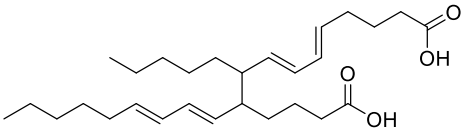


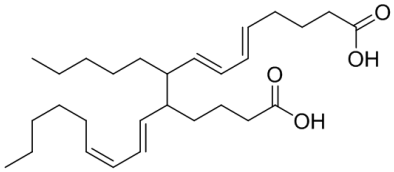
(formula I),



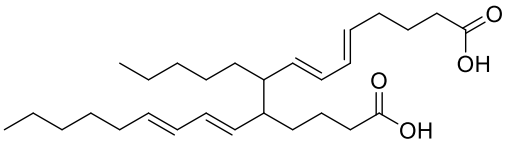
(formula III),

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

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



(formula I),



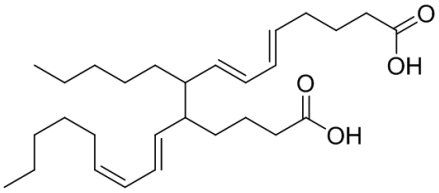
(formula III),

or a salt, ester, or labelled compound thereof, wherein the compound is a metabolic tracer or an *in vitro* or *in vivo* diagnostic agent or reference standard.

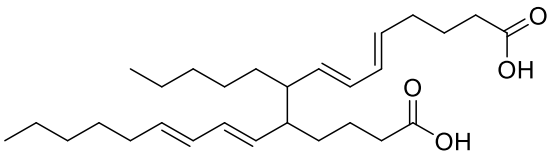
In a further example, the compound may an isolated compound.

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 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 example, there is provided herein a metabolic tracer composition comprising isotopically labelled compound or compounds as described above. In another 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.

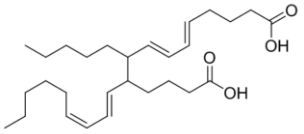
In yet another example, there is provided herein an *in vitro* or *in vivo* diagnostic agent comprising isotopically labelled compound or compounds as described above. In another example, the present disclosure refers to an *in vitro* or *in vivo* diagnostic agent comprising the isotopically labelled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

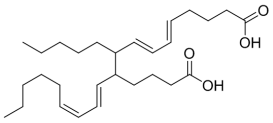
measuring a GTA detection signal from the sample, the GTA detection signal representative of the GTA level in the sample; and

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



(Formula I).

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

or methods as defined above.

In another 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;

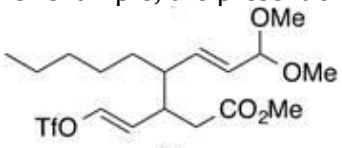
a composition or compositions as defined above; a diagnostic agent as defined above; and an antibody or antibodies as defined above; and, optionally, further comprising a set of instructions for performing a method or methods as defined above.

In another 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

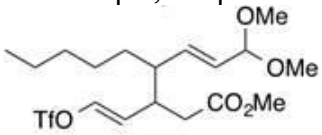
an antibody as disclosed herein; and, optionally, further comprising a set of instructions for performing a method as disclosed herein.

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

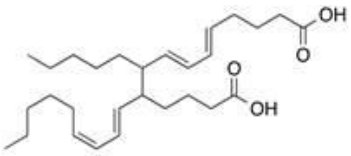


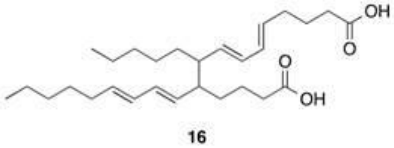
24 , or a labelled derivative thereof.

