favoured analytical approach would be to spike a known quantity of a stable isotope-labelled version of the analyte of interest into the sample being tested, and then determine the ratio of the endogenous analyte to the labelled standard. This ratio may then be used to extrapolate a quantitative assessment of the target analyte in the sample.

In addition to being difficult to obtain even in small quantities, the gastric tract acid GTA-446 has not been fully characterized, has not been synthetically prepared, and labelled derivatives have not been generated. Furthermore, until now, GTA-446 ($C_{28}H_{46}O_4$) was thought to be a single long-chain fatty acid containing four unsaturations, a single carboxylic acid moiety, and two hydroxy moieties.

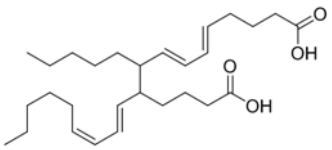
The quantification of GTA-446 has previously been further limited to primarily tandem mass spectrometry analyses, since enzyme-linked immunosorbent assay (ELISA)-based quantitation assays have not been performed due to the lack of suitable antibody. Although many diagnostic platforms are based on the ELISA principle, the lack of a specific anti-GTA-446 antibody represents a limiting factor for GTA-446 detection and quantification. Production of a specific antibody requires sufficient quantities of pure compound antigen, which has been limited by the unavailability of synthetic GTA-446.

Alternative, additional, and/or improved sources of GTA-446 and/or derivatives thereof, and/or methods for quantification thereof in a sample, are desirable.

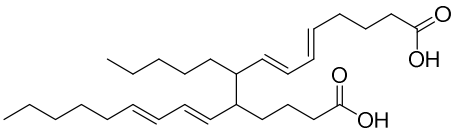
SUMMARY

It is an object of the disclosure to provide compounds having the structure of formula I, or salts, esters, prodrugs, or labelled derivatives thereof, or compounds related thereto, which may be of use in assays for quantifying GTA levels, such as GTA-446 levels, in a sample and/or in assays for the diagnosis of colorectal cancer in a subject.

In one ~~aspect~~example, the present disclosure refers to a compound having the structure of formula I or formula III:



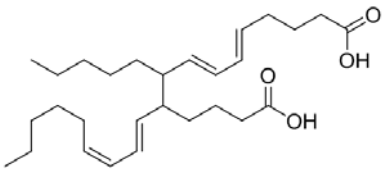
(formula I),



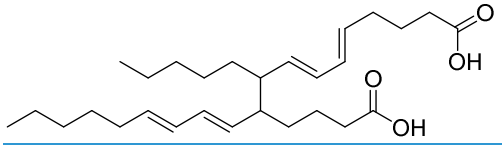
(formula III),

or a salt, ester, prodrug, or labelled derivative thereof.

In one aspect, the present disclosure refers to a compound having the structure of formula I or formula III:



(formula I),



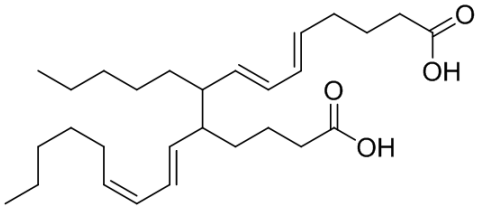
(formula III),

or a salt, ester, or labelled compound thereof, wherein the compound is an analytical standard compound.

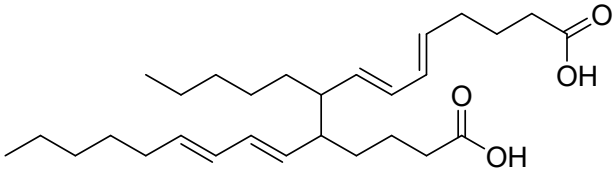
In a further example, the compound may an isolated compound.

In another example of the above compound or compounds, the compound may be a synthetically prepared compound, an analytical standard compound, or both.

In another ~~aspect~~example, the present disclosure refers to an isotopically labelled compound, the isotopically labelled compound comprising one or more isotopic labels incorporated within the structure of formula I or formula III:



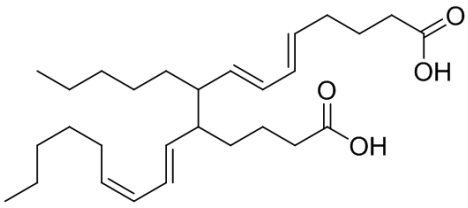
(formula I),



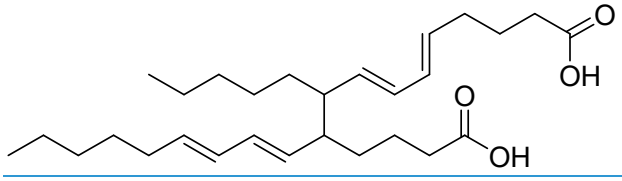
(formula III),

or a salt, ester, or prodrug thereof.

In another aspect, the present disclosure refers to an isotopically labelled compound, the isotopically labelled compound comprising one or more isotopic labels incorporated within the structure of formula I or formula III:



(formula I),



(formula III),

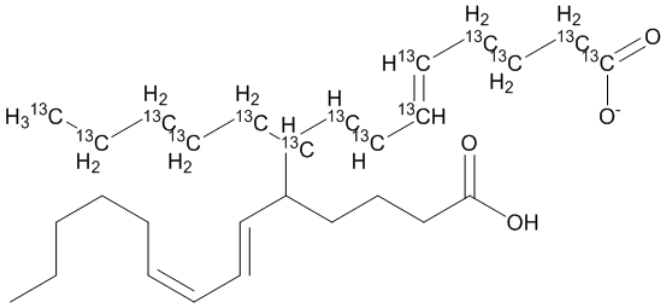
or a salt, or ester thereof.

In a further example of the isotopically labelled compound above, the one or more isotopic labels may be stable isotope labels, radioisotope labels, or a combination thereof.

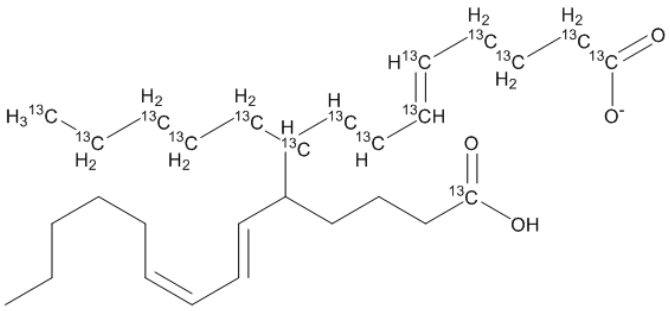
In still a further example of the isotopically labelled compound or compounds above, the one or more isotopic labels may be selected from the group consisting of deuterium (^{2}H) and ^{13}C.

In still a further example of the isotopically labelled compound or compounds above, the one or more isotopic labels may be selected from the group consisting of tritium (^{3}H) and ^{14}C.

In still another example, the isotopically labelled compound may be:



,



,

In another example, there is provided herein a metabolic tracer composition comprising isotopically labelled compound or compounds as described above. In another ~~aspect~~example, the present disclosure refers to a metabolic tracer composition comprising the isotopically labelled compound as described herein.

In still another example, there is provided herein a composition comprising any of the compound or compounds above, and an excipient, carrier, or diluent. In another aspect, the present disclosure refers to a composition comprising the compound as disclosed herein, and an excipient, carrier, or diluent, in particular wherein the composition is a metabolic tracer composition or an *in vitro* or *in vivo* diagnostic agent comprising the isotopically labelled compound as disclosed herein.

In yet another example, there is provided herein an *in vitro* or *in vivo* diagnostic agent comprising isotopically labelled compound or compounds as described above. In another ~~aspect~~example, the present disclosure refers to an *in vitro* or *in vivo* diagnostic agent comprising the isotopically labelled compound as disclosed herein.

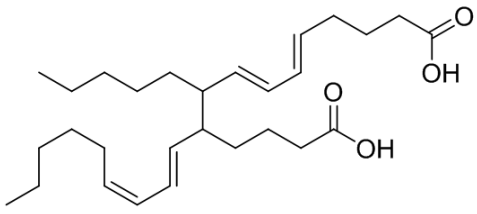
In another example, there is provided herein a composition comprising any of the compound or compounds above, and an excipient, carrier, or diluent. In another ~~aspect~~example, the present disclosure refers to a composition comprising the compound as disclosed herein, and an excipient, carrier, or diluent.

In yet another aspect, the present disclosure refers to a method for determining a level of a gastric tract acid (GTA) in a sample, said method comprising:

measuring a GTA detection signal from the sample, the GTA detection signal representative of the GTA level in the sample; and

quantifying the level of the GTA in the sample by comparing the measured GTA detection signal with a calibration reference.

In a further example of the above method, the GTA may be



(formula I).

In yet another example of the above method or methods, the GTA detection signal may be measured by mass spectrometry.

In still another example of the above method or methods, the calibration reference may comprise a standard curve prepared using known quantities of compound or compounds as

defined above.

In yet another example of the above method or methods, the calibration reference may be obtained by :

spiking the sample with a known quantity of isotopically labelled compound or compounds as defined above; and

measuring an internal standard signal from the sample, the internal standard signal being representative of the known quantity of the isotopically labelled compound spiked into the sample.

In a further example of the above method or methods, the internal standard signal may be measured by mass spectrometry.

In yet another example of the above method or methods, the method may further comprise a step of determining a ratio of the GTA level in the sample, as represented by the measured GTA detection signal, to the known quantity of isotopically labelled compound spiked into the sample, as represented by the internal standard signal.

In yet another example of the above method or methods, the calibration reference may comprise an isotope dilution curve (IDC) generated from a series of mixtures of varying GTA/isotopically labelled compound ratios and concentrations, to which said ratio is compared.

