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.

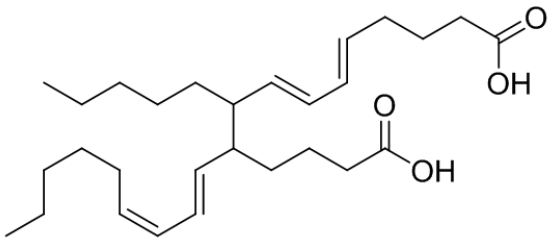
The quantification of GTA-446 has previously been further limited to primarily tandem mass spectrometry analyses, since enzyme-linked immunosorbent assay (ELISA)-based quantitation assays have not been performed due to the lack of suitable antibody. Although many diagnostic platforms are based on the 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.

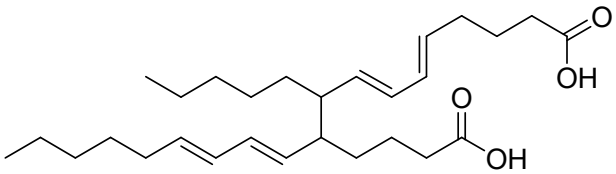
SUMMARY

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



(formula I),



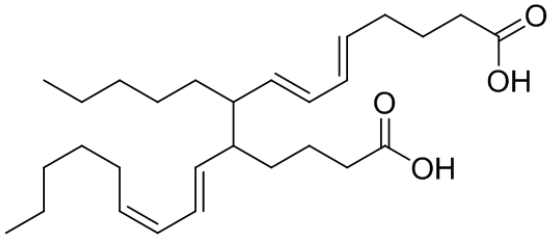
(formula III),

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

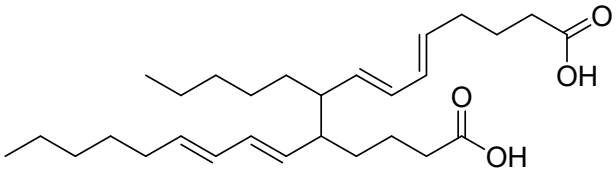
In a further example, the compound may an isolated compound.

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, 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),

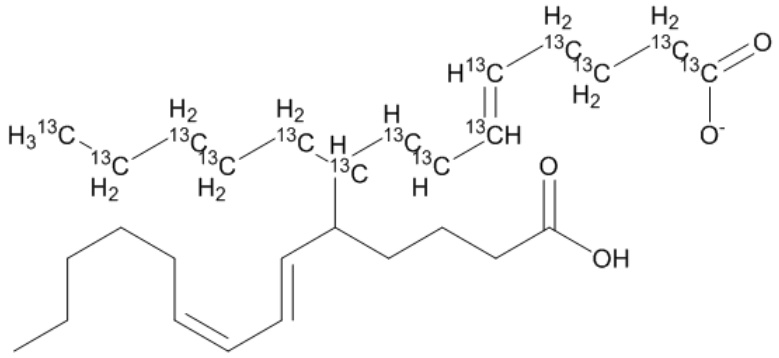
or a salt, ester, or prodrug thereof.

In 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.

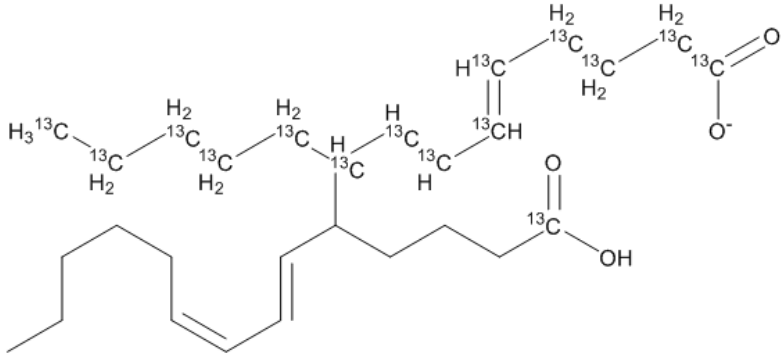
In still a further example of the isotopically labelled compound or compounds above, the one or more isotopic labels may be selected from the group consisting of deuterium (^{2}H) and ^{13}C.

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.

