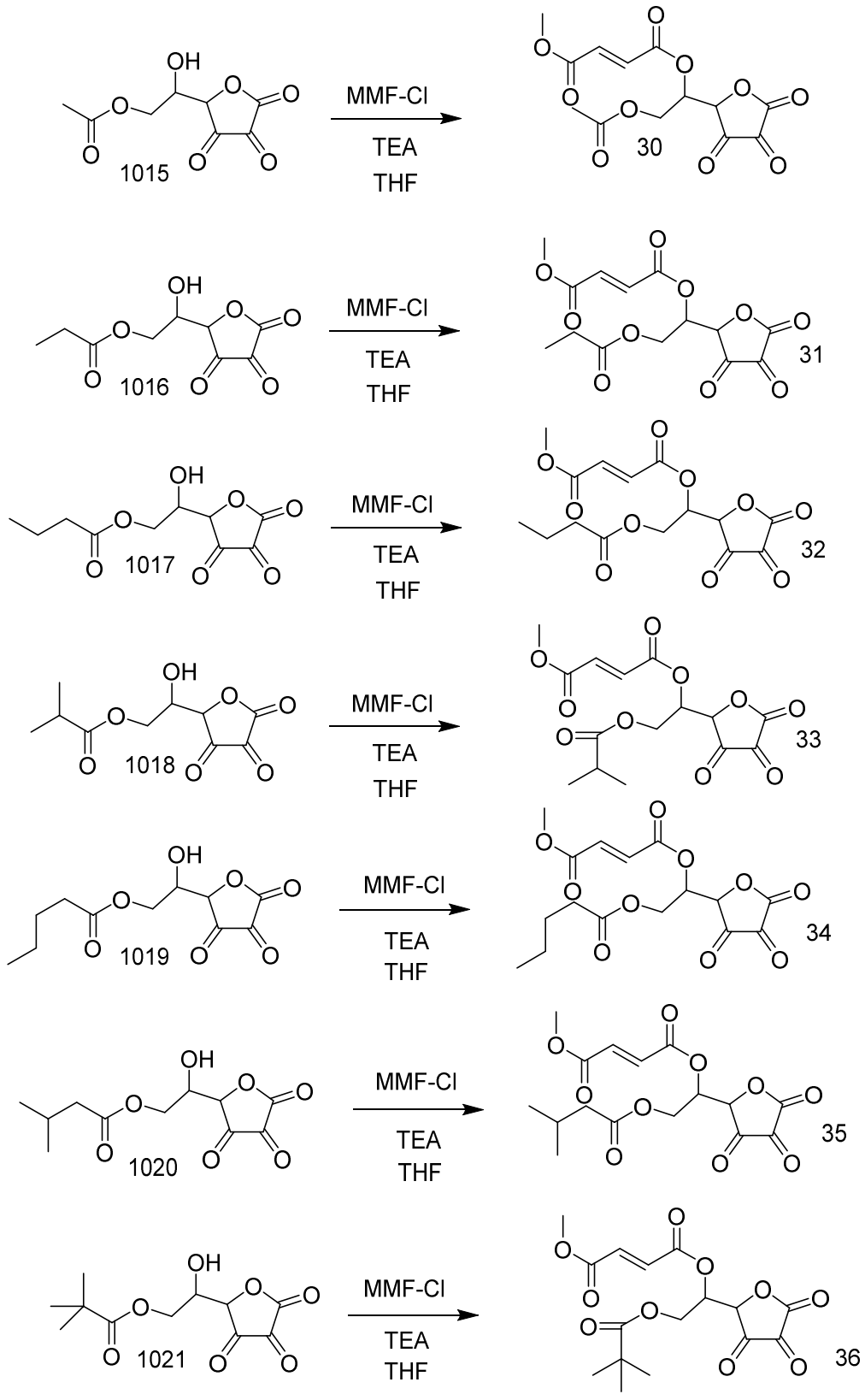

OH
O
O
O
O
O
O
1015
MMF-Cl
TEA
THF
30
1016
31
1017
32
1018
33
1019
34
1020
35
1021
36

Figure 8

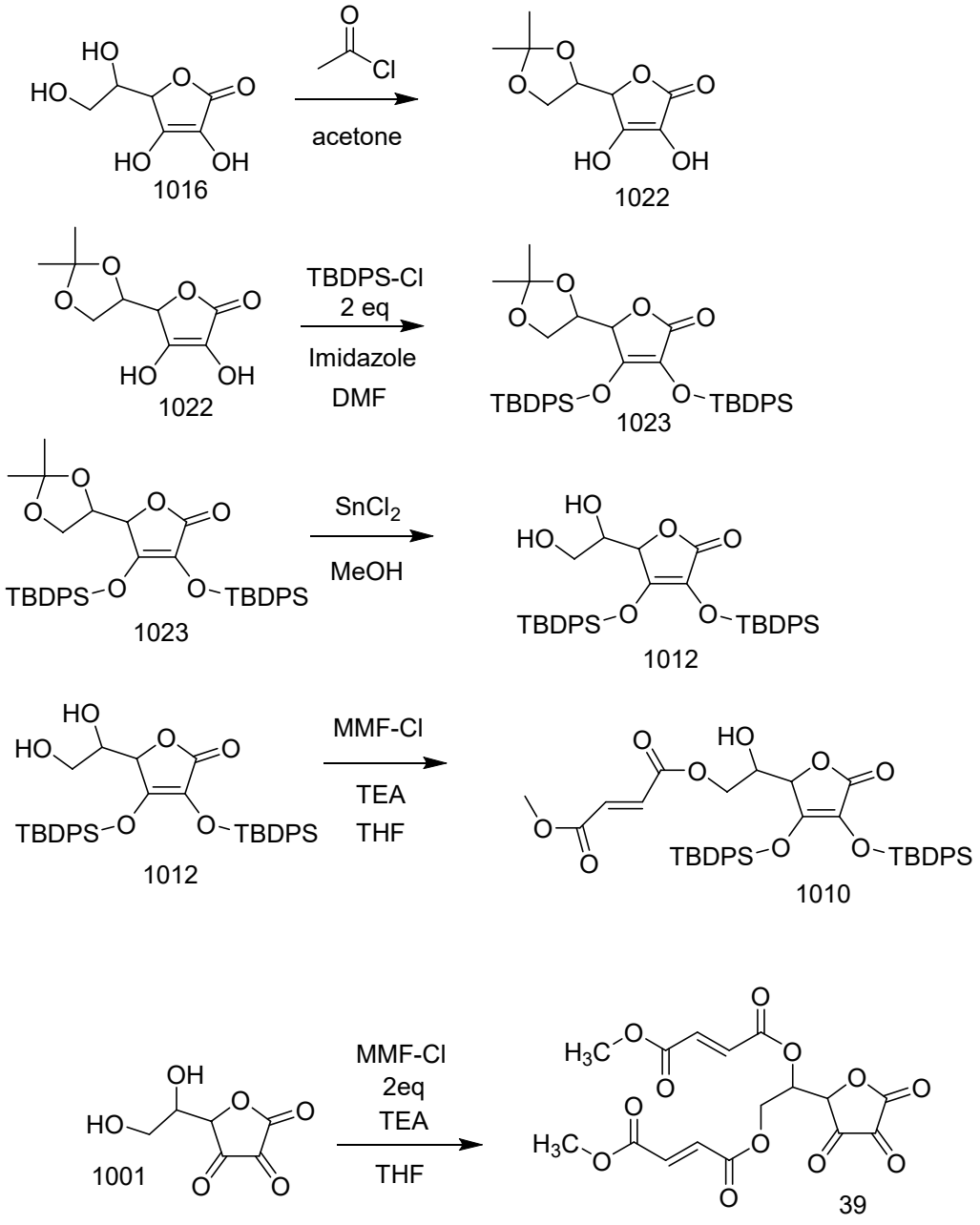

1016
acetone
1022
TBDPS-Cl
2 eq
Imidazole
DMF
1023
SnCl2
MeOH
1012
MMF-Cl
TEA
THF
1010
1001
MMF-Cl
2eq
TEA
THF
39