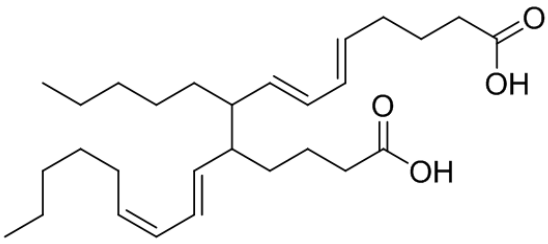
In still another example of the above method or methods, the IDC may be generated from a series of mixtures in which GTA content is varied over a fixed amount of isotopically labelled compound.

In another example of the above method or methods, the fixed amount of the isotopically labelled compound may be substantially the same as the known quantity of the isotopically labelled compound which is spiked into the sample.

In another example, there is provided herein a use of compound or compounds as defined above for determining a level of a gastric tract acid (GTA) in a sample. In another aspect, the present disclosure refers to use of the compound as disclosed herein for (i) determining a level of a gastric tract acid (GTA) in a sample; (ii) for generating a calibration reference for use in determining a level of a gastric tract acid (GTA) in a sample; or (iii) as an internal standard for use in determining a level of a gastric tract acid (GTA) in a sample.

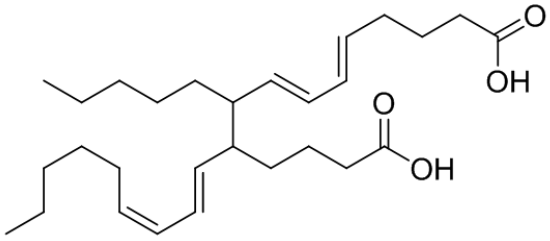
In a further example,

the GTA may be:



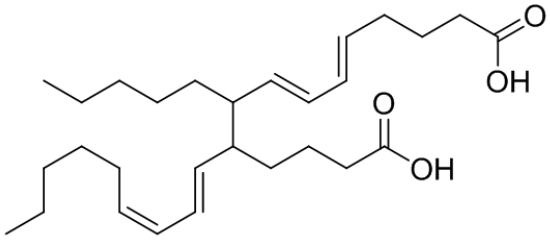
(formula I).

In yet another example, there is provided herein a use of compound or compounds as defined above for generating a calibration reference for use in determining a level of a gastric tract acid (GTA) in a sample. In another ~~aspect~~example, the present disclosure refers to use of the compound as disclosed herein for generating a calibration reference for use in determining a level of a gastric tract acid (GTA) in a sample. In a further example, the GTA may be:



(formula I).

In another example, there is provided herein a use of compound or compounds as defined above as an internal standard for use in determining a level of a gastric tract acid (GTA) in a sample. In another ~~aspect~~example, the present disclosure refers to use of the compound as disclosed herein as an internal standard for use in determining a level of a gastric tract acid (GTA) in a sample. In a further example, the GTA may be:



(formula I).

In another aspect, the present disclosure refers to a diagnostic method for identifying a subject as

measuring an internal standard signal from the sample, the internal standard signal being representative of the known quantity of the isotopically labelled compound spiked into the sample.

In a further example, the internal standard signal may be measured by mass spectrometry.

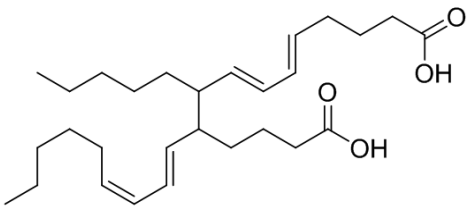
In yet another example of the above method or methods, the method may further comprise a step of determining a ratio of the GTA level in the sample, as represented by the measured GTA detection signal, to the known quantity of isotopically labelled compound spiked into the sample, as represented by the internal standard signal.

In still another example, of the above method or methods, the calibration reference may comprise an isotope dilution curve (IDC) generated from a series of mixtures of varying GTA/isotopically labelled compound ratios and concentrations, to which said ratio is compared.

In another example, the IDC may be generated from a series of mixtures in which GTA content is varied over a fixed amount of isotopically labelled compound.

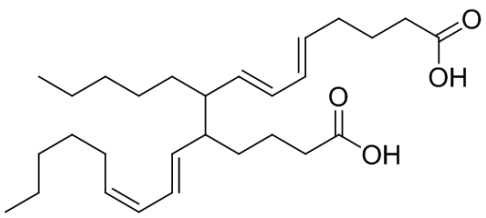
In yet another example of the above method or methods, the fixed amount of the isotopically labelled compound may be substantially the same as the known quantity of the isotopically labelled compound which is spiked into the sample.

In another example, there is provided herein a use of compound or compounds as defined above in a diagnostic method for identifying a subject as having, or being at risk of developing, a colorectal cancer associated with altered levels of a gastric tract acid (GTA) which is:



(formula I).

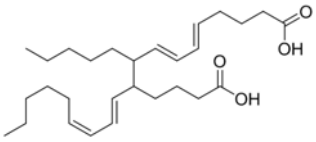
In another ~~aspect~~example, the present disclosure refers to use of the compound as disclosed herein in a diagnostic method for identifying a subject as having, or being at risk of developing, a colorectal cancer associated with altered levels of a gastric tract acid (GTA) which is:



(formula I).

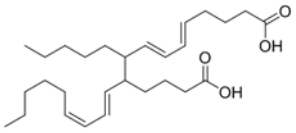
In still another example, there is provided herein a use of compound or compounds as

defined above for generating a calibration reference for use in a diagnostic method for identifying a subject as having, or being at risk of developing, a colorectal cancer associated with altered levels of a gastric tract acid (GTA) which is:



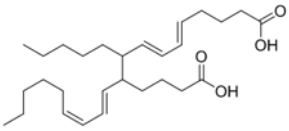
(formula I).

In another ~~aspect~~example, the present disclosure refers to use of the compound as disclosed herein for generating a calibration reference for use in a diagnostic method for identifying a subject as having, or being at risk of developing, a colorectal cancer associated with altered levels of a gastric tract acid (GTA) which is:



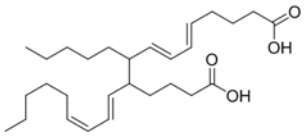
(formula I).

In another example, there is provided herein a use of the compound or compounds defined above as an internal standard for use in a diagnostic method for identifying a subject as having, or being at risk of developing, a colorectal cancer associated with altered levels of a gastric tract acid (GTA) which is:



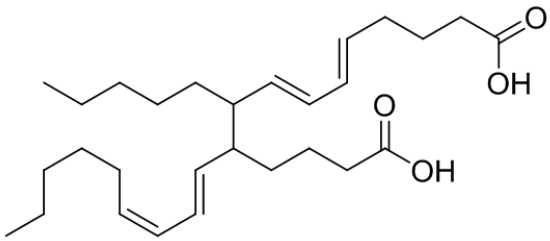
(formula I).

In another ~~aspect~~example, the present disclosure refers to use of the compound as disclosed herein as an internal standard for use in a diagnostic method for identifying a subject as having, or being at risk of developing, a colorectal cancer associated with altered levels of a gastric tract acid (GTA) which is:



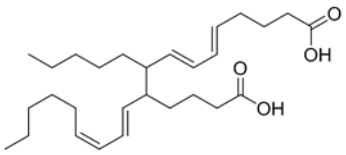
(formula I).

In another ~~aspect~~example, the present disclosure refers to an antibody, or antigen-binding fragment thereof, which specifically binds a compound of formula I:

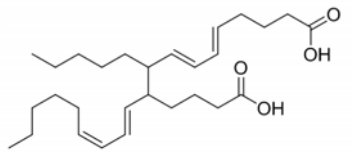


(formula I).

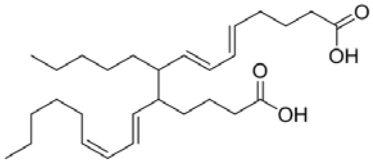
In another example, the antibody may be a monoclonal or a polyclonal antibody.
In another example, there is provided herein a use of the compound or compounds defined above as an antigen for preparing an antibody which specifically binds to an antigenic epitope of the compound. In another ~~aspect~~example, the present disclosure refers to use of a compound as disclosed herein as an antigen for preparing an antibody which specifically binds to an antigenic epitope of the compound.
In yet another example, there is provided herein a use of the antibody or antibodies defined above for detecting or quantifying a level of a gastric tract acid (GTA) in a sample by immunoassay, wherein the GTA is:



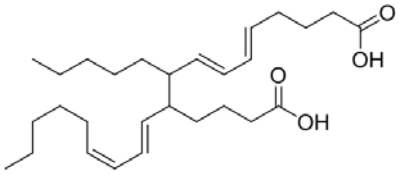
(formula I).
In another ~~aspect~~example, the present disclosure refers to use of the antibody as disclosed herein for detecting or quantifying a level of a gastric tract acid (GTA) in a sample by immunoassay, wherein the GTA is:



(formula I).
In still another example, there is provided herein a use of the antibody or antibodies defined above in a diagnostic method for identifying a subject as having, or being at risk of developing, a colorectal cancer associated with altered levels of a gastric tract acid (GTA) which is:



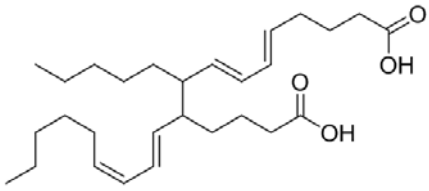
(formula I).
In another ~~aspect~~example, the present disclosure refers to use of the antibody as disclosed herein in a diagnostic method for identifying a subject as having, or being at risk of developing, a colorectal cancer associated with altered levels of a gastric tract acid (GTA) which is:



(formula I).
In another ~~aspect~~example, the present disclosure refers to a method for determining a level of a gastric tract acid (GTA) in a sample, said method comprising:

measuring a level of the GTA in the sample using an immunoassay employing an antibody, or antigen-binding fragment thereof, which specifically binds to the GTA;

wherein the GTA is:

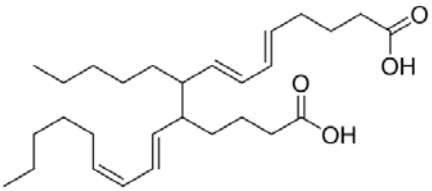


(formula I).

