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[Continued on next page]

(54) Title: SUBSTITUTED FULLERENES AS MRI CONTRAST AGENTS

(57) Abstract: A contrast agent for enhancing contrast in in vivo magnetic resonance imaging, comprising a water-soluble, non-zero spin isotope-enriched substituted fullerene having a fullerene core or heterofullerene core and at least one substituent group bonded to at least one carbon of the fullerene core. A method of magnetic resonance imaging a sample, comprising (i) administering to the sample a plurality of substituted fullerenes; and (ii) detecting magnetic resonance signals from the sample. In the method, some or all of the plurality of substituted fullerenes may be non-zero spin isotope-enriched, but need not be. In the method, some or all of the plurality of substituted fullerenes may be hyperpolarized, but need not be.

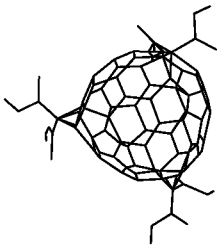


FIG. 1A

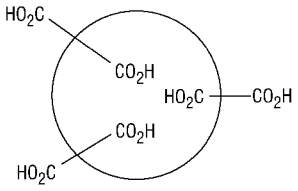


FIG. 1B

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OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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SUBSTITUTED FULLERENES AS MRI CONTRAST AGENTS

BACKGROUND OF THE INVENTION

The present invention relates generally to the field of magnetic resonance imaging (MRI). In particular, the present invention relates to a method of dynamic nuclear polarization with substituted fullerenes as (MRI) contrast agents.

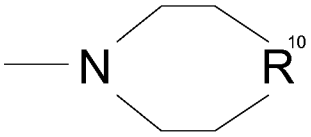
SUMMARY OF THE INVENTION

In one embodiment, the present invention relates to a contrast agent for enhancing contrast in magnetic resonance imaging, comprising a water-soluble, non-zero spin isotope-enriched substituted fullerene having a fullerene core or heterofullerene core and at least one substituent group bonded to at least one carbon of the fullerene core, wherein the substituent group is selected from the group consisting of (i) m ($>CX^1X^2$) groups bonded to the fullerene core, wherein: (i-a) m is an integer from 1 to 6, inclusive, (i-b) each X^1 and X^2 is independently selected from –H; -COOH; -$CONH_2$; -CONHR'; -$CONR'_2$; -COOR'; -CHO; -$(CH_2)_dOR^{11}$; a peptidyl moiety; -R; -RCOOH; -$RCONH_2$; -RCONHR'; -$RCONR'_2$; -RCOOR'; -RCHO; -$R(CH_2)_dOR^{11}$; a heterocyclic moiety; a branched moiety comprising one or more terminal -OH, -NH_2, triazole, tetrazole, or sugar groups; or a salt thereof, wherein each R is a hydrocarbon moiety having from 1 to about 6 carbon atoms and each R' is independently a hydrocarbon moiety having from 1 to about 6 carbon atoms, an aryl-containing moiety having from 6 to about 18 carbon atoms, a hydrocarbon moiety having from 1 to about 6 carbon atoms and a terminal carboxylic acid or alcohol, or an aryl-containing moiety having from 6 to about 18 carbon atoms and a terminal carboxylic acid or alcohol, and d is an integer from 0 to about 20, and each R^{11} is independently –H, a charged moiety, or a polar moiety;

(ii) p -X^3 groups bonded to the fullerene core, wherein (ii-a) p is an integer from 1 to 18, inclusive; and (ii-b) each -X^3 is independently selected from:

-$N^+(R^2)(R^3)(R^4)$, wherein R^2, R^3, and R^4 are independently -H or -$(CH_2)_d$-CH_3, wherein d is an integer from 0 to about 20;

$-N^{+}(R^{2})(R^{3})(R^{8})$, wherein R^{2} and R^{3} are independently -H or $-(CH_2)_d-CH_3$, wherein d is an integer from 0 to about 20, and each R^{8} is independently $-(CH_2)_f-SO_3^{-}$, $-(CH_2)_f-PO_4^{-}$, or $-(CH_2)_f-COO^{-}$, wherein f is an integer from 1 to about 20;