Figure 9

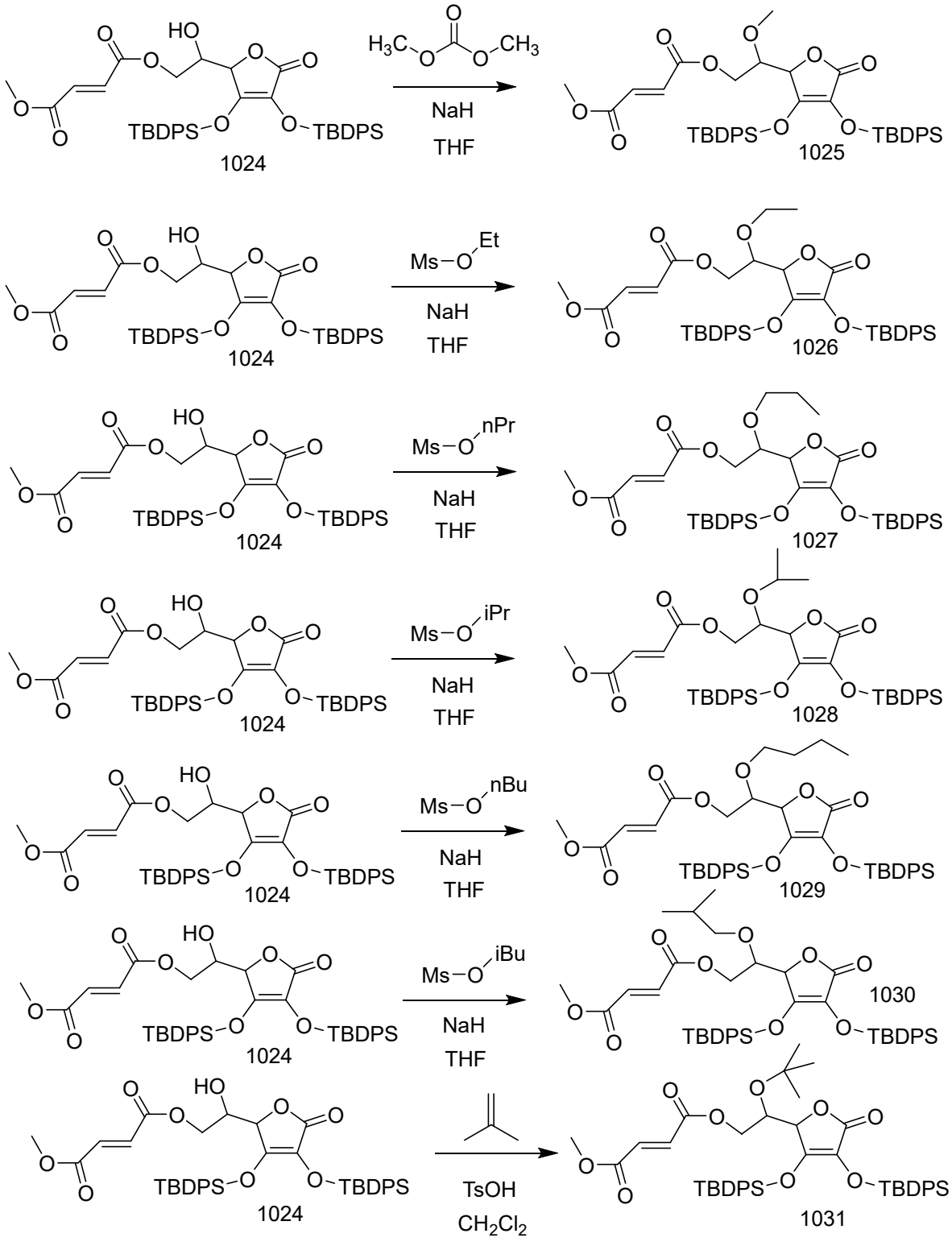

HO
TBDPS
TBDPS
1024
H3C
CH3
NaH
THF
1025
Ms
Et
1026
nPr
1027
iPr
1028
nBu
1029
iBu
1030
TsOH
CH2Cl2
1031