In another aspect, the present disclosure refers to a method for determining a level of a gastric tract acid (GTA) in a sample, said method comprising:

measuring a level of the GTA in the sample using an immunoassay employing an antibody, or antigen-binding fragment thereof, which specifically binds to the GTA; in particular wherein the immunoassay comprises an enzyme-linked immunosorbent assay (ELISA);

wherein the GTA is:



(formula I); in particular wherein the method further comprises a step of using a control sample comprising a compound as defined herein as a positive control in the immunoassay; in particular wherein the method further comprises a step of using a standard curve to extrapolate the level of the GTA in the sample, the standard curve having been generated using a plurality of known quantities of a compound as defined herein.

In a further example of the above method, the immunoassay may comprise an enzyme-linked immunosorbent assay (ELISA).

In another example of the above method or methods, the method may further comprise a step of using a control sample comprising compound or compounds as defined above as a positive control in the immunoassay.

In yet another example of the above method or methods, the method may further comprise a step of using a standard curve to extrapolate the level of the GTA in the sample, the standard curve having been generated using a plurality of known quantities of a compound or compounds as defined above.

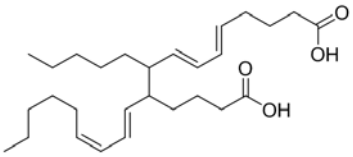
In another ~~aspect~~example, the present disclosure refers to a diagnostic method for identifying a subject as having, or being at risk of developing, colorectal cancer, said method comprising:

determining a level of a gastric tract acid (GTA) in a sample obtained from the subject by

measuring a level of the GTA in the sample using an immunoassay employing an antibody, or antigen-binding fragment thereof, which specifically binds to the GTA; and

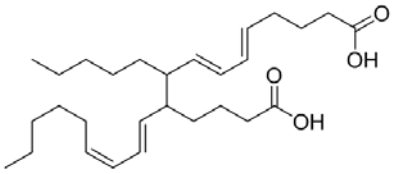
identifying the subject as having, or being at risk of developing, colorectal cancer when the determined level of the GTA in the sample is reduced in comparison to a healthy control group,

wherein the GTA is:



(formula I).

In another aspect, the present disclosure refers to a diagnostic method for identifying a subject as having, or being at risk of developing, colorectal cancer, said method comprising:
determining a level of a gastric tract acid (GTA) in a sample obtained from the subject by measuring a level of the GTA in the sample using an immunoassay employing an antibody, or antigen-binding fragment thereof, which specifically binds to the GTA; in particular wherein the immunoassay comprises an enzyme-linked immunosorbent assay (ELISA); and
identifying the subject as having, or being at risk of developing, colorectal cancer when the determined level of the GTA in the sample is reduced in comparison to a healthy control group,
wherein the GTA is:



(formula I), in particular wherein the method further comprises a step of using a control sample comprising a compound as defined herein as a positive control in the immunoassay; in particular wherein the step of determining the level of the GTA in the sample further comprises using a standard curve to extrapolate the level of the GTA in the sample, the standard curve having been generated using a plurality of known quantities of a compound as defined herein.

In a further example, the immunoassay may comprise an enzyme-linked immunosorbent assay (ELISA).
In another example of the above method or methods, the method may further comprise a step of using a control sample comprising a compound or compounds as defined above as a positive control in the immunoassay.
In still another example of the above method or methods, the step of determining the level of the GTA in the sample may further comprise using a standard curve to extrapolate the level of the GTA in the sample, the standard curve having been generated using a plurality of known quantities of a compound or compounds as defined above.
In another example, there is provided herein a kit for quantifying a level of a gastric tract acid (GTA) in a sample, the kit comprising at least one of:

a compound or compounds as defined above;
a metabolic tracer as defined above;
a composition or compositions as defined above;
a diagnostic agent as defined above; and
an antibody or antibodies as defined above;
and, optionally, further comprising a set of instructions for performing a method

or methods as defined above.

In another ~~aspect~~example, the present disclosure refers to a kit for quantifying a level of a gastric tract acid (GTA) in a sample, the kit comprising at least one of: a compound as disclosed herein;

a metabolic tracer as disclosed herein; a composition as disclosed herein; a diagnostic agent as disclosed herein; and an antibody as disclosed herein; and, optionally, further comprising a set of instructions for performing a method as disclosed herein.

In another example, there is provided herein a diagnostic kit for identifying a subject as having, or being at risk of developing, colorectal cancer, the kit comprising at least one of:

a compound or compounds as defined above; a metabolic tracer as defined above;

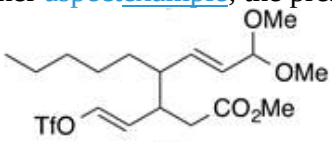
a composition or compositions as defined above; a diagnostic agent as defined above; and an antibody or antibodies as defined above; and, optionally, further comprising a set of instructions for performing a method or methods as defined above.

In another ~~aspect~~example, the present disclosure refers to a diagnostic kit for identifying a subject as having, or being at risk of developing, colorectal cancer, the kit comprising at least one of: a compound as disclosed herein; a metabolic tracer as disclosed herein;

a composition as disclosed herein; a diagnostic agent as disclosed herein; and

an antibody as disclosed herein; and, optionally, further comprising a set of instructions for performing a method as disclosed herein.

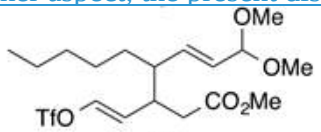
In another ~~aspect~~example, the present disclosure refers to a compound having the formula:



24

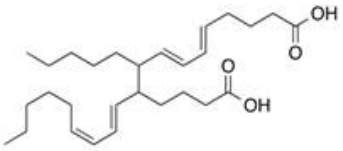
, or a labelled derivative thereof.

In another aspect, the present disclosure refers to a compound having the formula:



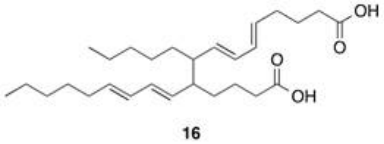
24

, or a labelled compound thereof; in particular wherein the compound is for use in the synthesis of a compound having the formula:



15

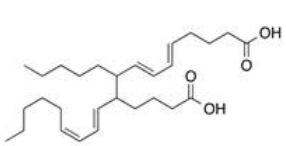
or



16

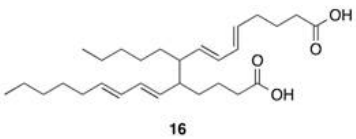
, or a salt, ester, or labelled compound thereof.

In still another ~~aspect~~example, the present disclosure refers to use of the compound as disclosed herein in the synthesis of a compound having the formula:



15

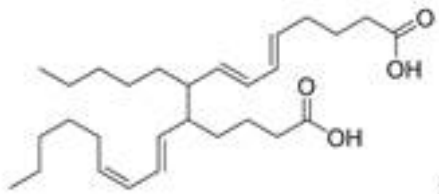
or



16

, or a salt, ester, prodrug, or labelled derivative thereof.

In another ~~aspect~~example, the present disclosure refers to a method for synthesizing a compound having formula (I):

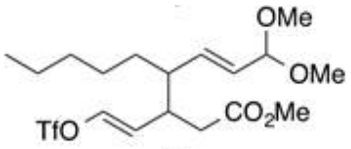


(formula I),

or an isotopically labelled derivative thereof,

said method comprising:

providing a compound having the formula



24 ;

performing a Sonagashira coupling of the compound with 1-heptyne;

performing a reduction with Lindlar's catalyst;

performing a methyl ester reduction;

performing a reaction with methanesulfonyl chloride;

performing a mesylate displacement with dimethyl malonate;

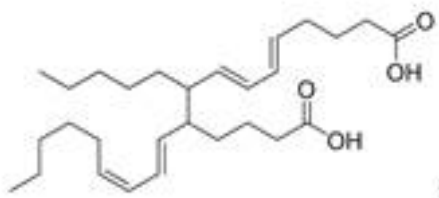
performing an acid treatment for simultaneous acetal cleavage, ester hydrolysis, and decarboxylation; and

performing a Wittig reaction to yield the compound of formula I, or an isotopically labelled derivative thereof,

wherein the compound of formula 24, or at least one reactant in the method, comprises at

least one isotopically labelled atom which is incorporated into the resulting compound of formula I when an isotopically labelled derivative of formula I is synthesized.

In another example, the present disclosure refers to a method for synthesizing a compound having formula (I):



(formula I),

or an isotopically labelled compound thereof,

said method comprising:

providing a compound according to claim 16;

performing a Sonagashira coupling of the compound with 1-heptyne;

performing a reduction with Lindlar's catalyst;

performing a methyl ester reduction;

performing a reaction with methanesulfonyl chloride;

performing a mesylate displacement with dimethyl malonate;

performing an acid treatment for simultaneous acetal cleavage, ester hydrolysis, and decarboxylation; and

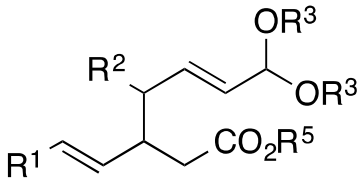
performing a Wittig reaction to yield the compound of formula I, or an isotopically labelled compound thereof; in particular wherein the Wittig reaction comprises reaction with (triphenylphosphoranylidene) acetaldehyde or (4-carboxybutyl)triphenylphosphonium bromide,

wherein the compound as disclosed herein, or at least one reactant in the method, comprises at

least one isotopically labelled atom which is incorporated into the resulting compound of formula I when an isotopically labelled compound of formula I is synthesized.

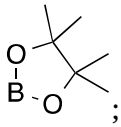
In a further example, the Wittig reaction may comprise reaction with (triphenylphosphoranylidene) acetaldehyde or (4-carboxybutyl)triphenylphosphonium bromide.