, wherein each R^{10} is independently >O, $>C(R^{2})(R^{3})$, $>CHN^{+}(R^{2})(R^{3})(R^{4})$, or $>CHN^{+}(R^{2})(R^{3})(R^{8})$;

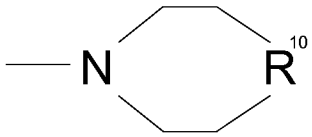
$-C(R^{5})(R^{6})(R^{7})$, wherein R^{5}, R^{6}, and R^{7} are independently -COOH, -H, -CH(=O), $-CH_2OH$, or a peptidyl moiety;

$-C(R^{2})(R^{3})(R^{8})$,

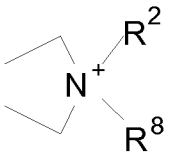
$-(CH_2)_e-COOH$, wherein e is an integer from 1 to about 6

$-(CH_2)_e-CONH_2$, or

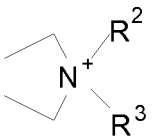
$-(CH_2)_e-COOR'$;

wherein when the $-X^{3}$ group is selected from , the substituted fullerene can further comprise from 1 to 6 >O groups;

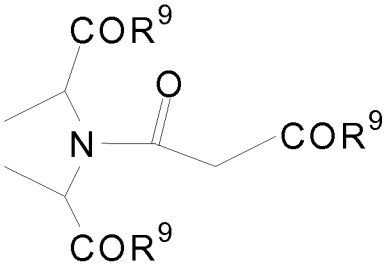
(iii) q $-X^{4}-$ groups bonded to the fullerene core, wherein (iii-a) q is an integer from 1 to 6, inclusive; and (iii-b) each $-X^{4}-$ group is independently:



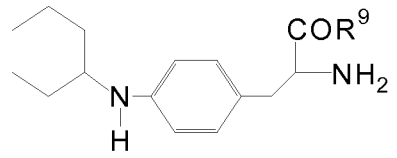
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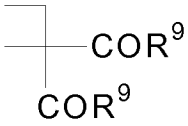
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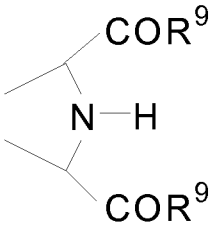
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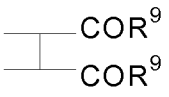
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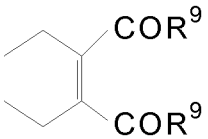
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; and

(iv), r dendrons bonded to the fullerene core and s nondendrons bonded to the fullerene core, wherein: (iv-a) r is an integer from 1 to 6, inclusive; (iv-b) s is an integer from 0 to 18, inclusive; (iv-b) each dendron has at least one protic group which imparts water solubility, and (iv-d) each nondendron independently comprises at least one drug, amino acid, peptide, nucleotide, vitamin, or organic moiety.

In a further embodiment, the present invention relates to a contrast agent as described above, and further comprising a sterile carrier.

In one embodiment, the present invention relates to a method of magnetic resonance imaging a tissue or organ of a mammal, comprising (i) administering to the mammal a plurality of non-zero spin isotope-enriched substituted fullerenes; and (ii) detecting magnetic resonance signals from the tissue or organ of the mammal.

In another embodiment, the present invention relates to a method of magnetic resonance imaging a tissue or organ of a mammal, comprising (i) administering to the mammal a plurality of substituted fullerenes; and (ii) detecting magnetic resonance signals from the tissue or organ of the mammal.

Any method of the present invention may further comprise hyperpolarizing a plurality of the substituted fullerenes, to yield a plurality of hyperpolarized substituted fullerenes.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

Figure 1A shows an exemplary substituted fullerene in structural formula, and Figure 1B shows the same substituted fullerene in a schematic formula.