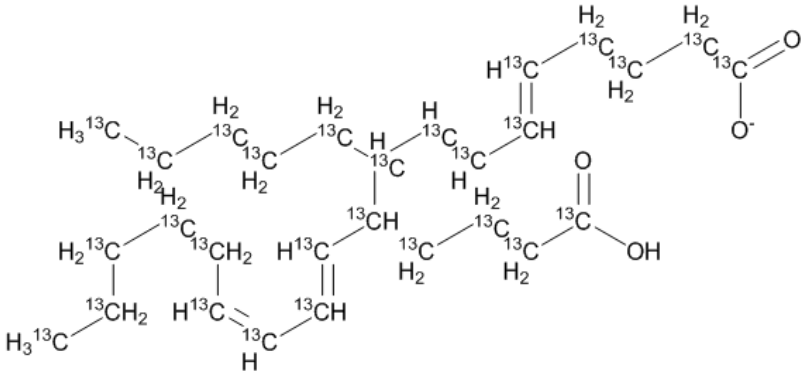
In still another example, the isotopically labelled compound may be:



,



,



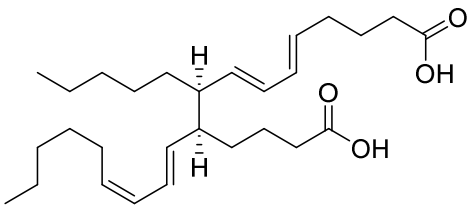
,

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

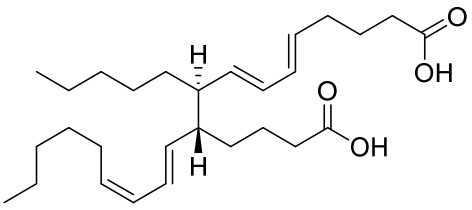
or a salt, ester, or prodrug thereof.

In yet another example, the isotopically labelled compound or compounds above may be an analytical standard compound.

In another example of the compound or compounds above, the compound may be a compound of:



(formula Ia);



(formula Ib);

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

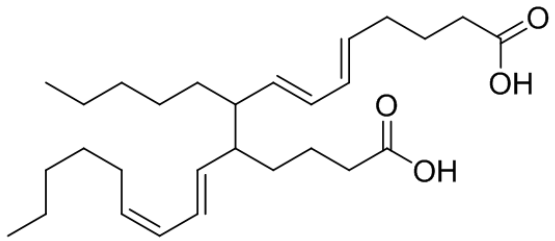
In another example, there is provided herein a composition comprising any of the compound or compounds above, and an excipient, carrier, or diluent. In another aspect, the present disclosure refers to a composition comprising the compound as disclosed herein, and an excipient, carrier, or diluent.

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.

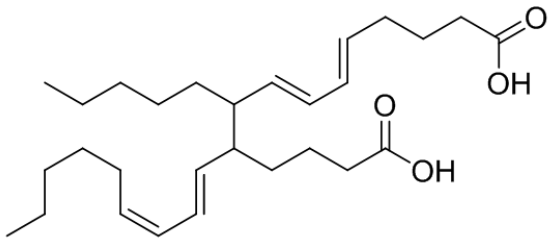
In still another example of the above method or methods, the IDC may be generated from a series of mixtures in which GTA content is varied over a fixed amount of isotopically labelled compound.

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 determining a level of a gastric tract acid (GTA) in a sample.

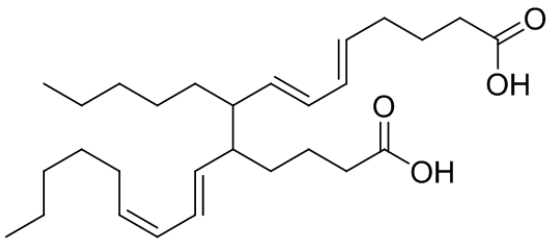
In a further example,

the GTA may be:



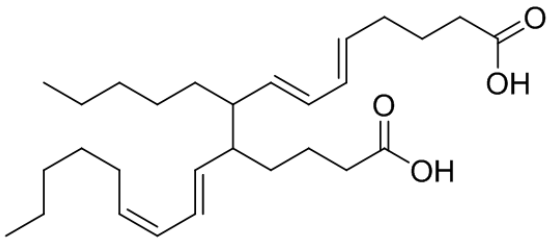
(formula I).

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, 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, 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

having, or being at risk of developing, colorectal cancer, said method comprising:

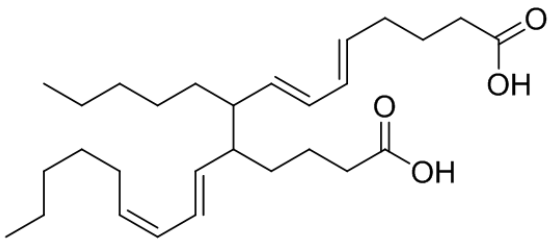
determining a level of a gastric tract acid (GTA) in a sample obtained from the subject by

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).