In yet another ~~aspect~~example, the present disclosure refers to a compound having formula D:



(Formula D),

wherein:

R^1 is $-Sn(R^{10})_3$, -OTf, -Cl, -Br, -I, $-B(OH)_2$ or 

;

R^2 is optionally substituted saturated or unsaturated C_1-C_{20} alkyl, saturated or unsaturated C_2-C_{20} alkenyl, or saturated or unsaturated C_2-C_{20} alkynyl;

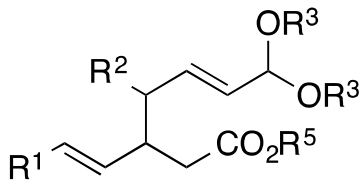
each R^3 is, independently, optionally substituted C_1-C_6 alkyl, or the R^3 groups together form an optionally substituted ethylene or propylene group bridging the attached oxygen atoms to form a five- or six-membered ring;

R^5 is optionally substituted C_1-C_6 alkyl; and

R^{10} is optionally substituted C_1-C_6 alkyl,

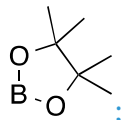
or a labelled derivative thereof.

In another aspect, the present disclosure refers to a compound having formula D:



(Formula D),

wherein:

R^1 is -Sn(R^{10})$_3$, -OTf, -Cl, -Br, -I, -B(OH)$_2$ or 

;

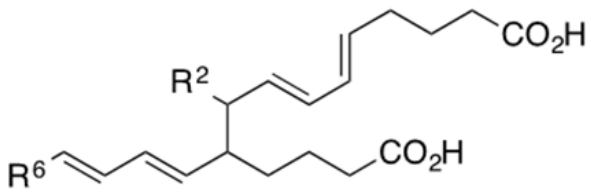
R^2 is optionally substituted saturated or unsaturated C_1-C_{20} alkyl, saturated or unsaturated C_2-C_{20} alkenyl, or saturated or unsaturated C_2-C_{20} alkynyl;

each R^3 is, independently, optionally substituted C_1-C_6 alkyl, or the R^3 groups together form an optionally substituted ethylene or propylene group bridging the attached oxygen atoms to form a five- or six-membered ring;

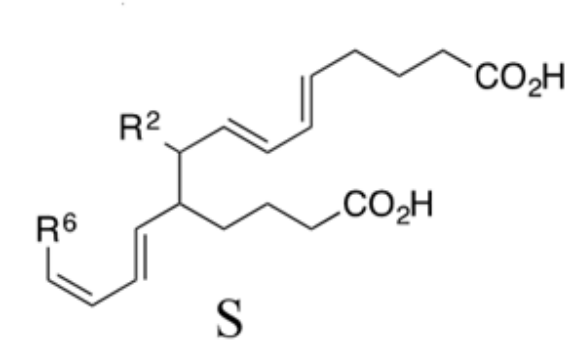
R^5 is optionally substituted C_1-C_6 alkyl; and

R^{10} is optionally substituted C_1-C_6 alkyl,

or a labelled compound thereof, in particular wherein the compound is for use in the synthesis of a gastric tract acid (GTA), or a compound thereof; in particular wherein the GTA or the compound thereof is a compound of formula N or S:



N , or



S , or a salt, ester, or labelled compound thereof,

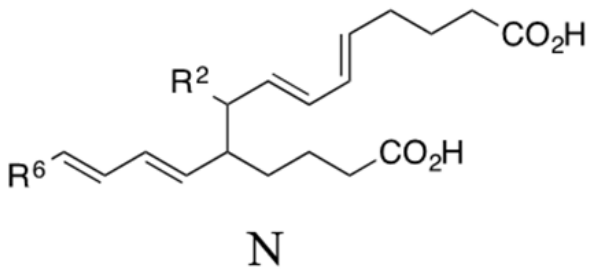
wherein

R2 is optionally substituted saturated or unsaturated C1-C20 alkyl, saturated or unsaturated C2-C20 alkenyl, or saturated or unsaturated C2-C20 alkynyl; and

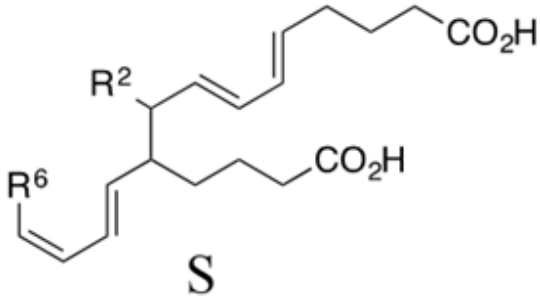
R6 is optionally substituted saturated or unsaturated C1-C20 alkyl, saturated or unsaturated C2-C20 alkenyl, or saturated or unsaturated C2-C20 alkynyl.

In still another example, there is provided herein a use of the compound of formula D in the synthesis of a gastric tract acid (GTA), or a derivative thereof. In another ~~aspect~~example, the present disclosure refers to use of the compound as disclosed herein in the synthesis of a gastric tract acid (GTA), or a derivative thereof.

In certain examples, the GTA or the derivative thereof may be a compound of formula N or S:



N , or



S ,

or a salt, ester, prodrug, or labelled derivative thereof,

wherein

R^2 is optionally substituted saturated or unsaturated C_1-C_{20} alkyl, saturated or unsaturated C_2-C_{20} alkenyl, or saturated or unsaturated C_2-C_{20} alkynyl; and

R^6 is optionally substituted saturated or unsaturated C_1-C_{20} alkyl, saturated or unsaturated C_2-C_{20} alkenyl, or saturated or unsaturated C_2-C_{20} alkynyl.

In another ~~aspect~~example, the present disclosure refers to a method for synthesizing a compound of formula N or S as defined above, or an isotopically labelled derivative thereof, said method comprising:

providing a compound of formula D as disclosed herein;

performing a coupling reaction and, optionally, a reduction, to replace the R^1 group with an optionally substituted saturated or unsaturated alkyl, saturated or unsaturated alkenyl, or saturated or unsaturated alkynyl;

converting the R^5-containing ester to a hydroxyl group;
converting the hydroxyl group to a leaving group;
displacing the leaving group with a dialkyl malonate;
performing acetal hydrolysis, ester hydrolysis, and decarboxylation, forming an aldehyde; and
performing a coupling reaction at the aldehyde to yield the compound of formula N or S, or an isotopically labelled derivative thereof,

wherein the compound of formula D as disclosed herein, or at least one reactant in the method, comprises at least one isotopically labelled atom which is incorporated into the resulting compound of formula N or S when an isotopically labelled derivative of formula N or S is synthesized.

In another aspect, the present disclosure refers to a method for synthesizing a compound of formula N or S as defined in claim 18, or an isotopically labelled compound thereof, said method comprising:

providing a compound as disclosed herein;
performing a coupling reaction and, optionally, a reduction, to replace the R^1 group with an optionally substituted saturated or unsaturated alkyl, saturated or unsaturated alkenyl, or saturated or unsaturated alkynyl;
converting the R^5-containing ester to a hydroxyl group;
converting the hydroxyl group to a leaving group;
displacing the leaving group with a dialkyl malonate;
performing acetal hydrolysis, ester hydrolysis, and decarboxylation, forming an aldehyde; and
performing a coupling reaction at the aldehyde to yield the compound of formula N or S, or an isotopically labelled compound thereof,

wherein the compound as disclosed herein, or at least one reactant in the method, comprises at least one isotopically labelled atom which is incorporated into the resulting compound of formula N or S when an isotopically labelled compound of formula N or S is synthesized.

In another ~~aspect~~example, the present disclosure refers to a compound or method as described herein.

BRIEF DESCRIPTION OF DRAWINGS

FIGURE 1 shows a typical total ion flow injection chromatogram (TIC) of a regular serum organic extract in water saturated ethyl acetate in negative APCI before any method development. This Figure shows how low GTA-446 in a comprehensive serum matrix;

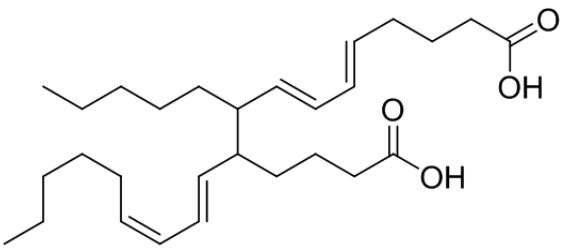
FIGURE 2 shows a full scan column chromatogram (RP-18) of a regular serum organic extracts in water saturated ethyl acetate showing GTA elution window of 16-18 minutes using methanol water gradient;

FIGURE 3 shows a comparison of CRC 446 (MRM 445/383) levels in the organic phase between monophasic extractions (MeOH-1 – MeOH-3) versus biphasic extractions (N-1 – N3). Monophasic extraction was implemented based on this data;

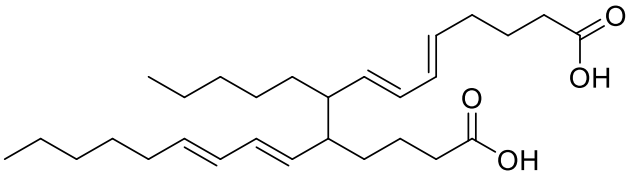
FIGURE 4 shows a total ion current flow injection chromatogram of the upper organic phase using the monophasic extraction followed by phase separation. GTA 446 is observed as m/z 445.3;

WHAT IS CLAIMED IS:

1. A compound having the structure of formula I or formula III:



(formula I),



(formula III),

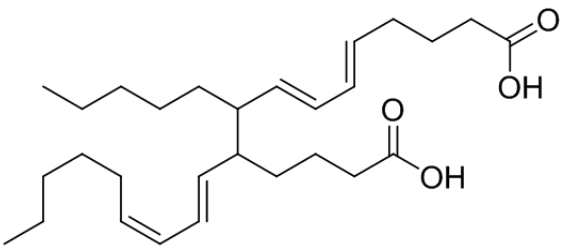
or a salt, ester, ~~prodrug,~~ or labelled ~~derivative~~ compound thereof, wherein the compound is an analytical standard compound.