Figure 10

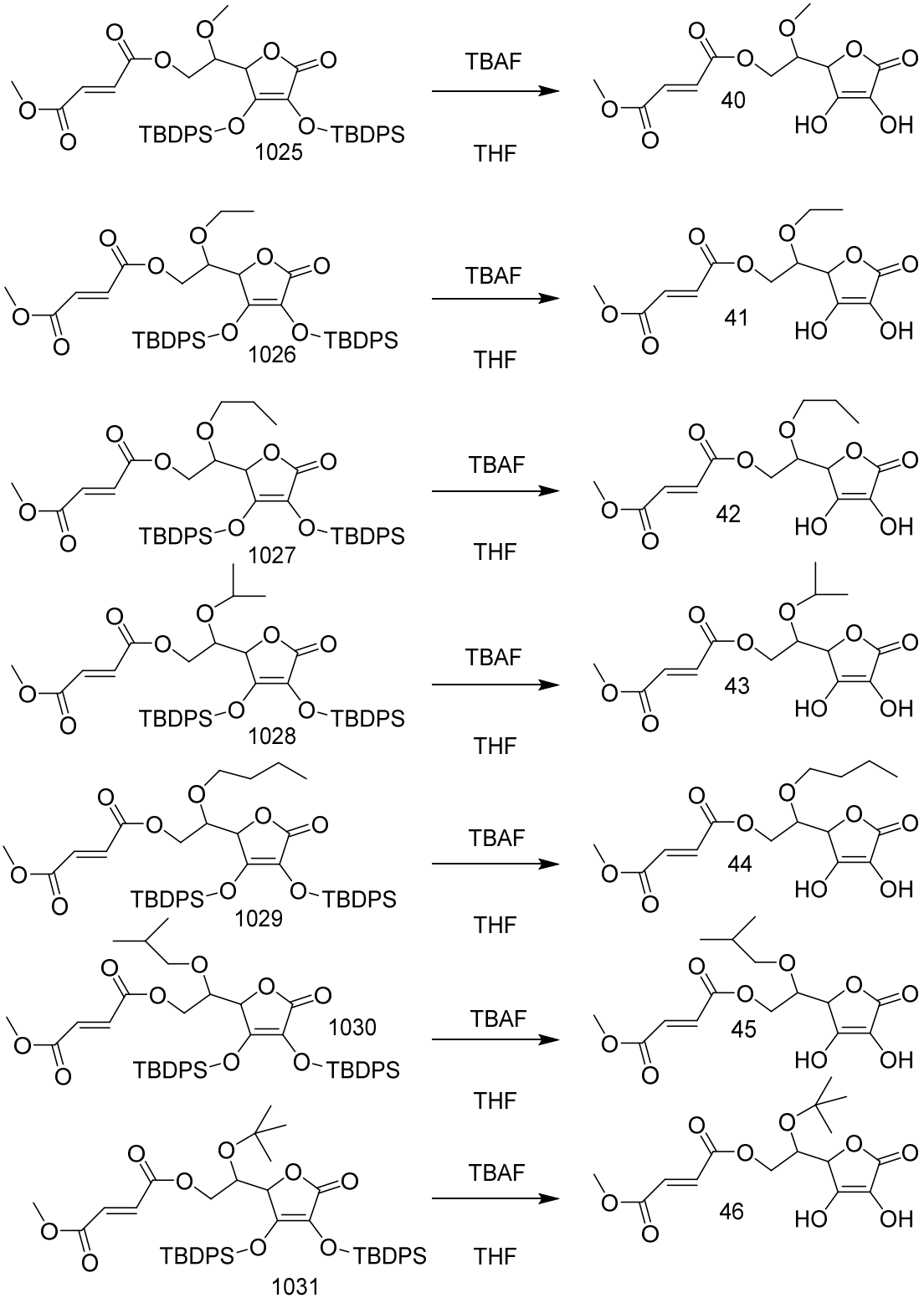

TBAF
THF
TBDPS
TBDPS
1025
40
1026
41
1027
42
1028
43
1029
44
1030
45
1031
46
HO
OH

Figure 11

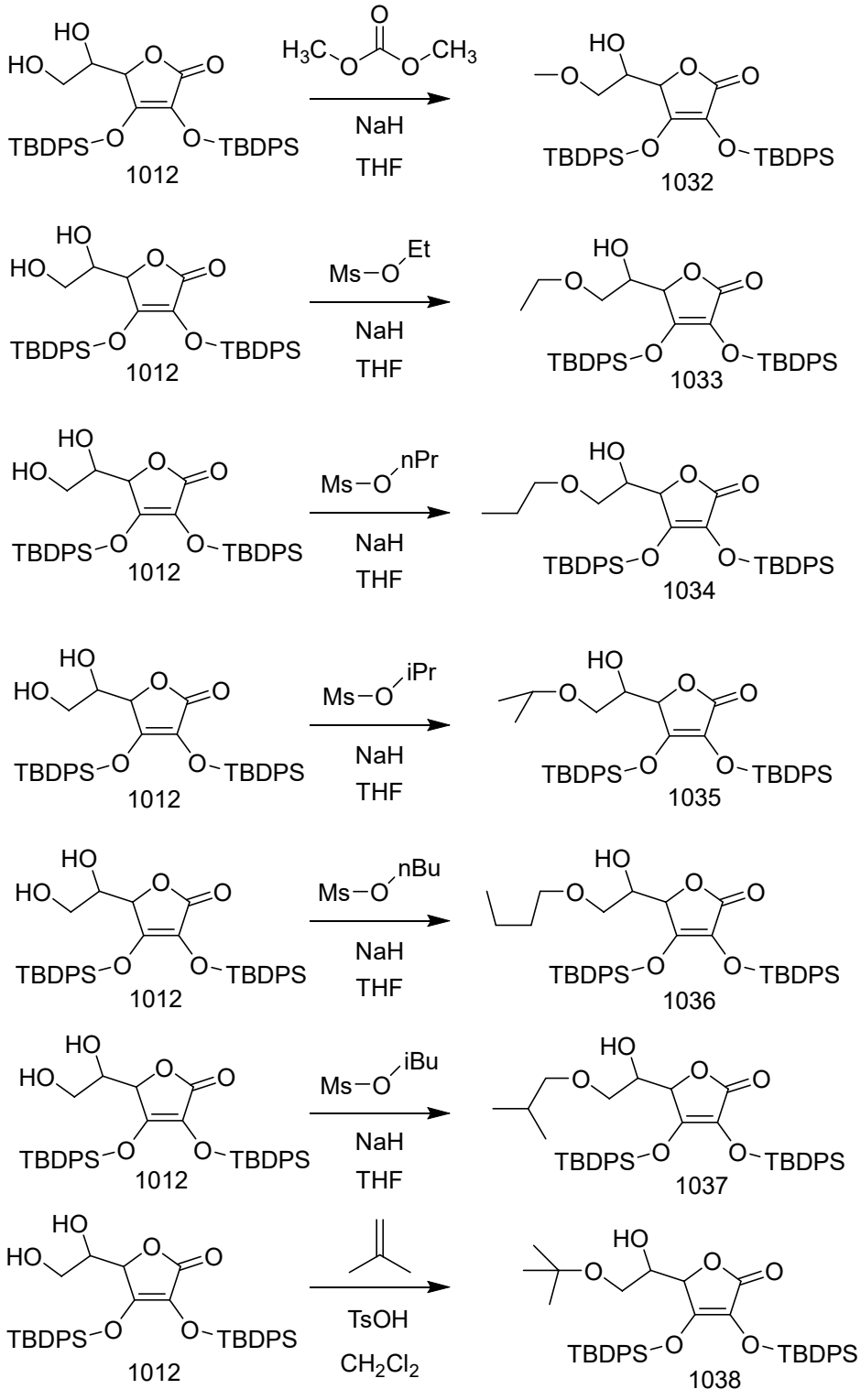

HO
HO
O
O
TBDPS
O
O
TBDPS
1012
H3C
O
O
CH3
NaH
THF
1032
Ms
O
Et
1033
nPr
1034
iPr
1035
nBu
1036
iBu
1037
TsOH
CH2Cl2
1038