In a further example, the GTA detection signal may be measured by mass spectrometry.

In yet 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 another example of the above method or methods, the step of determining the level of the GTA in the sample obtained from the subject may comprise:

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, the internal standard signal may be measured by mass spectrometry.

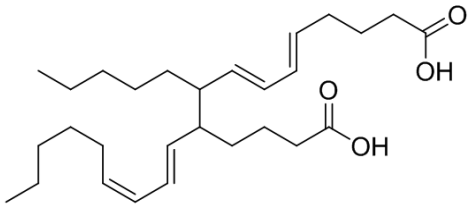
In yet another example of the above method or methods, the method may further comprise a step of determining a ratio of the GTA level in the sample, as represented by the measured GTA detection signal, to the known quantity of isotopically labelled compound spiked into the sample, as represented by the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

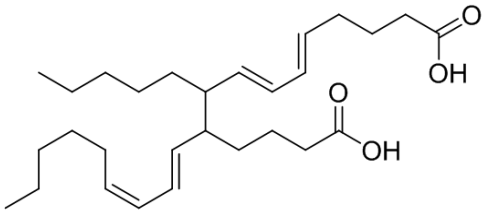
In yet another example of the above method or methods, the fixed amount of the isotopically labelled compound may be substantially the same as the known quantity of the isotopically labelled compound which is spiked into the sample.

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).

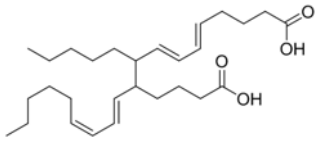
In another aspect, the present disclosure refers to use of the compound as disclosed herein in a diagnostic method for identifying a subject as having, or being at risk of developing, a colorectal cancer associated with altered levels of a gastric tract acid (GTA) which is:



(formula I).

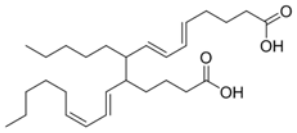
In still another example, there is provided herein a use of compound or compounds as

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:



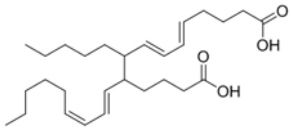
(formula I).

In another aspect, 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:



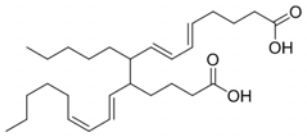
(formula I).

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:



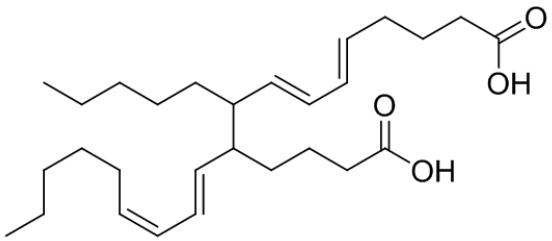
(formula I).

In another aspect, 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:



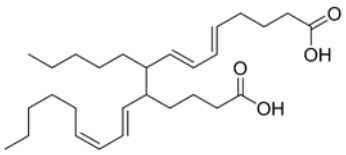
(formula I).

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

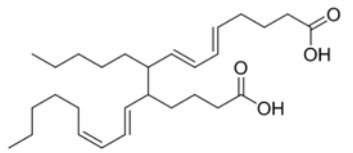


(formula I).

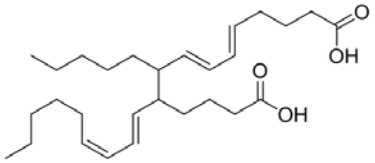
In another example, the antibody may be a monoclonal or a polyclonal antibody.
In another example, there is provided herein a use of the compound or compounds defined above as an antigen for preparing an antibody which specifically binds to an antigenic epitope of the compound. In another aspect, the 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:



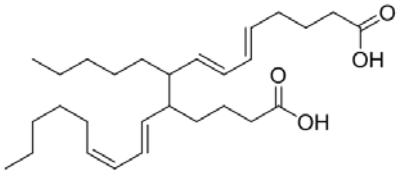
(formula I).
In another aspect, the present disclosure refers to use of the antibody as disclosed herein for detecting or quantifying a level of a gastric tract acid (GTA) in a sample by immunoassay, wherein the GTA is:



(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:



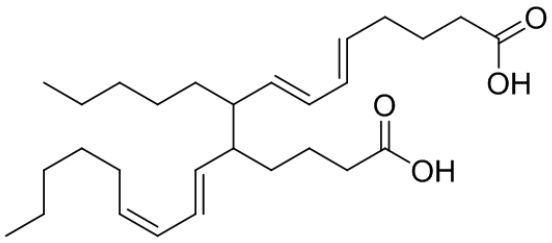
(formula I).
In another aspect, the present disclosure refers to use of the antibody as disclosed herein in a diagnostic method for identifying a subject as having, or being at risk of developing, a colorectal cancer associated with altered levels of a gastric tract acid (GTA) which is:



(formula I).
In another aspect, 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 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.