2. The compound of claim 1, wherein the compound is an isolated compound or a synthetically prepared compound.

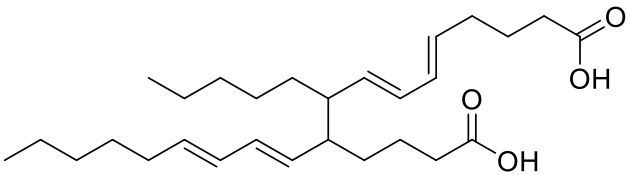
~~3. The compound of claim 1 or 2, wherein the compound is a synthetically prepared compound.~~

~~4. The compound of any one of claims 1-3, wherein the compound is an analytical standard compound.~~

~~5.~~ ~~3.~~ An isotopically labelled compound, the isotopically labelled compound comprising one or more isotopic labels incorporated within the structure of formula I or formula III:



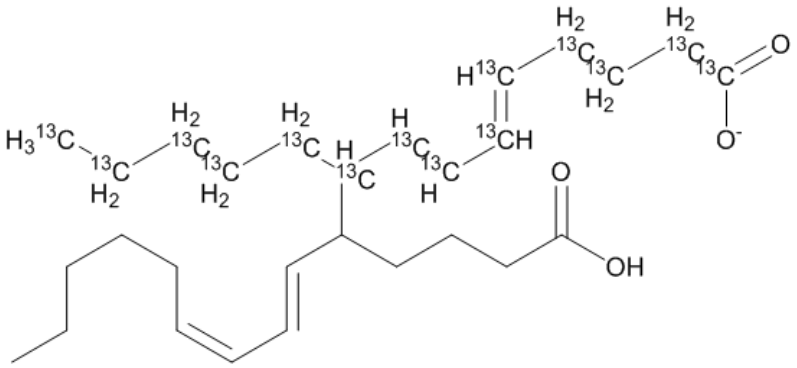
(formula I),

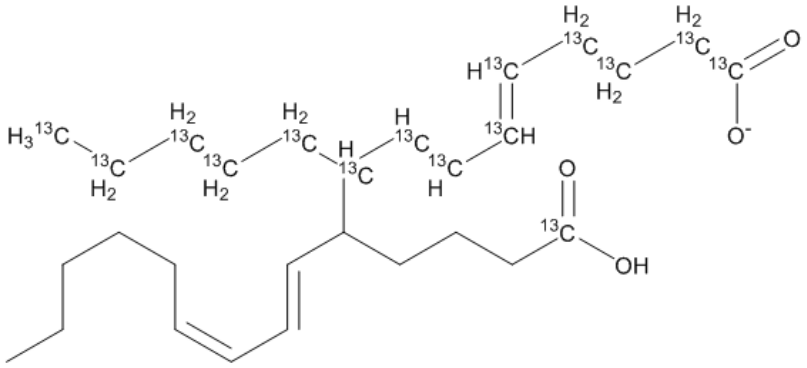


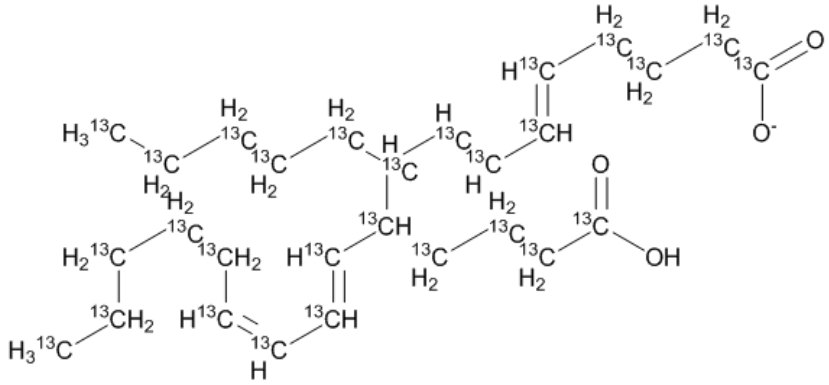
(formula III),

or a salt, or ester~~, or prodrug~~ thereof.

~~6.~~ ~~4.~~ The isotopically labelled compound of claim ~~5~~3, wherein the one or more isotopic labels are stable isotope labels, radioisotope labels, or a combination thereof, in particular wherein the one or more isotopic labels are selected from the group consisting of deuterium (2H) and 13C, or wherein the one or more isotopic labels are selected from the group consisting of tritium (3H) and 14C, in particular wherein the isotopically labelled compound is:~~.~~





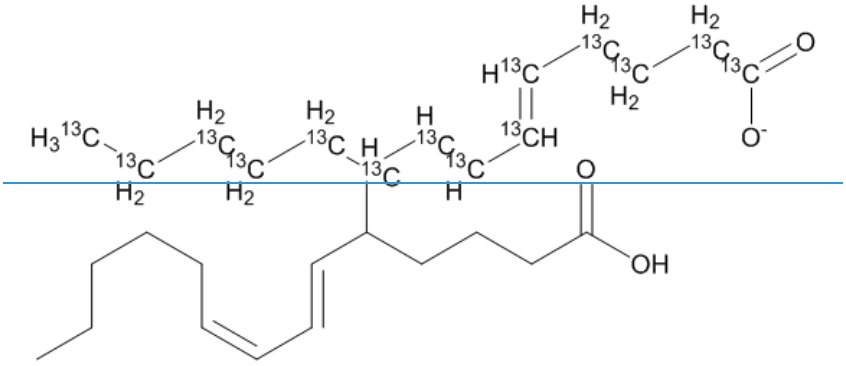


or a compound thereof in which all carbon-carbon double bonds are in trans configuration, or a salt, or ester thereof.

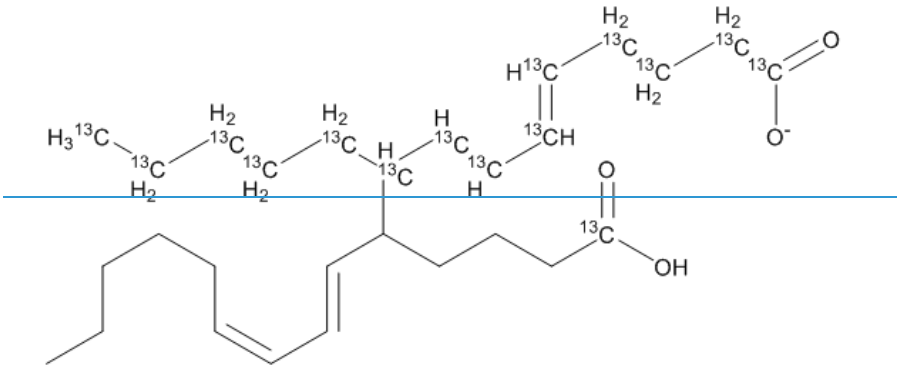
~~7. The isotopically labelled compound of claim 5 or 6, wherein the one or more isotopic labels are selected from the group consisting of deuterium (^{2}H) and ^{13}C.~~

8. The isotopically labelled compound of claim 5 or 6, wherein the one or more isotopic labels are selected from the group consisting of tritium (^{3}H) and ^{14}C.

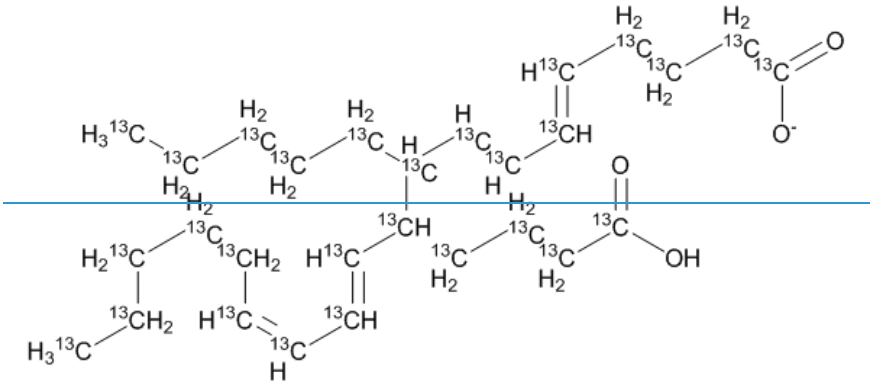
9. The isotopically labelled compound of any one of claims 5-7, wherein the isotopically labelled compound is:



;



;



~~or a derivative thereof in which all carbon-carbon double bonds are in trans configuration.~~

~~or a salt, ester, or prodrug thereof.~~

~~10. The isotopically labelled compound of any one of claims 5-9, wherein the compound is an analytical standard compound.~~

~~11. A metabolic tracer composition comprising the isotopically labelled compound of any one of claims 5-9.~~

~~12.~~ 5. A composition comprising the compound of any one of claims 1-~~9~~4, and an excipient, carrier, or diluent, in particular wherein the composition is a metabolic tracer composition or an *in vitro* or *in vivo* diagnostic agent comprising the isotopically labelled compound of claim 3 or 4.

~~13. An *in vitro* or *in vivo* diagnostic agent comprising the isotopically labelled compound of~~

~~any one of claims 5-9.~~

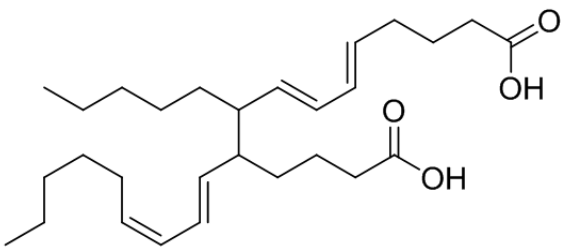
~~14. A composition comprising the compound of any one of claims 1-9, and an excipient, carrier, or diluent.~~

~~15.~~ 6. A method for determining a level of a gastric tract acid (GTA) in a sample, said method comprising:

measuring a GTA detection signal from the sample, the GTA detection signal representative of the GTA level in the sample; and

quantifying the level of the GTA in the sample by comparing the measured GTA detection signal with a calibration reference.