Figure 12

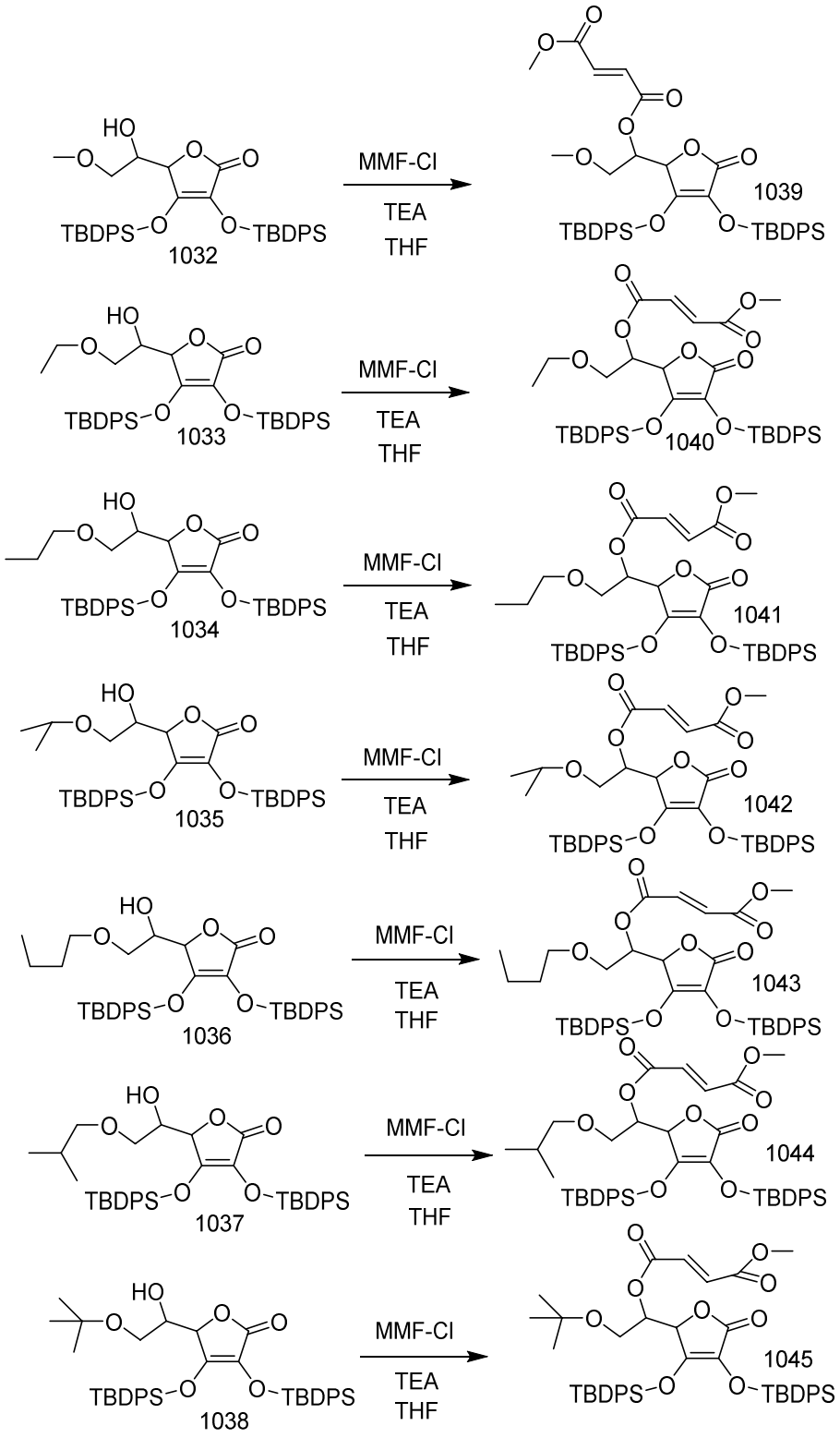

HO
TBDPS
TBDPS
1032
MMF-CI
TEA
THF
1039
1033
1040
1034
1041
1035
1042
1036
1043
1037
1044
1038
1045

Figure 13

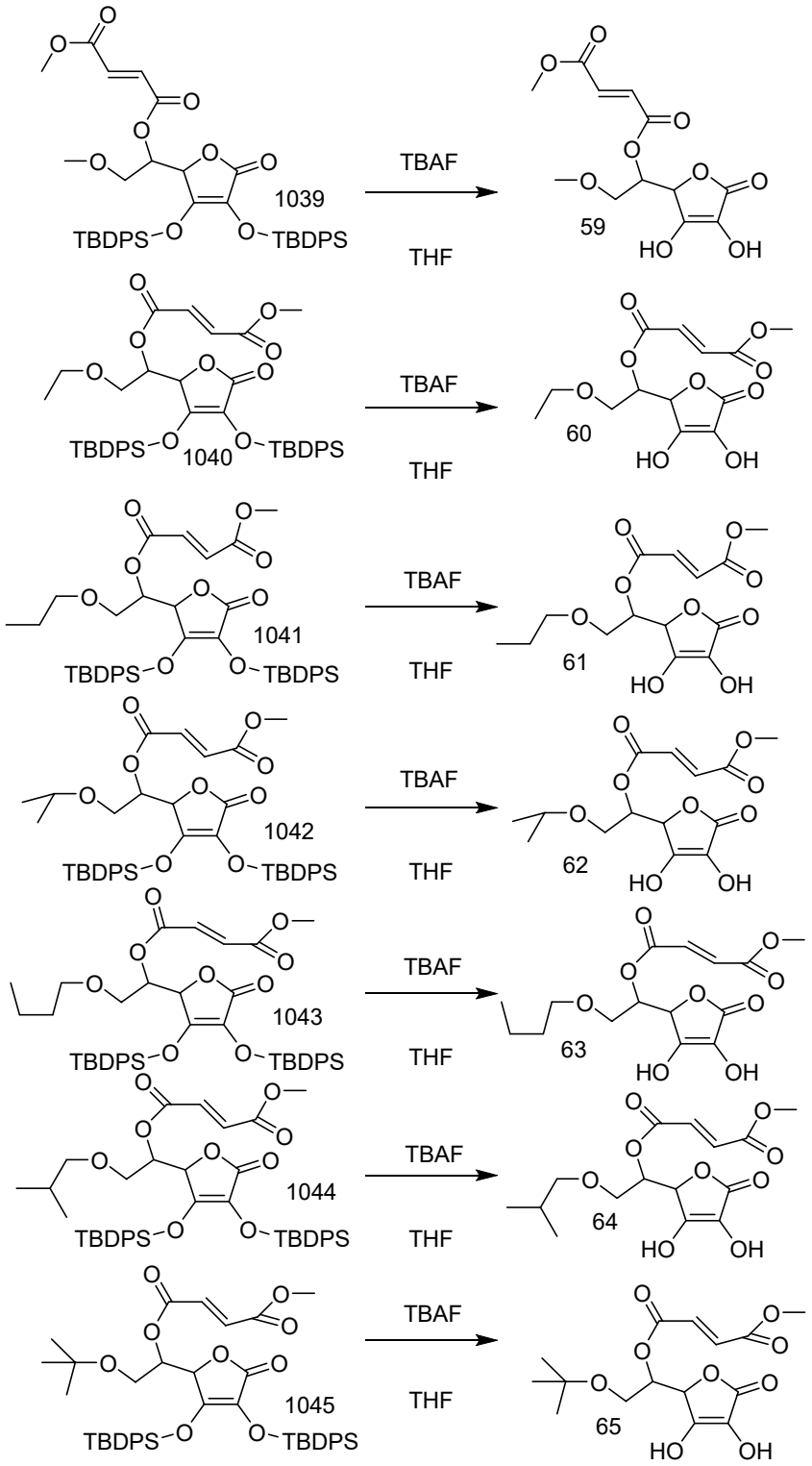

1039
TBDPS
TBDPS
TBAF
THF
59
HO
OH
1040
60
1041
61
1042
62
1043
63
1044
64
1045
65

Figure 14

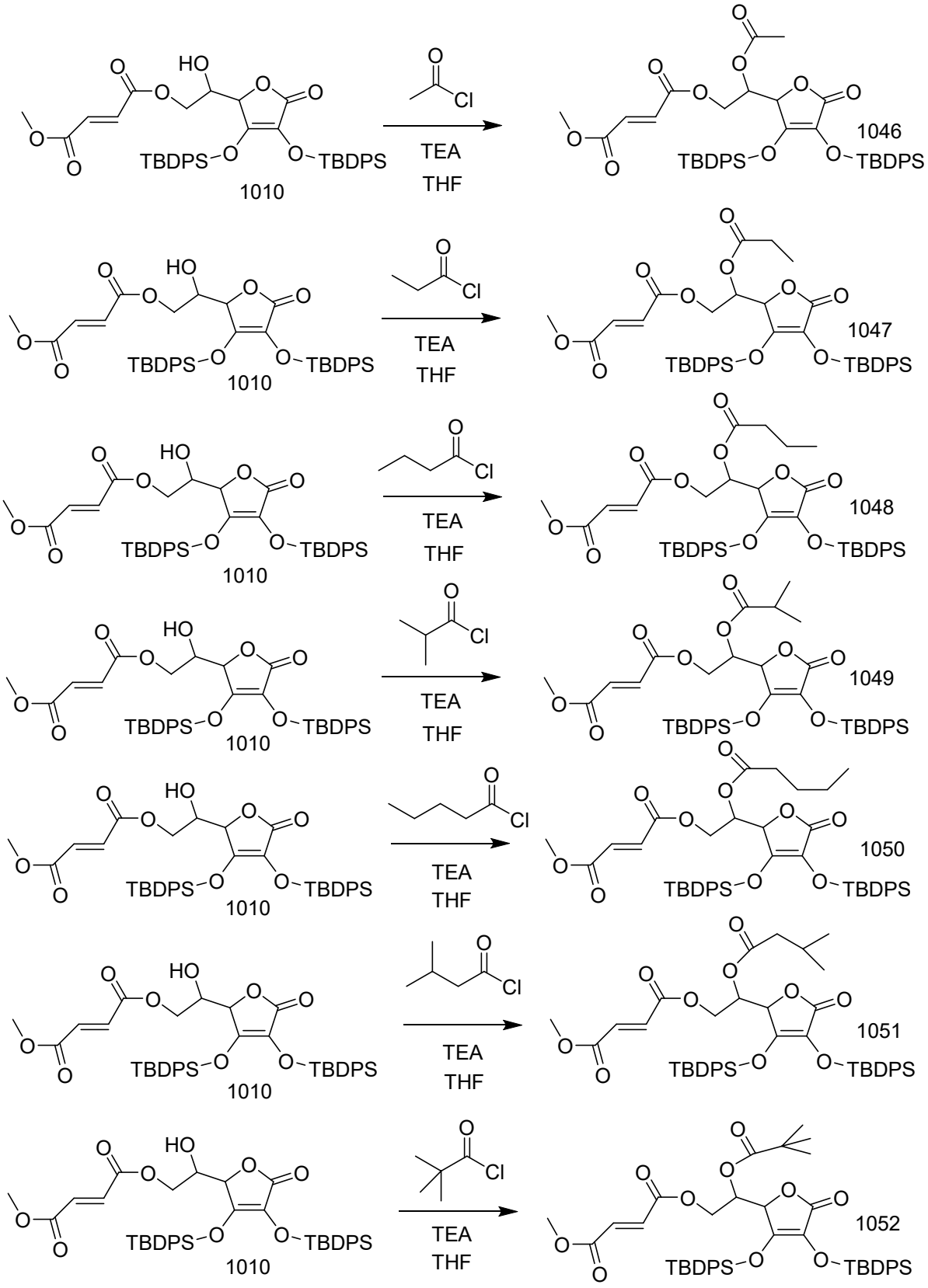
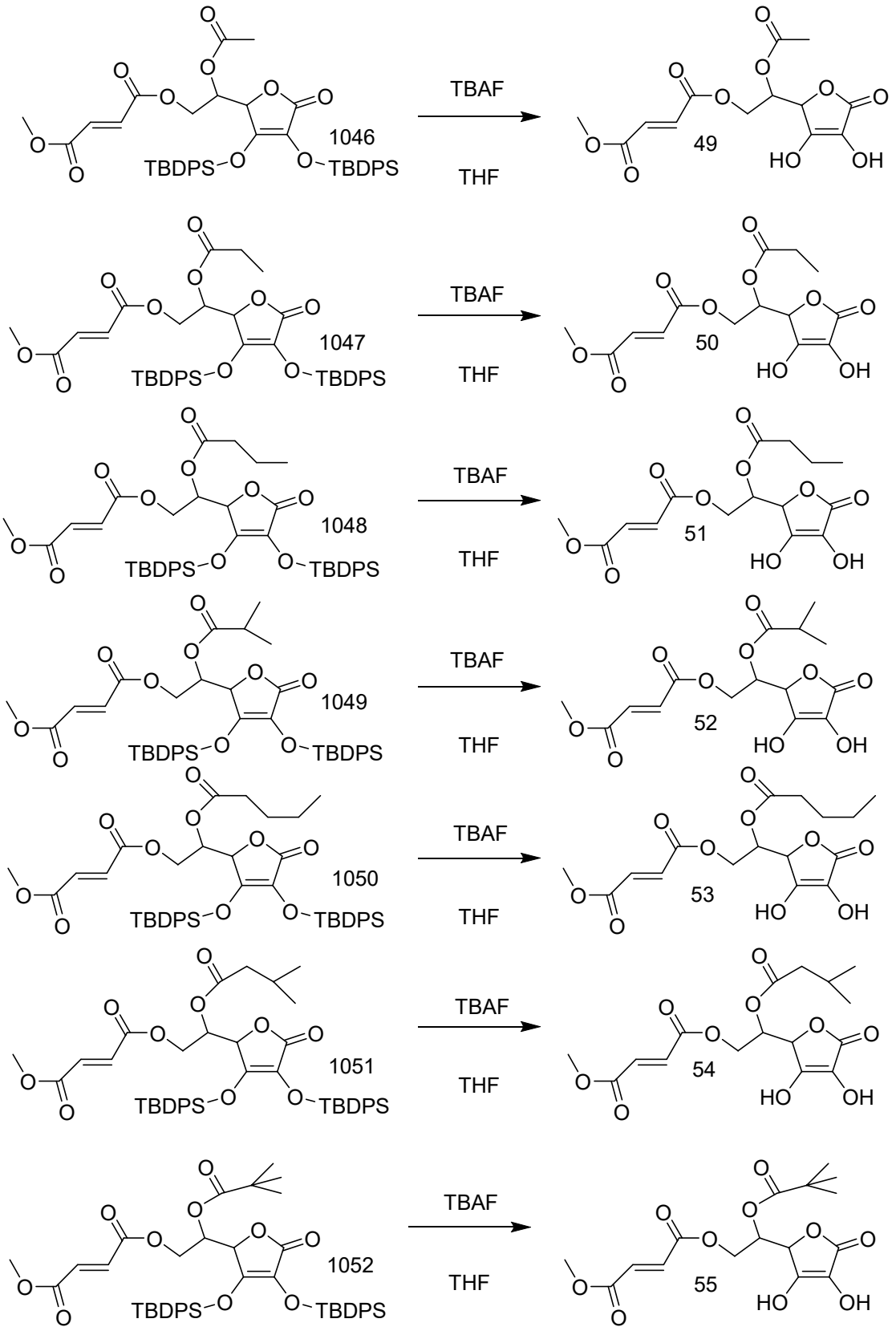