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



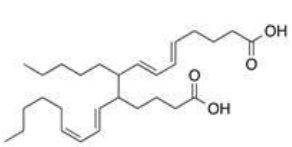
24 , or a labelled compound thereof; in particular wherein the compound is for use in the synthesis of a compound having the formula:

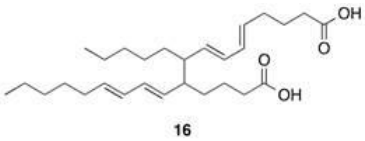


15 or 

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

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

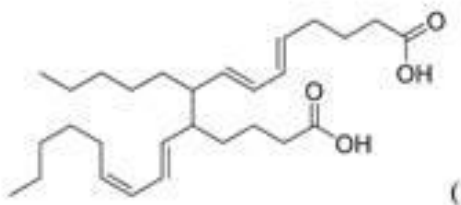


15 or 

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

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.

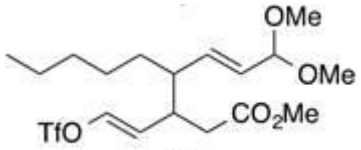
In another aspect, the present disclosure refers to a method for synthesizing a compound having formula (I):



(formula I),

or an isotopically labelled compound thereof,

said method comprising:

providing a compound of the formula 

24 or a labelled compound thereof;

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

performing a reduction with Lindlar's catalyst;

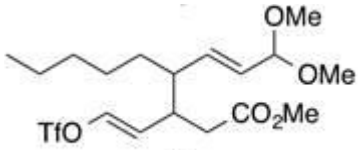
performing a methyl ester reduction;

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,

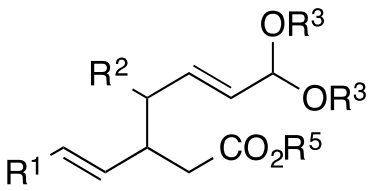
wherein the compound of the formula 

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 compound of formula I is synthesized.

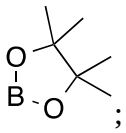
In a further example, the Wittig reaction may comprise reaction with (triphenylphosphoranylidene) acetaldehyde or (4-carboxybutyl)triphenylphosphonium bromide.

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

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 example, the present disclosure refers to a method for synthesizing a compound of formula N or S as defined herein, 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 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;

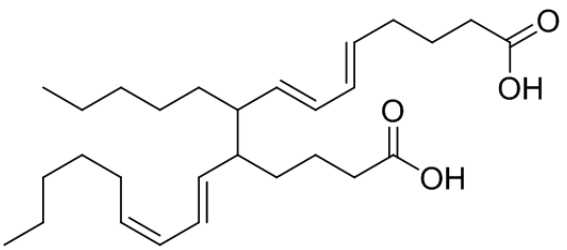
FIGURE 2 shows a full scan column chromatogram (RP-18) of a regular serum organic extracts in water saturated ethyl acetate showing GTA elution window of 16-18 minutes using methanol water gradient;

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;

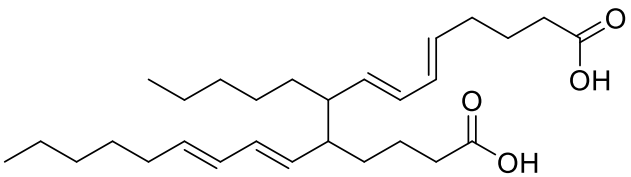
FIGURE 4 shows a total ion current flow injection chromatogram of the upper organic phase using the monophasic extraction followed by phase separation. GTA 446 is observed as m/z 445.3;

WHAT IS CLAIMED IS:

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



(formula I),

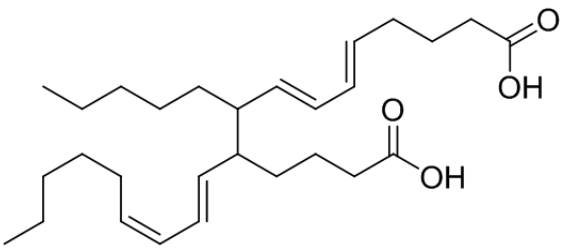


(formula III),

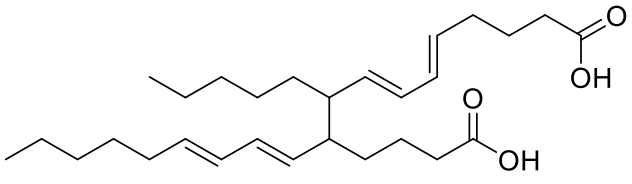
or a salt, ester, or labelled compound thereof, wherein the compound is a metabolic tracer or an *in vitro* or *in vivo* diagnostic agent or reference standard.