Figure 2 shows the decarboxylation of C3 to C3-penta-acid and thence to C3-tetra-acid.

Figure 3 shows the decarboxylation of C3-tetra-acid to C3-tris-acid.

Figure 4 shows the chirality of C3.

Figure 5 shows the effect of C3 chirality on isomers formed by decarboxylation.

Figure 6 shows exemplary substituted fullerenes according to one embodiment of the present invention.

Figure 7A and 7B show two exemplary substituted fullerenes.

Figures 8A-8G show seven exemplary dendrofullerenes.

Figure 9 shows dendrofullerene DF-1.

Figures 10A-10H show various substituted fullerenes, including DF-1 Mini (Figure 10F, ref. no. **1212**).

Figure 11 shows a ^{13}C spectrum of a fullerene sample acquired with 1 scan at 4.7T MR scanner, B_1=50 kHz, and HHLW=5 Hz.

Figure 12 shows inversion on the NMR resonance with variable inversion delay.

Figure 13 shows a ^{13}C spectrum of unlabelled, hyperpolarized PW75.

Figure 14 shows a ^{13}C spectrum of unlabelled, hyperpolarized dendrimer (the derivatizing reagent minus fullerene).

Figure 15 shows a ^{13}C spectrum of unlabelled, hyperpolarized, derivatized fullerene PW75. The inset shows an expanded ^{13}C spectrum of the hyperpolarized derivatized fullerene (PW75), in which individual assignments were made.

Figure 16 shows the structure of FB03.

Figures 17-18 show the solid-state polarization of FB03 and PW75.

Figure 19 shows the structure of PW75.

Figure 20 shows the different carbon types in the fullerene-dendrimer complex (PW75).

Figure 21: (A) Single scan hyperpolarized ^{13}C NMR spectrum of ^{13}C PW75. This single shot spectrum has over 10,000 fold sensitivity enhanced over conventional ^{13}C NMR spectrum. The general assignment is based on the peak positions of the different functional groups (see inset,B) in this dendrimer-fullerene complex.

Figure 22: Symmetry splitting in ^{13}C PW75 by the dendrimer chain, leads to formation of multiplets in fullerene resonances. Shown here is the aromatic region of the hyperpolarized ^{13}C NMR spectrum of ^{13}C PW75 demonstrating the resonances from the fullerene aromatic carbons and the quaternary carbon of the fullerene-dendrimer junction.

Figure 23: Three dimensional structure of fullerene (1) and two distinct structural components of fullerene; sumanene (2) and corannulene (3).

Figure 24 shows the ^{13}C NMR spectrum of fullerene.

Figure 25 shows the ^{13}C NMR spectrum of PW75.

Figure 26 shows the ^{13}C NMR spectrum of dendrimer.

Figure 27 shows the ^{13}C NMR spectrum of C_{60}.

Figure 28 shows the ^{13}C NMR spectrum of C_{70}.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

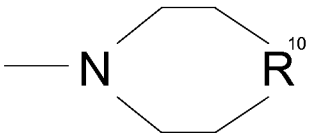
The particulars shown herein are by way of example and for the purposes of illustrative discussion of the embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the present invention. In this regard, no attempt is made to show structural and experimental details of the present invention in more detail than is necessary for the fundamental understanding of the present invention, the description taken with the drawings making apparent to those skilled in the art how several forms of the present invention may be embodied in practice.