HO
TBDPS
TBDPS
1010
TEA
THF
1046
1047
1048
1049
1050
1051
1052

Figure 15


1046
TBDPS
TBDPS
TBAF
THF
49
HO
OH
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TBDPS
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TBAF
THF
50
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OH
1048
TBDPS
TBDPS
TBAF
THF
51
HO
OH
1049
TBDPS
TBDPS
TBAF
THF
52
HO
OH
1050
TBDPS
TBDPS
TBAF
THF
53
HO
OH
1051
TBDPS
TBDPS
TBAF
THF
54
HO
OH
1052
TBDPS
TBDPS
TBAF
THF
55
HO
OH

Figure 16

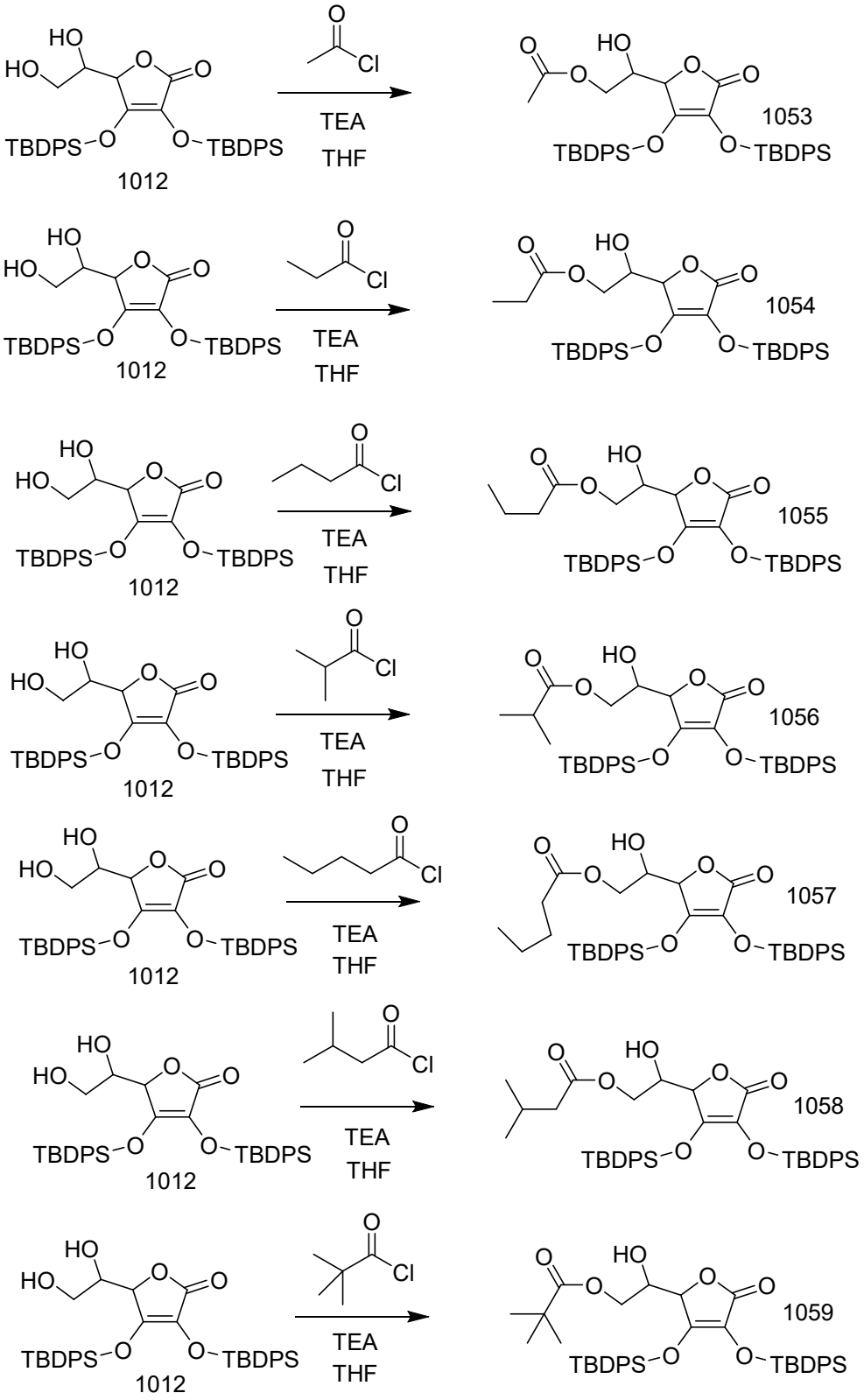
HO
HO
O
O
TBDPS
O
O
TBDPS
1012
O
Cl
TEA
THF
1053
1054
1055
1056
1057
1058
1059

Figure 17

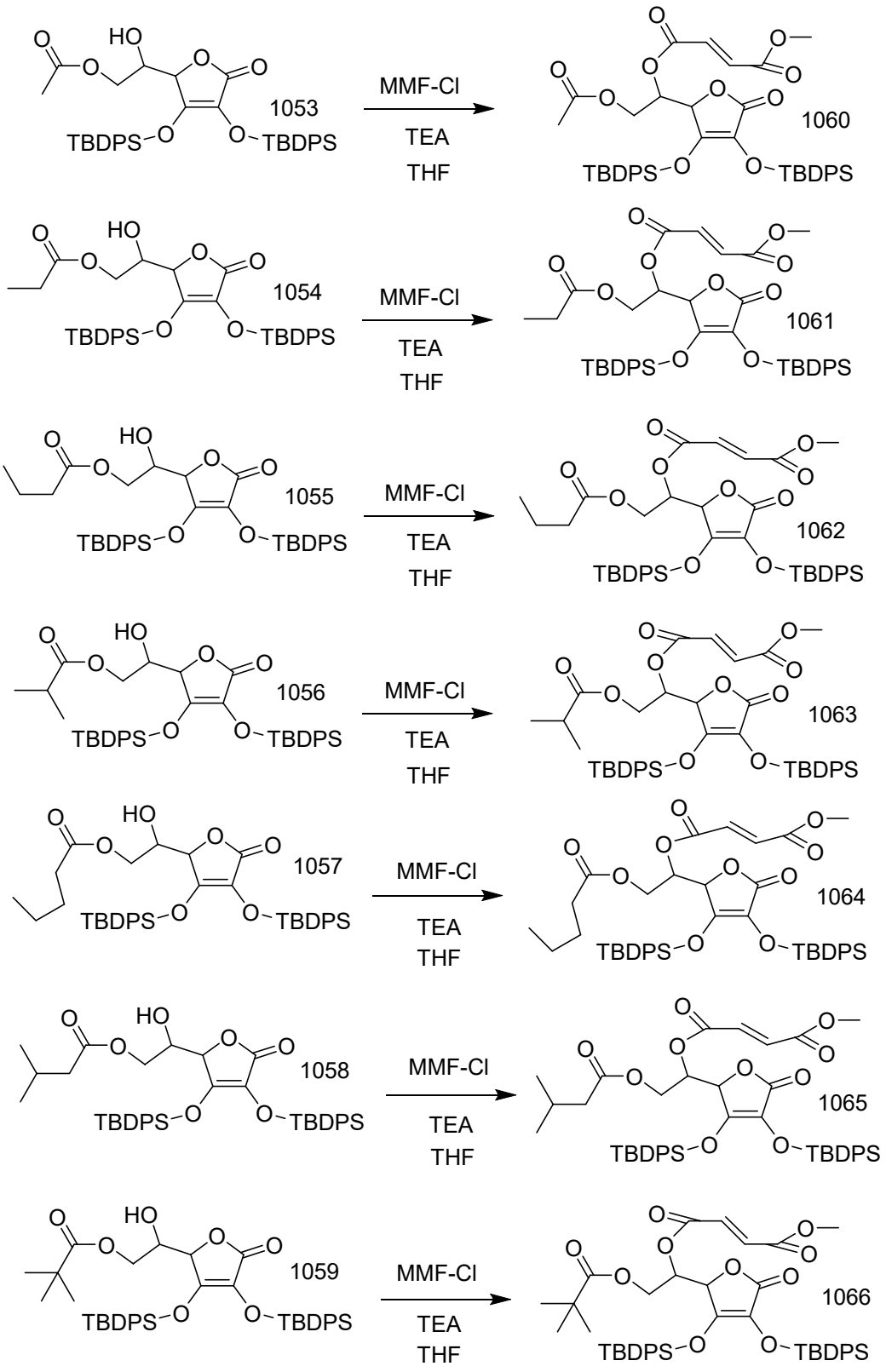
1053
MMF-Cl
TEA
THF
1060
1054
MMF-Cl
TEA
THF
1061
1055
MMF-Cl
TEA
THF
1062
1056
MMF-Cl
TEA
THF
1063
1057
MMF-Cl
TEA
THF
1064
1058
MMF-Cl
TEA
THF
1065
1059
MMF-Cl
TEA
THF
1066