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

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:



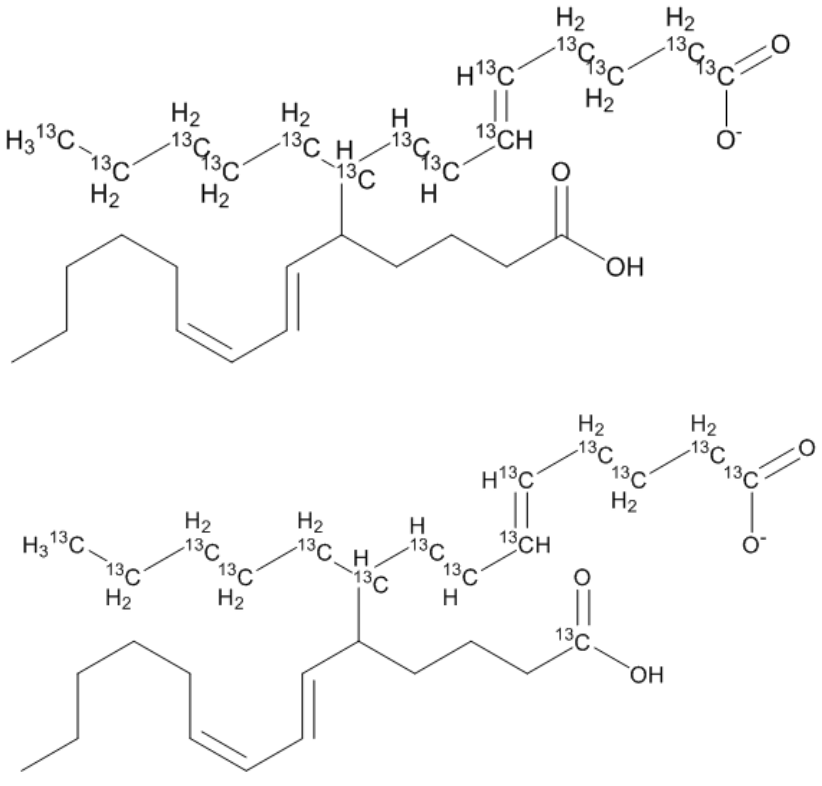
(formula I),

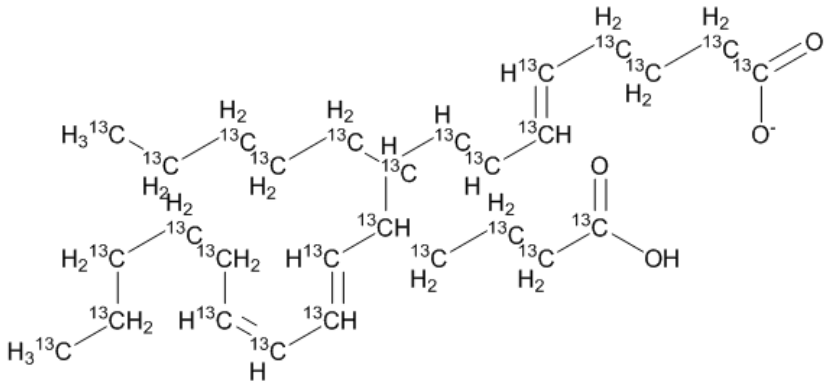


(formula III),

or a salt, or ester thereof.