~~16.~~ 7. The method according to claim ~~15~~6, wherein the GTA is



(formula I); in particular, wherein the calibration reference comprises a standard curve prepared using known quantities of a compound as defined in claim 3 or 4; or wherein the calibration reference is obtained by spiking the sample with a known quantity of an isotopically labelled compound as defined in claim 3 or 4, and measuring an internal standard signal from the sample, the internal standard signal being representative of the known quantity of the isotopically labelled compound spiked into the sample; optionally wherein the GTA detection signal and/or the internal standard signal is measured by mass spectrometry.

~~17. The method of claim 15 or 16, wherein the GTA detection signal is measured by mass spectrometry.~~

~~18. The method of any one of claims 15-17, wherein the calibration reference comprises a standard curve prepared using known quantities of a compound as defined in any one of claims 5-10.~~

~~19. The method of any one of claims 15-17, wherein the calibration reference is obtained by :~~

~~spiking the sample with a known quantity of an isotopically labelled compound as defined in any one of claims 5-10; and~~

~~measuring an internal standard signal from the sample, the internal standard signal being representative of the known quantity of the isotopically labelled compound spiked into the sample.~~

~~20. The method of claim 19, wherein the internal standard signal is measured by mass spectrometry.~~

~~21.~~ 8. The method of claim ~~19 or 20~~7, further comprising a step of determining a ratio of the GTA level in the sample, as represented by the measured GTA detection signal, to the known quantity of isotopically labelled compound spiked into the sample, as represented by the internal standard signal; in particular wherein the calibration reference comprises an isotope dilution curve (IDC) generated from a series of mixtures of varying GTA/isotopically labelled compound ratios and concentrations, to which said ratio is compared; in particular

wherein the IDC is generated from a series of mixtures in which GTA content is varied over a fixed amount of isotopically labelled compound; in particular wherein the fixed amount of the isotopically labelled compound is substantially the same as the known quantity of the isotopically labelled compound which is spiked into the sample.

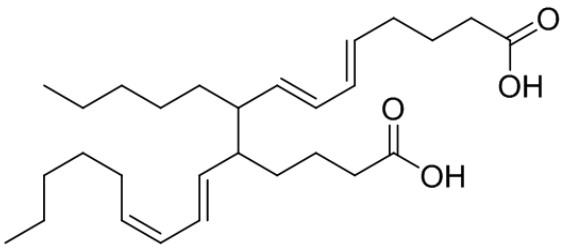
~~22. The method of claim 21, wherein the calibration reference comprises an isotope dilution curve (IDC) generated from a series of mixtures of varying GTA/isotopically labelled compound ratios and concentrations, to which said ratio is compared.~~

~~23. The method of claim 22, wherein the IDC is generated from a series of mixtures in which GTA content is varied over a fixed amount of isotopically labelled compound.~~

~~24. The method of claim 23, wherein the fixed amount of the isotopically labelled compound is substantially the same as the known quantity of the isotopically labelled compound which is spiked into the sample.~~

~~25.~~ 9. Use of the compound of any one of claims 1-~~10~~ 4 for (i) determining a level of a gastric tract acid (GTA) in a sample; (ii) for generating a calibration reference for use in determining a level of a gastric tract acid (GTA) in a sample; or (iii) as an internal standard for use in determining a level of a gastric tract acid (GTA) in a sample.

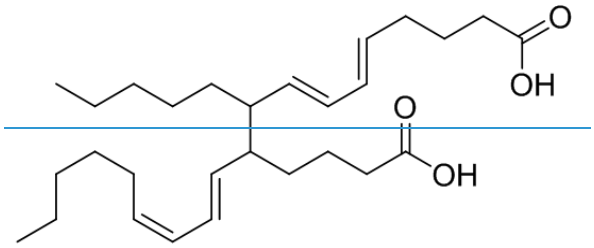
~~26.~~ 10. The use according to claim ~~25~~9, wherein the GTA is:



(formula I).

~~27. Use of the compound of any one of claims 1-10 for generating a calibration reference for use in determining a level of a gastric tract acid (GTA) in a sample.~~

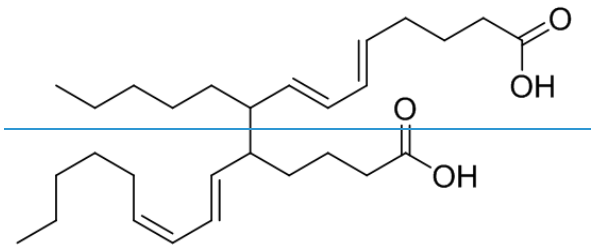
~~28. The use according to claim 27, wherein the GTA is:~~



~~(formula I).~~

~~29. Use of the compound of any one of claims 5-9 as an internal standard for use in determining a level of a gastric tract acid (GTA) in a sample.~~

~~30. The use according to claim 29, wherein the GTA is:~~



~~(formula I).~~

~~31.~~ 11. A diagnostic method for identifying a subject as having, or being at risk of developing, colorectal cancer, said method comprising:

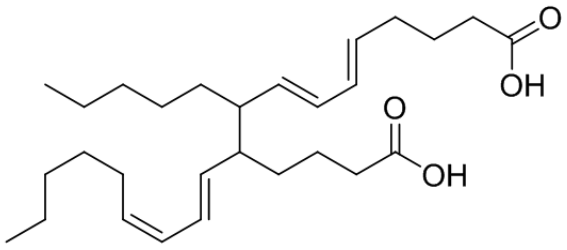
determining a level of a gastric tract acid (GTA) in a sample obtained from the subject by

measuring a GTA detection signal from the sample, the GTA detection signal representative of the GTA level in the sample; and

quantifying the level of the GTA in the sample by comparing the measured GTA detection signal with a calibration reference, and

identifying the subject as having, or being at risk of developing, colorectal cancer when the determined level of the GTA in the sample is reduced in comparison to a healthy control group,

wherein the GTA is:



(formula I).

~~32. The method of claim 31, wherein the GTA detection signal is measured by mass spectrometry.~~

~~33.~~ 12. The method of claim ~~31 or 32~~11, wherein the calibration reference comprises a standard curve prepared using known quantities of a compound as defined in claim 3 or 4; and/or wherein the step of determining the level of the GTA in the sample obtained from the subject comprises spiking the sample with a known quantity of an isotopically labelled compound as defined in any one of claims 3-5, and measuring an internal standard signal from the sample, the internal standard signal being representative of the known quantity of the isotopically labelled compound spiked into the sample; in particular wherein the GTA detection signal and/or the internal standard signal is measured by mass spectrometry~~in any one of claims 5-10~~.

~~34. The method of claim 31 or 32, wherein the step of determining the level of the GTA in the sample obtained from the subject comprises:~~

~~spiking the sample with a known quantity of an isotopically labelled compound as defined in any one of claims 5-10; and~~

~~measuring an internal standard signal from the sample, the internal standard signal being representative of the known quantity of the isotopically labelled compound spiked into the sample.~~

~~35. The method of claim 34, wherein the internal standard signal is measured by mass spectrometry.~~

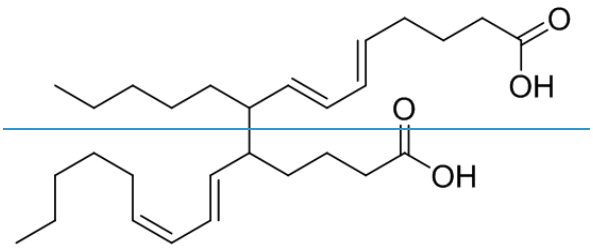
~~36.~~ 13. The method of claim ~~34 or 35~~13, wherein determining the level of the GTA in the sample obtained from the subject further ~~comprising~~ comprises a step of determining a ratio of the GTA level in the sample, as represented by the measured GTA detection signal, to the known quantity of isotopically labelled compound spiked into the sample, as represented by the internal standard signal; in particular wherein the calibration reference comprises an isotope dilution curve (IDC) generated from a series of mixtures of varying GTA/isotopically labelled compound ratios and concentrations, to which said ratio is compared; in particular wherein the IDC is generated from a series of mixtures in which GTA content is varied over a fixed amount of isotopically labelled compound; in particular wherein the fixed amount of the isotopically labelled compound is substantially the same as the known quantity of the isotopically labelled compound which is spiked into the sample.

~~37. The method of claim 36, wherein the calibration reference comprises an isotope dilution curve (IDC) generated from a series of mixtures of varying GTA/isotopically labelled compound ratios and concentrations, to which said ratio is compared.~~

~~38. The method of claim 37, wherein the IDC is generated from a series of mixtures in which GTA content is varied over a fixed amount of isotopically labelled compound.~~

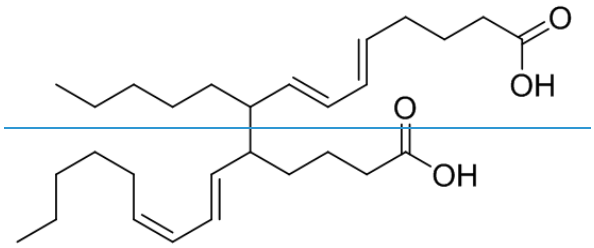
~~39. The method of claim 38, wherein the fixed amount of the isotopically labelled compound is substantially the same as the known quantity of the isotopically labelled compound which is spiked into the sample.~~

~~40. Use of the compound of any one of claims 1-10 in a diagnostic method for identifying a subject as having, or being at risk of developing, a colorectal cancer associated with altered levels of a gastric tract acid (GTA) which is:~~



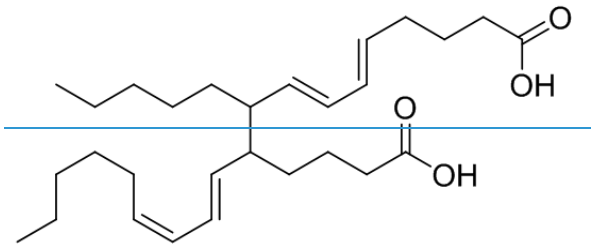
(formula I).