Figure 18

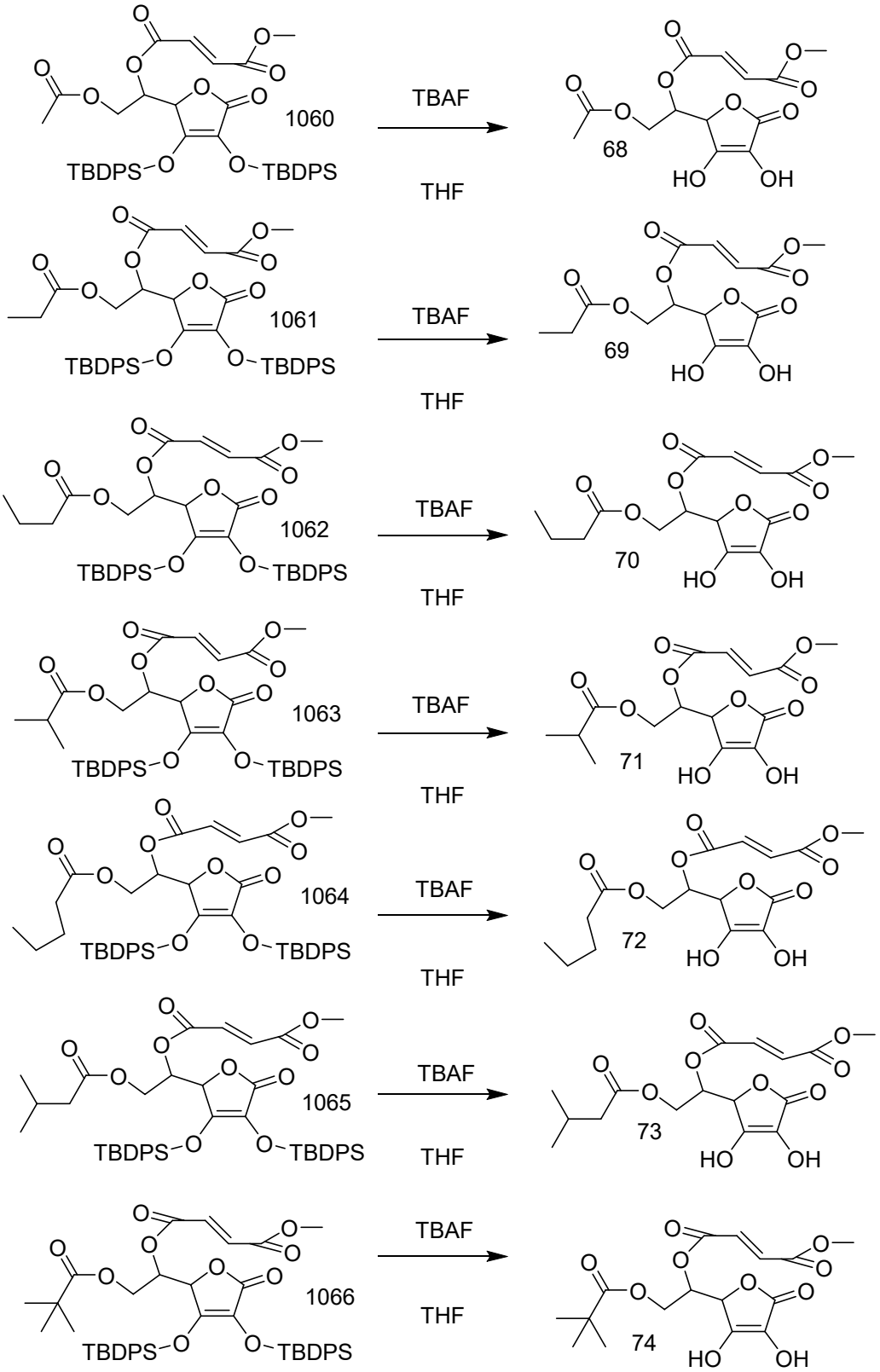
1060
TBAF
THF
68
1061
TBAF
THF
69
1062
TBAF
THF
70
1063
TBAF
THF
71
1064
TBAF
THF
72
1065
TBAF
THF
73
1066
TBAF
THF
74
TBDPS
TBDPS
HO
OH

Figure 19

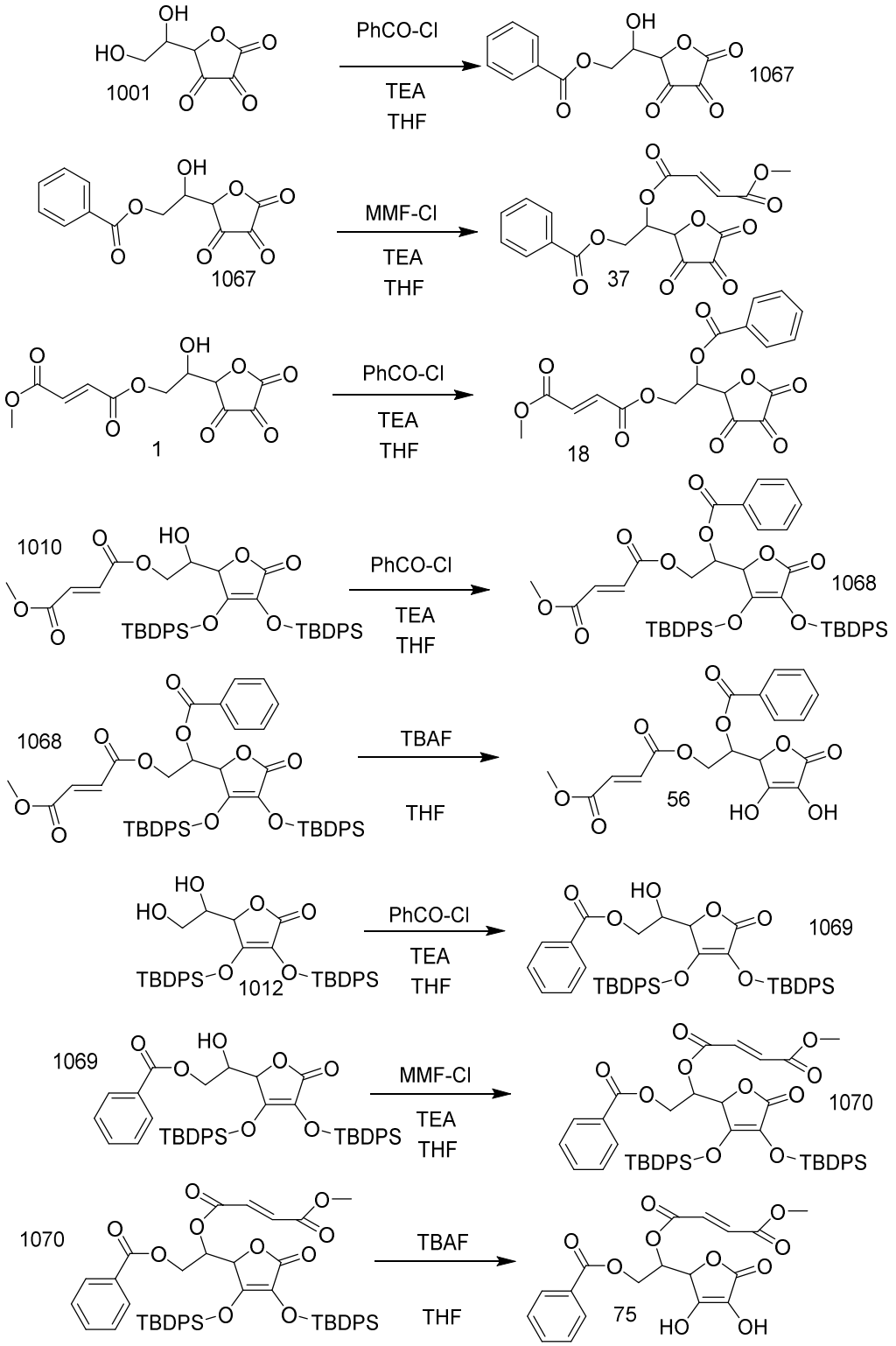
1001
PhCO-Cl
TEA
THF
1067
MMF-Cl
TEA
THF
37
1
PhCO-Cl
TEA
THF
18
1010
PhCO-Cl
TEA
THF
1068
TBAF
THF
56
1012
PhCO-Cl
TEA
THF
1069
1069
MMF-Cl
TEA
THF
1070
1070
TBAF
THF
75
TBDPS
HO
OH