4. The isotopically labelled compound of claim 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:





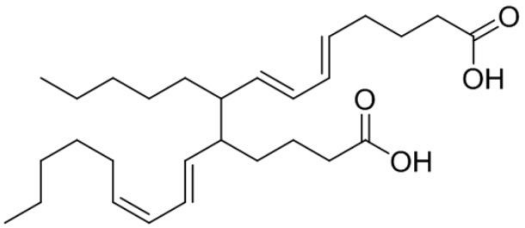
or a compound thereof in which all carbon-carbon double bonds are in trans configuration, or a salt, or ester thereof.

5. A composition comprising the compound as defined in any one of claims 1-4, 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.

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,

wherein the GTA is 

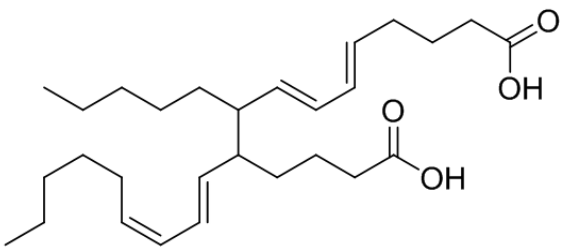
(Formula I).

7. The method according to claim 6, 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.

8. The method of claim 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.

9. Use of the compound as defined in any one of claims 1-4 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.

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



(formula I).

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

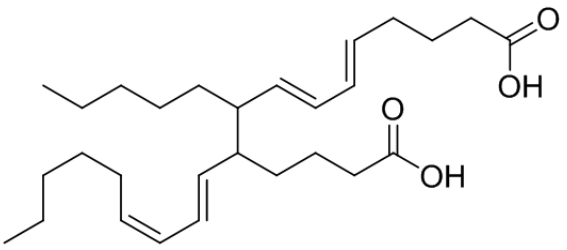
determining a level of a gastric tract acid (GTA) in a sample obtained from the subject by

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).

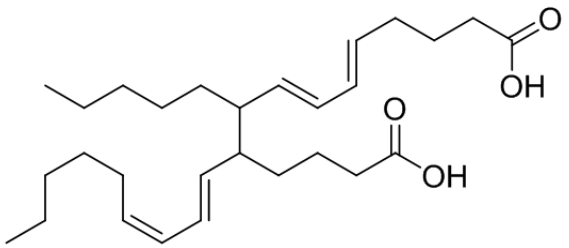
12. The method of claim 11, wherein the calibration reference comprises a standard curve prepared using known quantities of a compound as defined in claim 3 or 4; and/or wherein the step of determining the level of the GTA in the sample obtained from the subject comprises spiking the sample with a known 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.

13. The method of claim 13, wherein determining the level of the GTA in the sample obtained from the subject further 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.

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.

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

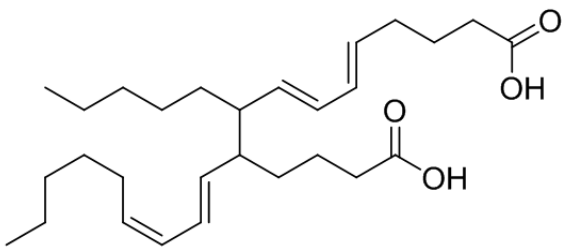
determining a level of a gastric tract acid (GTA) in a sample obtained from the subject by

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

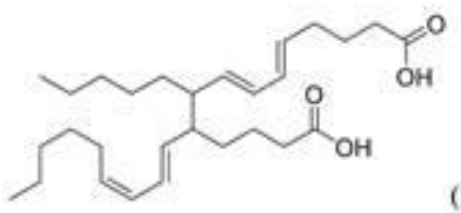
identifying the subject as having, or being at risk of developing, colorectal cancer when the determined level of the GTA in the sample is reduced in comparison to a healthy control group,

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.