41. Use of the compound of any one of claims 1-10 for generating a calibration reference for use in a diagnostic method for identifying a subject as having, or being at risk of developing, a colorectal cancer associated with altered levels of a gastric tract acid (GTA) which is:



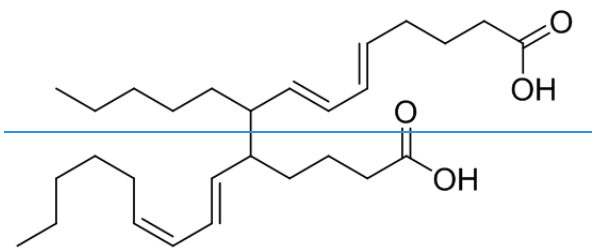
(formula I).

42. Use of the compound of any one of claims 5-9 as an internal standard for use in a diagnostic method for identifying a subject as having, or being at risk of developing, a colorectal cancer associated with altered levels of a gastric tract acid (GTA) which is:



(formula I).

~~43. An antibody, or antigen-binding fragment thereof, which specifically binds a compound of formula I:~~

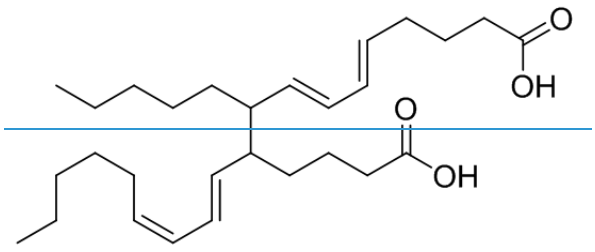


~~(formula I).~~

~~44. The antibody, or antigen-binding fragment thereof, of claim 43, wherein the antibody is a monoclonal or a polyclonal antibody.~~

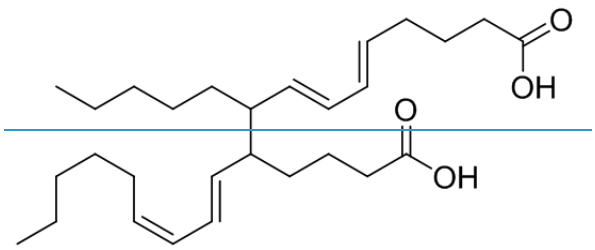
~~45. Use of a compound according to any one of claims 1-3 as an antigen for preparing an antibody which specifically binds to an antigenic epitope of the compound.~~

~~46. Use of the antibody of claim 43 or 44 for detecting or quantifying a level of a gastric tract acid (GTA) in a sample by immunoassay, wherein the GTA is:~~



~~(formula I).~~

~~47. Use of the antibody of claim 43 or 44 in a diagnostic method for identifying a subject as having, or being at risk of developing, a colorectal cancer associated with altered levels of a gastric tract acid (GTA) which is:~~

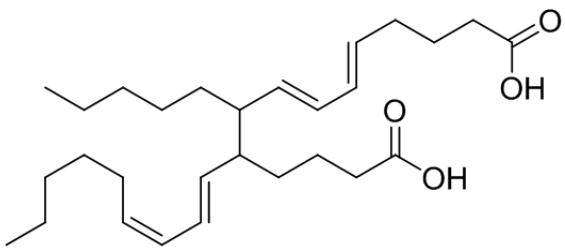


~~(formula I).~~

~~48.~~ 14. A method for determining a level of a gastric tract acid (GTA) in a sample, said method comprising:

measuring a level of the GTA in the sample using an immunoassay employing an antibody, or antigen-binding fragment thereof, which specifically binds to the GTA; in particular wherein the immunoassay comprises an enzyme-linked immunosorbent assay (ELISA);

wherein the GTA is:



(formula I); in particular wherein the method further comprises a step of using a control sample comprising a compound as defined in any one of claims 1-4 as a positive control in the immunoassay; in particular wherein the method further comprises a step of using a standard curve to extrapolate the level of the GTA in the sample, the standard

curve having been generated using a plurality of known quantities of a compound as defined in any one of claims 1-4.

~~49. The method of claim 48, wherein the immunoassay comprises an enzyme-linked immunosorbent assay (ELISA).~~

~~50. The method of claim 48 or 49, further comprising a step of using a control sample comprising a compound as defined in any one of claims 1-10 as a positive control in the immunoassay.~~

~~51. The method of any one of claims 48-49, further comprising a step of using a standard curve to extrapolate the level of the GTA in the sample, the standard curve having been generated using a plurality of known quantities of a compound as defined in any one of claims 1-10.~~

~~52.~~ 15. A diagnostic method for identifying a subject as having, or being at risk of developing, colorectal cancer, said method comprising:

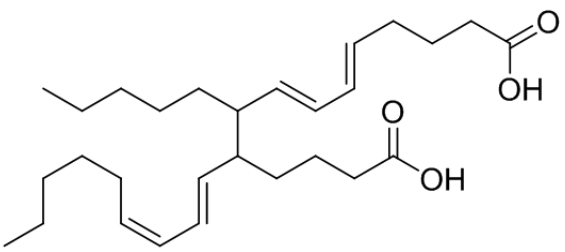
determining a level of a gastric tract acid (GTA) in a sample obtained from the subject by

measuring a level of the GTA in the sample using an immunoassay employing an antibody, or antigen-binding fragment thereof, which specifically binds to the GTA; in particular wherein the immunoassay comprises an enzyme-linked immunosorbent assay (ELISA); and

identifying the subject as having, or being at risk of developing, colorectal cancer

when the determined level of the GTA in the sample is reduced in comparison to a healthy control group,

wherein the GTA is:



(formula I), in particular wherein the method further comprises a step of using a control sample comprising a compound as defined in any one of claims 1-5 as a positive control in the immunoassay; in particular wherein the step of determining the level of the GTA in the sample further comprises using a standard curve to extrapolate the level of the GTA in the sample, the standard curve having been generated using a plurality of known quantities of a compound as defined in any one of claims 1-4.

~~53. The method of claim 52, wherein the immunoassay comprises an enzyme-linked immunosorbent assay (ELISA).~~

~~54. The method of claim 52 or 53, further comprising a step of using a control sample comprising a compound as defined in any one of claims 1-10 as a positive control in the immunoassay.~~

~~55. The method of any one of claims 52-54, wherein the step of determining the level of the GTA in the sample further comprises using a standard curve to extrapolate the level of the GTA in the sample, the standard curve having been generated using a plurality of known~~

quantities of a compound as defined in any one of claims 1-10.

56. A kit for quantifying a level of a gastric tract acid (GTA) in a sample, the kit comprising at least one of:

a compound according to any one of claims 1-10;

a metabolic tracer according to claim 11;

a composition according to claim 12 or 14;

a diagnostic agent according to claim 13; and

an antibody according to claim 43 or 44;

and, optionally, further comprising a set of instructions for performing a method as defined in any one of claims 15-24 and 48-51.

57. A diagnostic kit for identifying a subject as having, or being at risk of developing, colorectal cancer, the kit comprising at least one of:

a compound according to any one of claims 1-10;

a metabolic tracer according to claim 11;

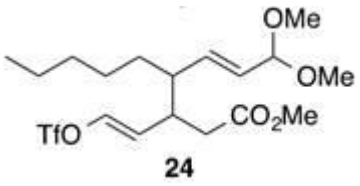
a composition according to claim 12 or 14;

a diagnostic agent according to claim 13; and

an antibody according to claim 43 or 44;

and, optionally, further comprising a set of instructions for performing a method as defined in any one of claims 31-39 and 52-55.

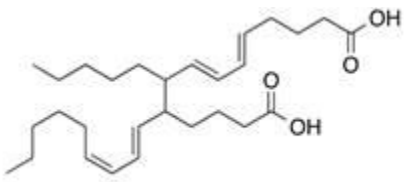
~~58~~16. A compound having the formula:



24

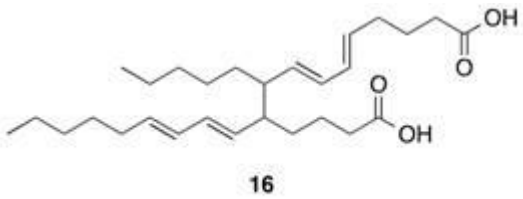
, or a labelled ~~derivative~~ compound thereof; in particular wherein the compound is for use in the synthesis of a compound having the formula:~~.~~

~~59. Use of the compound of claim 58 in the synthesis of a compound having the formula:~~



15

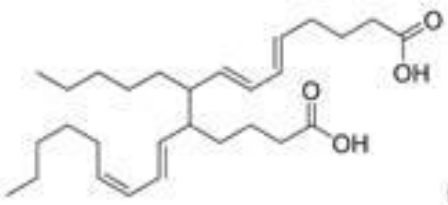
or



16

, or a salt, ester, ~~prodrug,~~ or labelled ~~derivative~~ compound thereof.

~~60~~17. A method for synthesizing a compound having formula (I):



(formula I),

or an isotopically labelled ~~derivative~~ compound thereof,

said method comprising:

providing a compound according to claim ~~58~~16;

performing a Sonagashira coupling of the compound with 1-heptyne;

performing a reduction with Lindlar's catalyst;

performing a methyl ester reduction;

performing a reaction with methanesulfonyl chloride;

performing a mesylate displacement with dimethyl malonate;

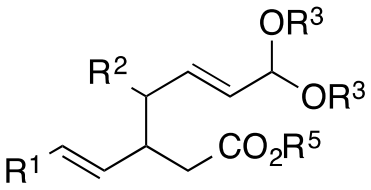
performing an acid treatment for simultaneous acetal cleavage, ester hydrolysis, and decarboxylation; and

performing a Wittig reaction to yield the compound of formula I, or an isotopically labelled ~~derivative~~compound thereof; in particular wherein the Wittig reaction comprises reaction with (triphenylphosphoranylidene) acetaldehyde or (4-carboxybutyl)triphenylphosphonium bromide,

wherein the compound according to claim ~~58~~16, or at least one reactant in the method, comprises at least one isotopically labelled atom which is incorporated into the resulting compound of formula I when an isotopically labelled ~~derivative~~ compound of formula I is synthesized.