Figure 20

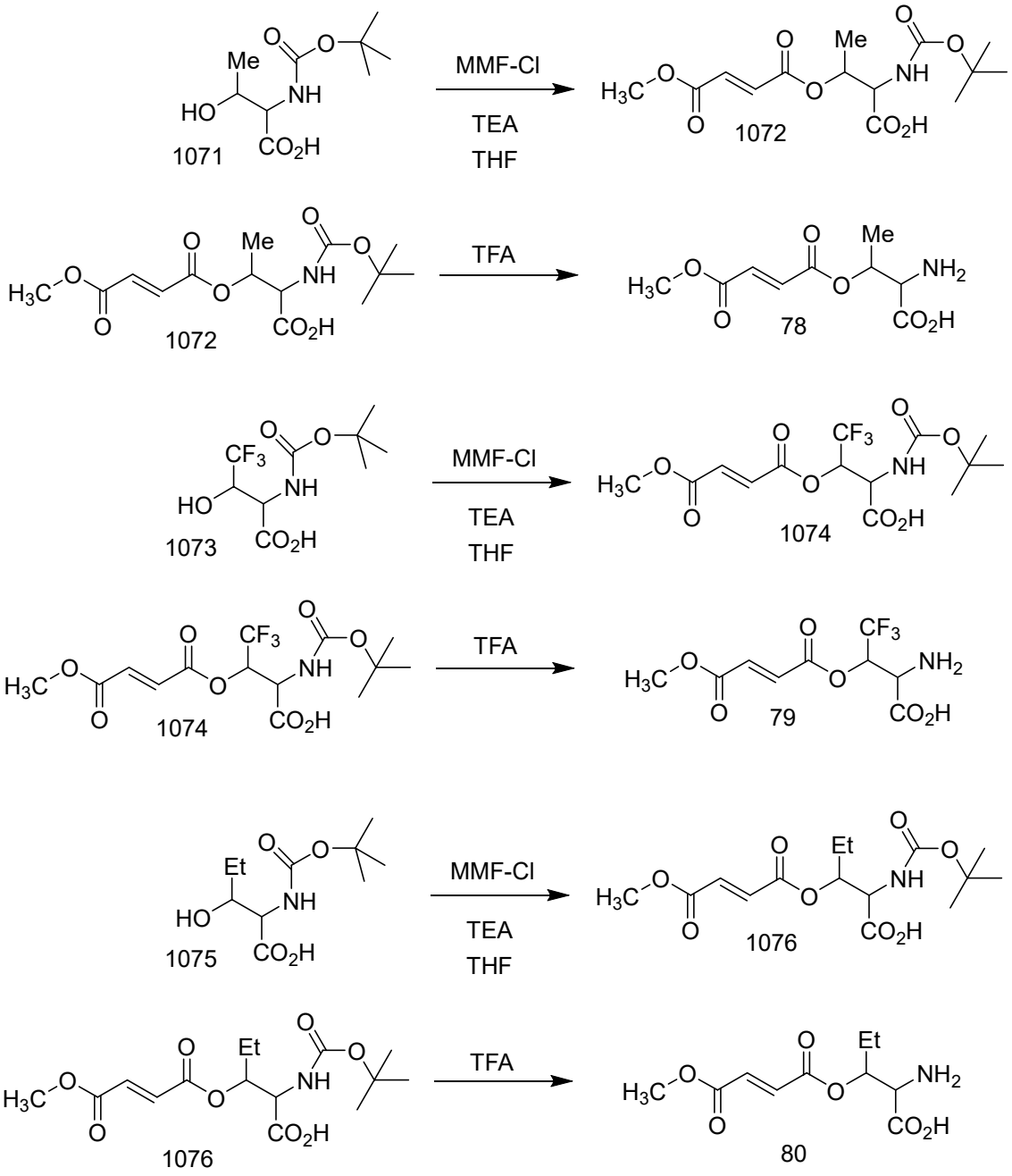

Figure 21

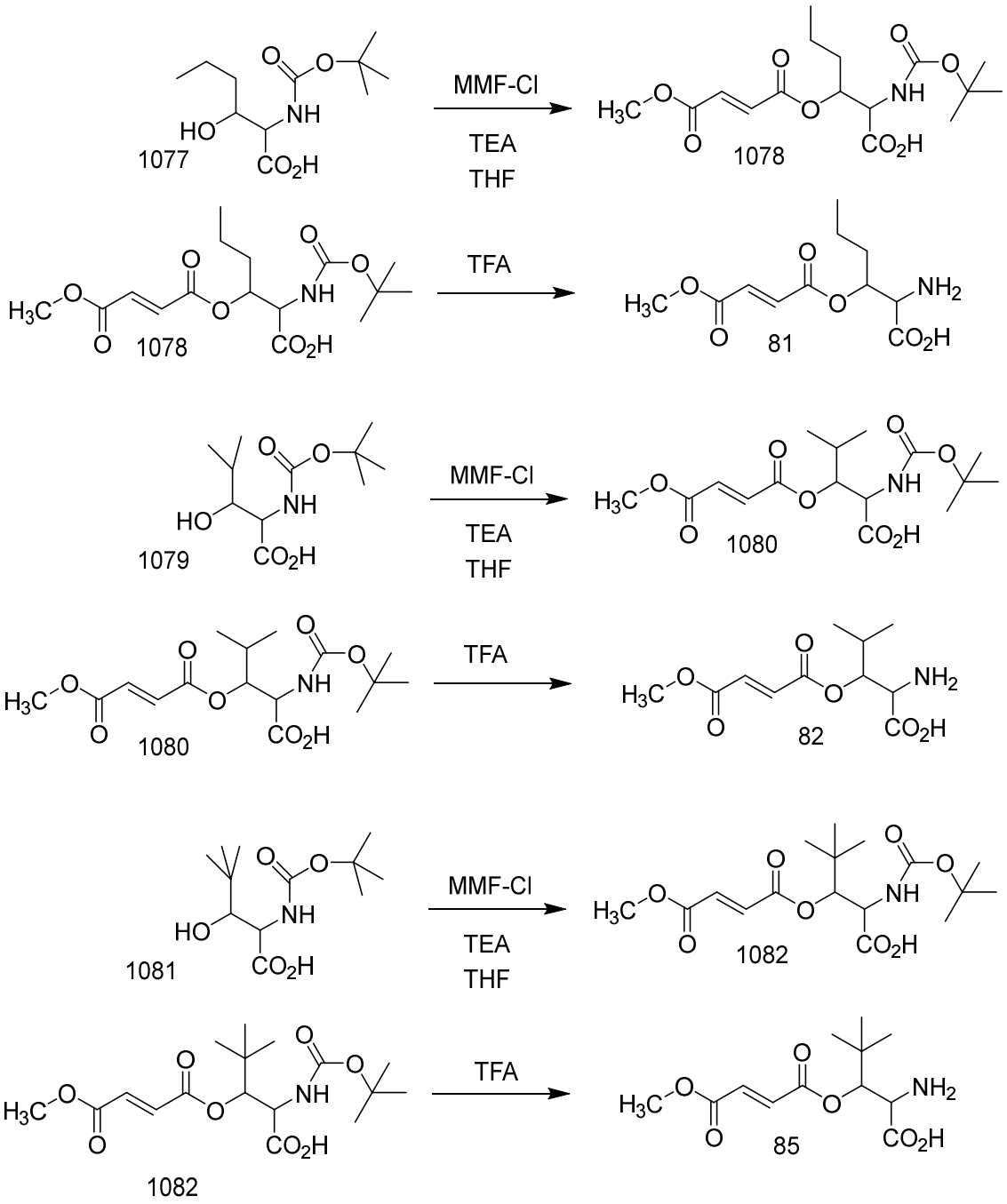

Figure 22