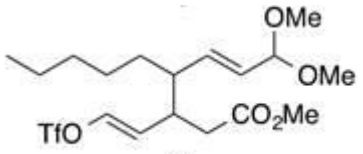
16. A method for synthesizing a compound having formula (I):



(formula I),

or an isotopically labelled compound thereof,

said method comprising:

providing a compound of the formula 

24 or a labelled compound thereof;

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

performing a reduction with Lindlar's catalyst;

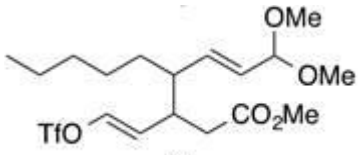
performing a methyl ester reduction;

performing a reaction with methanesulfonyl chloride;

performing a mesylate displacement with dimethyl malonate;

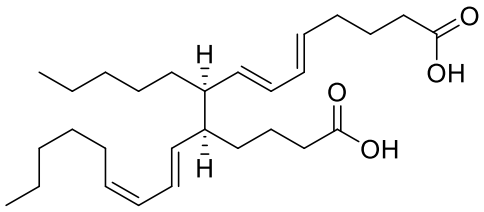
performing an acid treatment for simultaneous acetal cleavage, ester hydrolysis, and decarboxylation; and

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,

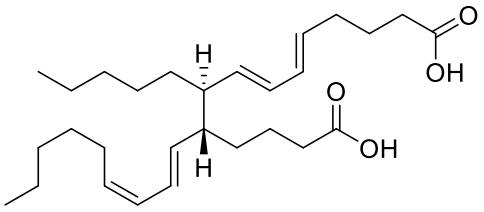
wherein the compound of the formula 

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 compound of formula I is synthesized.

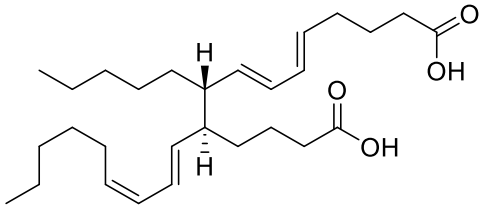
17. The composition of claim 1 or 2 or the compound of claim 3 or 4, wherein the compound is:



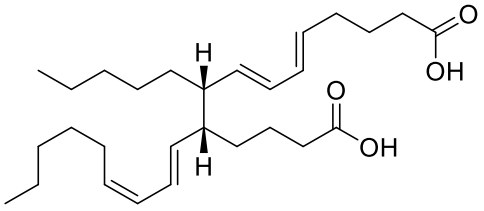
(formula Ia);



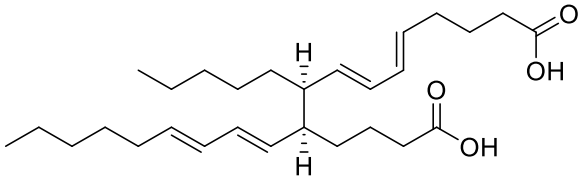
(formula Ib);



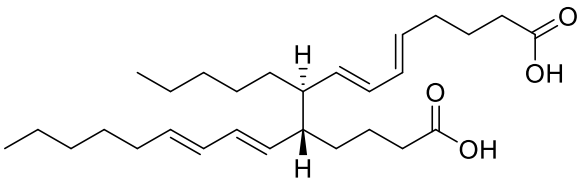
(formula Ic);



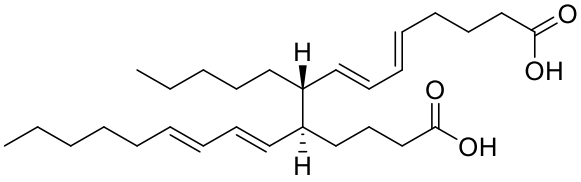
(formula Id);



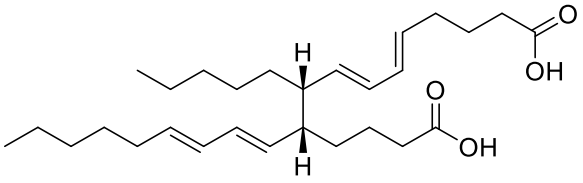
(formula IIIa);



(formula IIIb);



(formula IIIc); or



(formula IIId);

or any combination thereof;

or a salt, ester, or labelled compound thereof, wherein the compound is a metabolic tracer or an *in vitro* or *in vivo* diagnostic agent or reference standard.