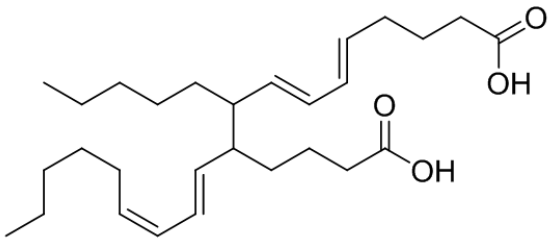
In another aspect, the present disclosure refers to a diagnostic method for identifying a subject as having, or being at risk of developing, colorectal cancer, said method comprising:

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 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, 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:

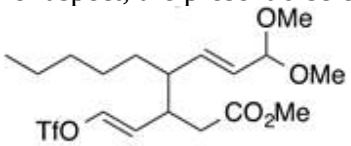
a compound or compounds as defined above;
a metabolic tracer as defined above;
a composition or compositions as defined above;
a diagnostic agent as defined above; and
an antibody or antibodies as defined above;
and, optionally, further comprising a set of instructions for performing a method or methods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

In another aspect, 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.

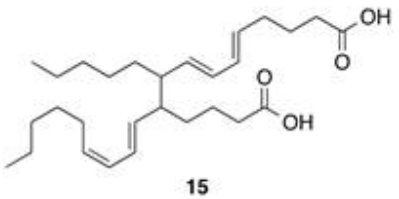
In another aspect, the present disclosure refers to a compound having the formula:



24

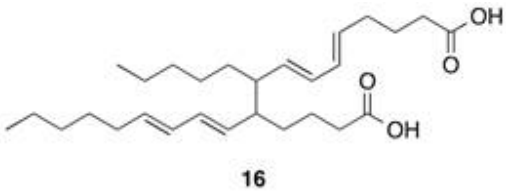
, or a labelled derivative thereof.

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



15

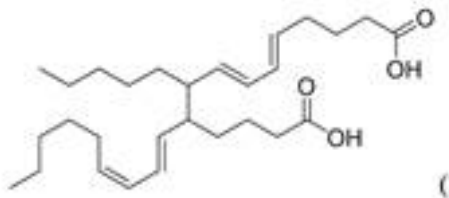
or



16

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

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

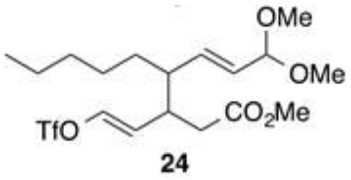


(formula I),

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

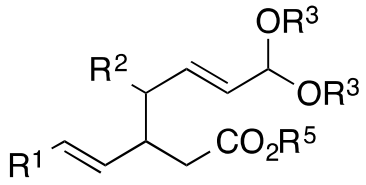
performing a Wittig reaction to yield the compound of formula I, or an isotopically labelled derivative thereof,

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.

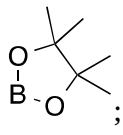
In a further example, the Wittig reaction may comprise reaction with (triphenylphosphoranylidene) acetaldehyde or (4-carboxybutyl)triphenylphosphonium bromide.

In yet 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;

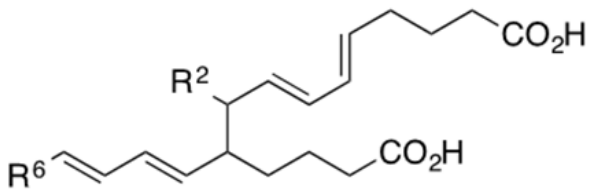
R^5 is optionally substituted C_1-C_6 alkyl; and

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

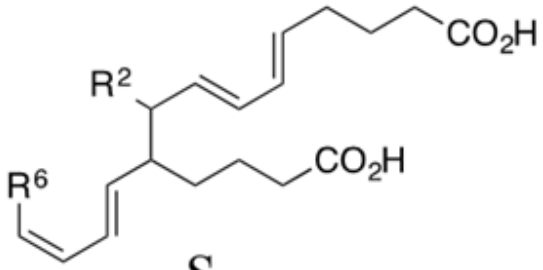
or a labelled derivative thereof.

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, 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, 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 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;