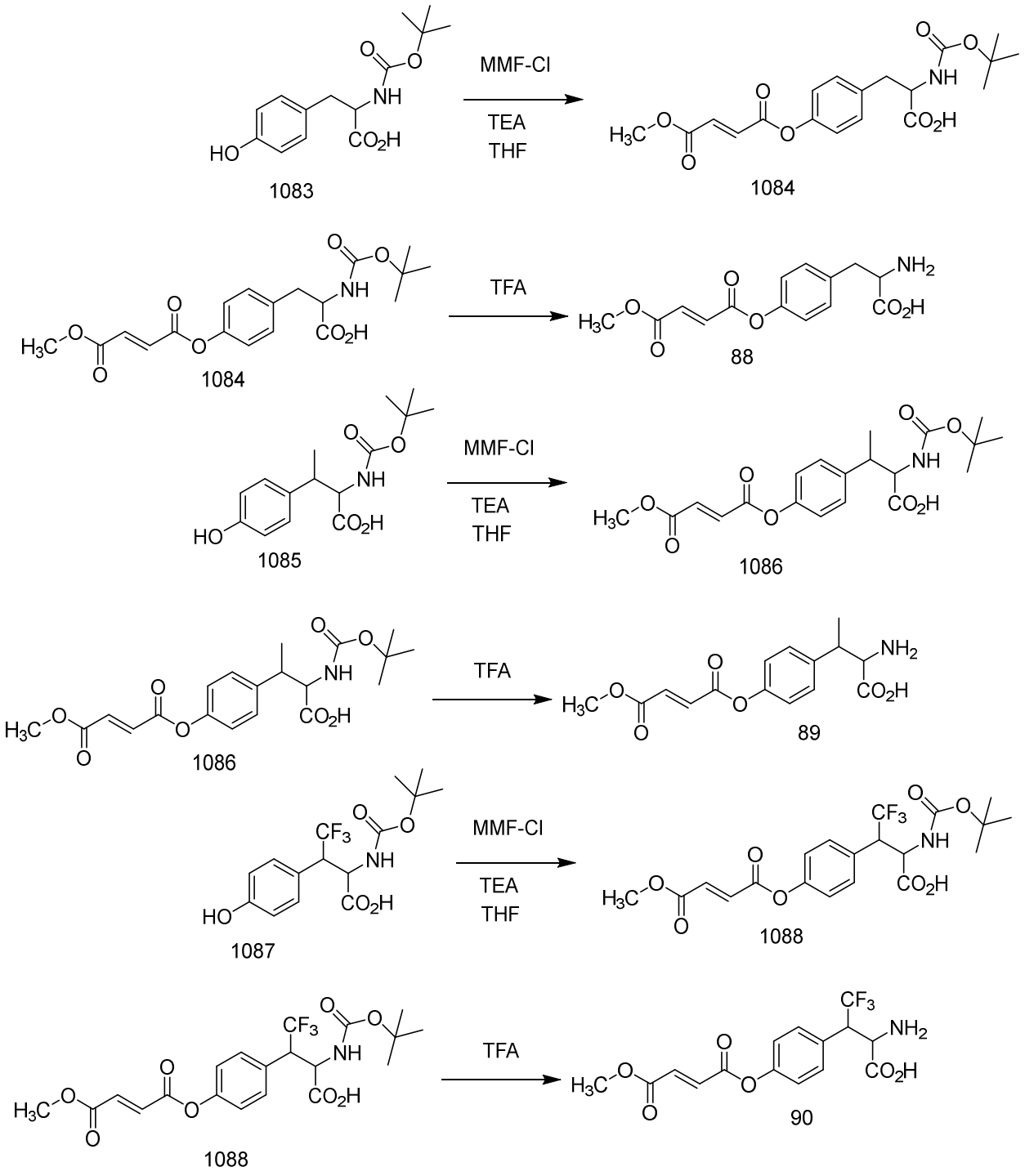

Figure 23

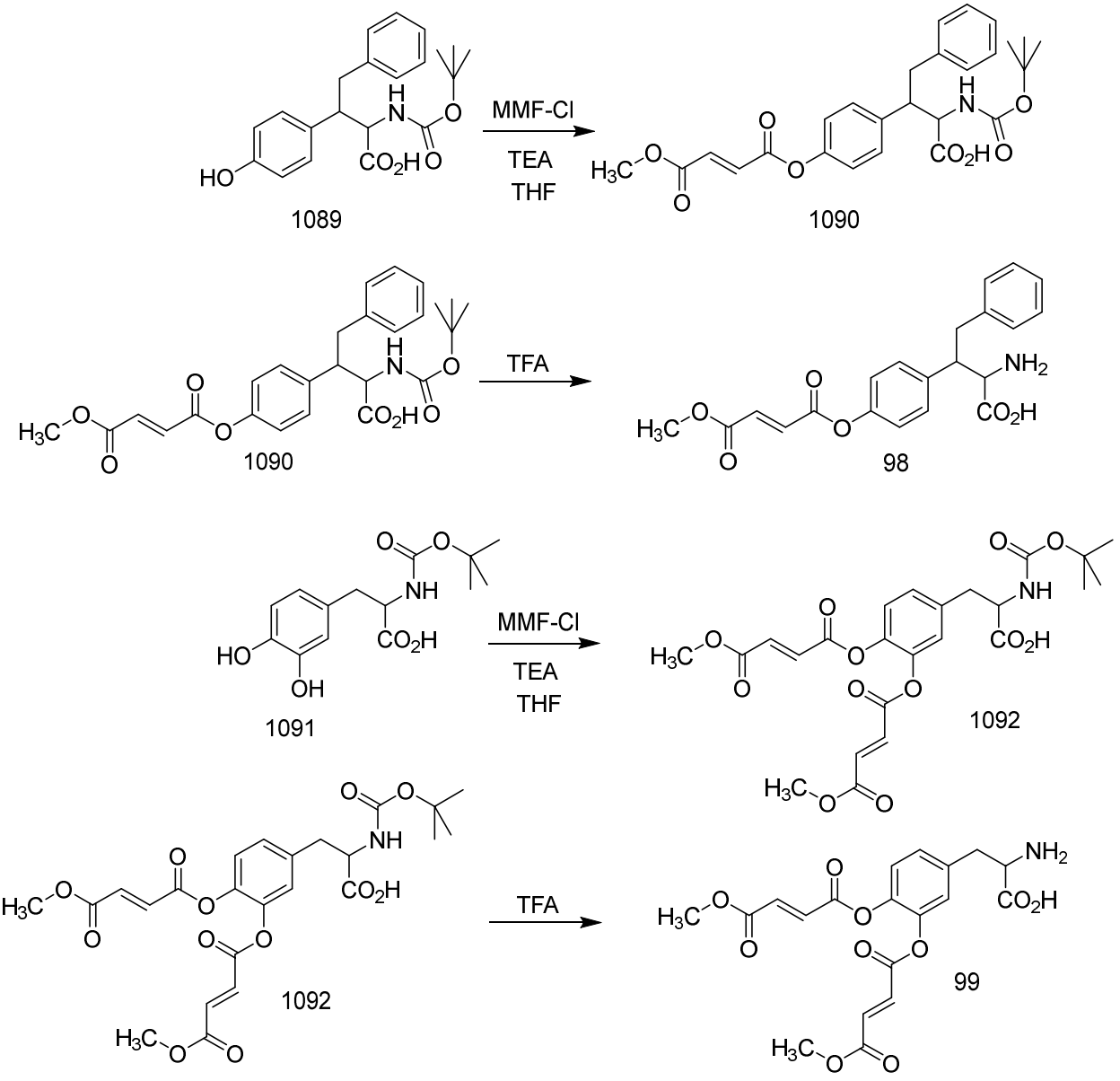
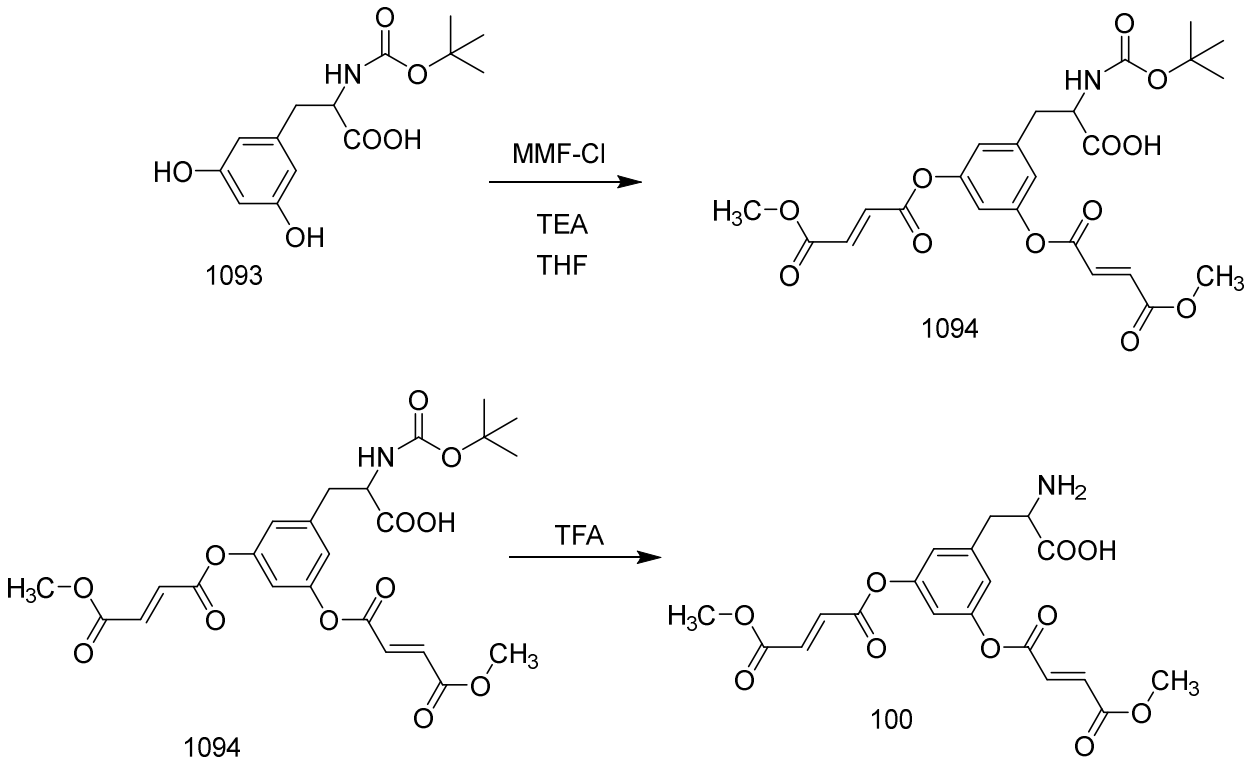
Figure 24