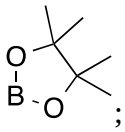
~~61. The method according to claim 60, wherein the Wittig reaction comprises reaction with (triphenylphosphoranylidene) acetaldehyde or (4-carboxybutyl)triphenylphosphonium bromide.~~

~~62~~18. A compound having formula D:



(Formula D),

wherein:

R^1 is -Sn(R^{10})$_3$, -OTf, -Cl, -Br, -I, -B(OH)$_2$ or 

;

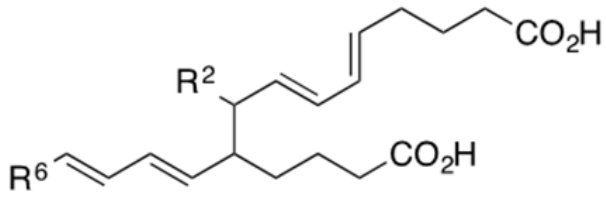
R^2 is optionally substituted saturated or unsaturated C_1-C_{20} alkyl, saturated or unsaturated C_2-C_{20} alkenyl, or saturated or unsaturated C_2-C_{20} alkynyl;

each R^3 is, independently, optionally substituted C_1-C_6 alkyl, or the R^3 groups together form an optionally substituted ethylene or propylene group bridging the attached oxygen atoms to form a five- or six-membered ring;

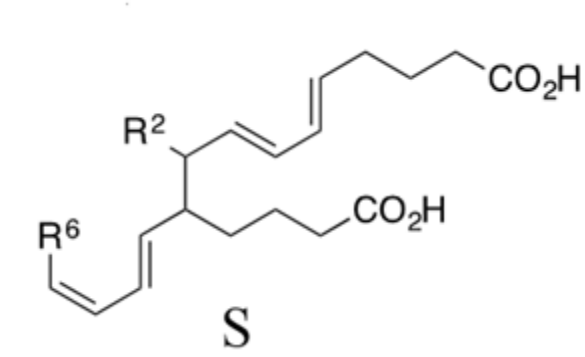
R^5 is optionally substituted C_1-C_6 alkyl; and

R^{10} is optionally substituted C_1-C_6 alkyl,

or a labelled ~~derivative~~ compound thereof, in particular wherein the compound is for use in the synthesis of a gastric tract acid (GTA), or a compound thereof; in particular wherein the GTA or the compound thereof is a compound of formula N or S:



N , or



S , or a salt, ester, or labelled compound thereof,

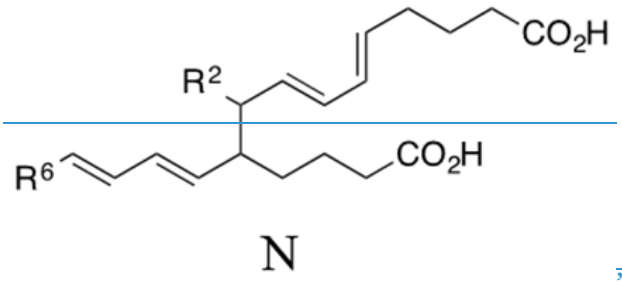
wherein

R2 is optionally substituted saturated or unsaturated C1-C20 alkyl, saturated or unsaturated C2-C20 alkenyl, or saturated or unsaturated C2-C20 alkynyl; and

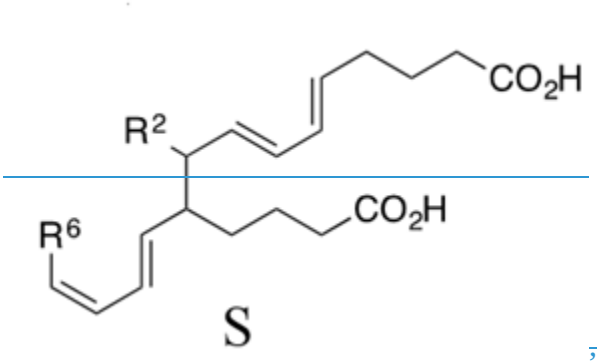
R6 is optionally substituted saturated or unsaturated C1-C20 alkyl, saturated or unsaturated C2-C20 alkenyl, or saturated or unsaturated C2-C20 alkynyl.

~~63. Use of the compound of claim 62 in the synthesis of a gastric tract acid (GTA), or a derivative thereof.~~

~~64. The use of claim 63, wherein the GTA or the derivative thereof is a compound of formula N or S:~~



N ~~, or~~



S

;

~~or a salt, ester, prodrug, or labelled derivative thereof,~~

~~wherein~~

~~R^2 is optionally substituted saturated or unsaturated C_1-C_{20} alkyl, saturated or unsaturated C_2-C_{20} alkenyl, or saturated or unsaturated C_2-C_{20} alkynyl; and~~

~~R^6 is optionally substituted saturated or unsaturated C_1-C_{20} alkyl, saturated or unsaturated C_2-C_{20} alkenyl, or saturated or unsaturated C_2-C_{20} alkynyl.~~

~~65~~19. A method for synthesizing a compound of formula N or S as defined in claim ~~64~~18, or an isotopically labelled ~~derivative~~ compound thereof, said method comprising:

providing a compound according to claim ~~62~~18;

performing a coupling reaction and, optionally, a reduction, to replace the R^1 group with an optionally substituted saturated or unsaturated alkyl, saturated or unsaturated alkenyl, or saturated or unsaturated alkynyl;

converting the R^5-containing ester to a hydroxyl group;

converting the hydroxyl group to a leaving group;

displacing the leaving group with a dialkyl malonate;

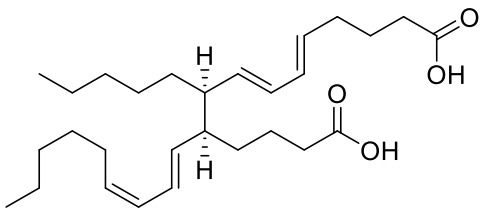
performing acetal hydrolysis, ester hydrolysis, and decarboxylation, forming an

aldehyde; and

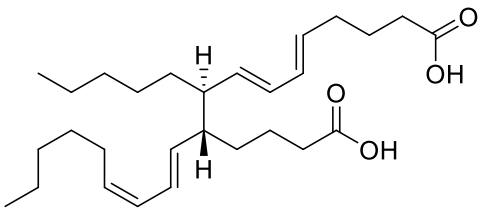
performing a coupling reaction at the aldehyde to yield the compound of formula N or S, or an isotopically labelled ~~derivative~~ compound thereof,

wherein the compound according to claim ~~62~~18, or at least one reactant in the method, comprises at least one isotopically labelled atom which is incorporated into the resulting compound of formula N or S when an isotopically labelled ~~derivative~~ compound of formula N or S is synthesized.

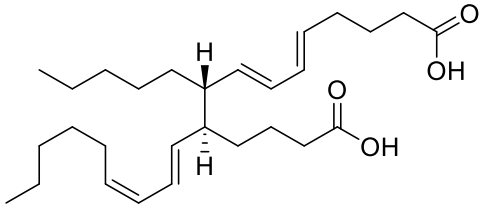
~~66~~20. The compound of any one of claims 1-~~10~~4, wherein the compound is:



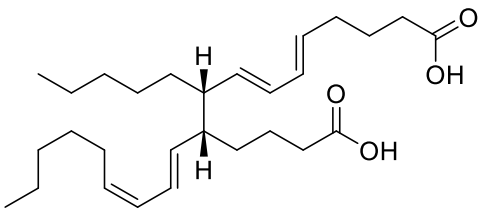
(formula Ia);



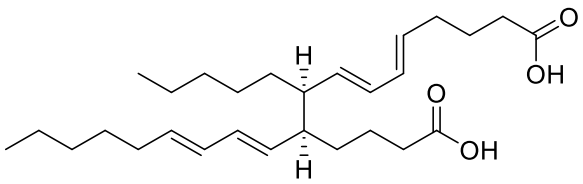
(formula Ib);



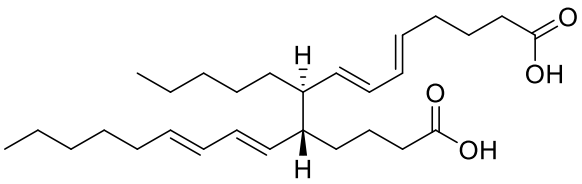
(formula Ic);



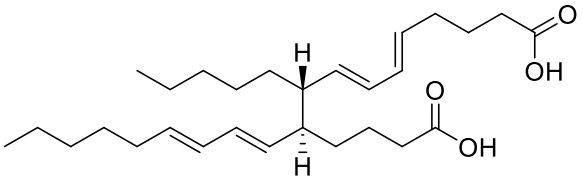
(formula Id);



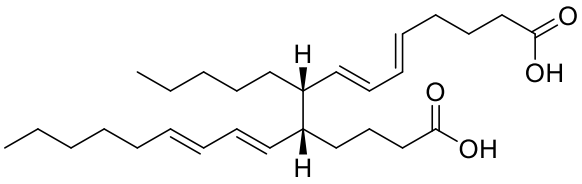
(formula IIIa);



(formula IIIb);



(formula IIIc); or



(formula IIId);

or any combination thereof;

or a salt, ester, ~~prodrug,~~ or labelled ~~derivative~~compound thereof.

~~67. A compound or method as described herein.~~