In one embodiment, the present invention relates to a contrast agent for enhancing contrast in magnetic resonance imaging, comprising a water-soluble, non-zero spin isotope-enriched substituted fullerene having a fullerene core or heterofullerene core and at least one substituent group bonded to at least one carbon of the fullerene core, wherein the substituent group is selected from the group consisting of (i) m ($>CX^1X^2$) groups bonded to the fullerene core, wherein: (i-a) m is an integer from 1 to 6, inclusive, (i-b) each X^1 and X^2 is independently selected from –H; -COOH; $-CONH_2$; -CONHR'; $-CONR'_2$; -COOR'; -CHO; $-(CH_2)_dOR^{11}$; a peptidyl moiety; -R; -RCOOH; $-RCONH_2$; -RCONHR'; $-RCONR'_2$; -RCOOR'; -RCHO; $-R(CH_2)_dOR^{11}$; a heterocyclic moiety; a branched moiety comprising one or more terminal -OH, $-NH_2$, triazole, tetrazole, or sugar groups; or a salt thereof, wherein each R is a hydrocarbon moiety having from 1 to about 6 carbon atoms and each R' is independently a hydrocarbon moiety having from 1 to about 6 carbon atoms, an aryl-containing moiety having from 6 to about 18 carbon atoms, a hydrocarbon moiety having from 1 to about 6 carbon atoms and a terminal carboxylic acid or alcohol, or an aryl-containing moiety having from 6 to about 18 carbon atoms and a terminal carboxylic acid or alcohol, and d is an integer from 0 to about 20, and each R^{11} is independently –H, a charged moiety, or a polar moiety;

(ii) p $-X^3$ groups bonded to the fullerene core, wherein (ii-a) p is an integer from 1 to 18, inclusive; and (ii-b) each $-X^3$ is independently selected from:

$-N^+(R^2)(R^3)(R^4)$, wherein R^2, R^3, and R^4 are independently -H or $-(CH_2)_d-CH_3$, wherein d is an integer from 0 to about 20;

$-N^+(R^2)(R^3)(R^8)$, wherein R^2 and R^3 are independently -H or $-(CH_2)_d-CH_3$, wherein d is an integer from 0 to about 20, and each R^8 is independently $-(CH_2)_f-SO_3^-$, $-(CH_2)_f-PO_4^-$, or $-(CH_2)_f-COO^-$, wherein f is an integer from 1 to about 20;



, wherein each R^{10} is independently >O, $>C(R^2)(R^3)$, $>CHN^+(R^2)(R^3)(R^4)$, or $>CHN^+(R^2)(R^3)(R^8)$;

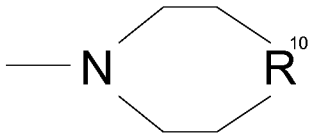
$-C(R^5)(R^6)(R^7)$, wherein R^5, R^6, and R^7 are independently -COOH, -H, -CH(=O), $-CH_2OH$, or a peptidyl moiety;

$-C(R^2)(R^3)(R^8)$,

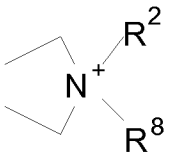
$-(CH_2)_e-COOH$, wherein e is an integer from 1 to about 6

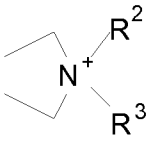
$-(CH_2)_e-CONH_2$, or

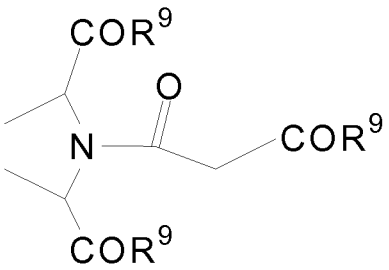
$-(CH_2)_e-COOR'$;

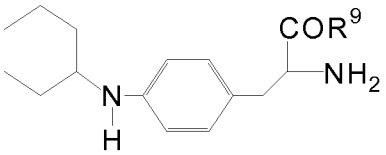
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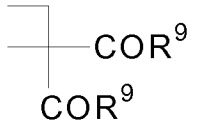
(iii) q $-X^4-$ groups bonded to the fullerene core, wherein (iii-a) q is an integer from 1 to 6, inclusive; and (iii-b) each $-X^4-$ group is independently:

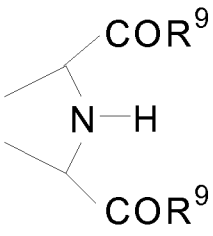
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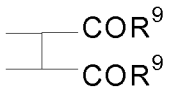
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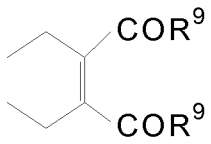
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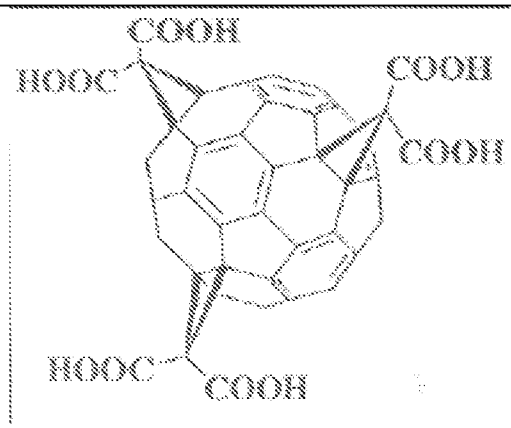
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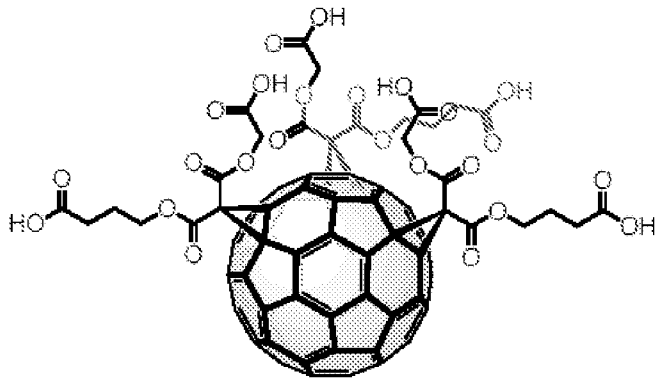
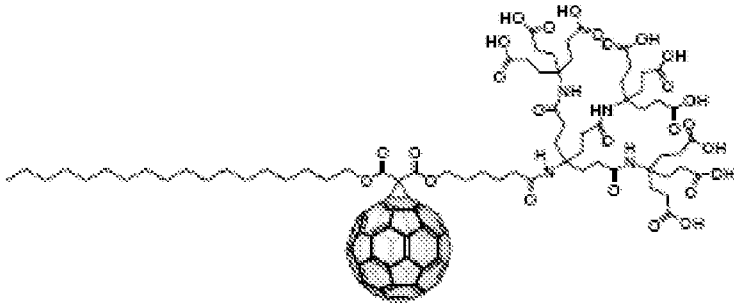
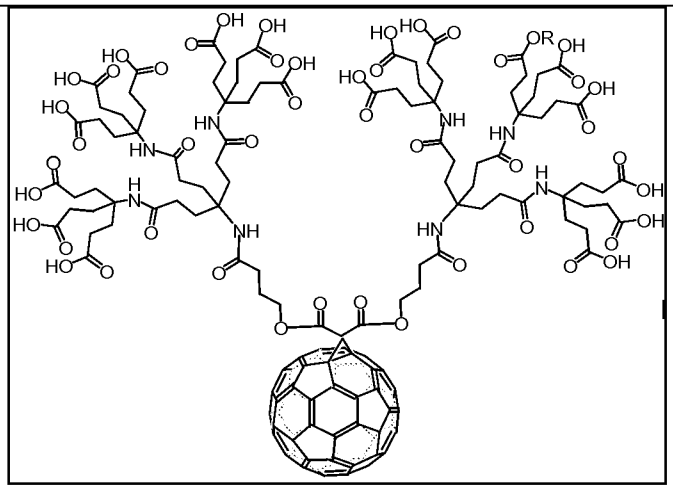
(iv), r dendrons bonded to the fullerene core and s nondendrons bonded to the fullerene core, wherein: (iv-a) r is an integer from 1 to 6, inclusive; (iv-b) s is an integer from 0 to 18, inclusive; (iv-b) each dendron has at least one protic group which imparts water solubility, and (iv-d) each nondendron independently comprises at least one drug, amino acid, peptide, nucleotide, vitamin, or organic moiety.

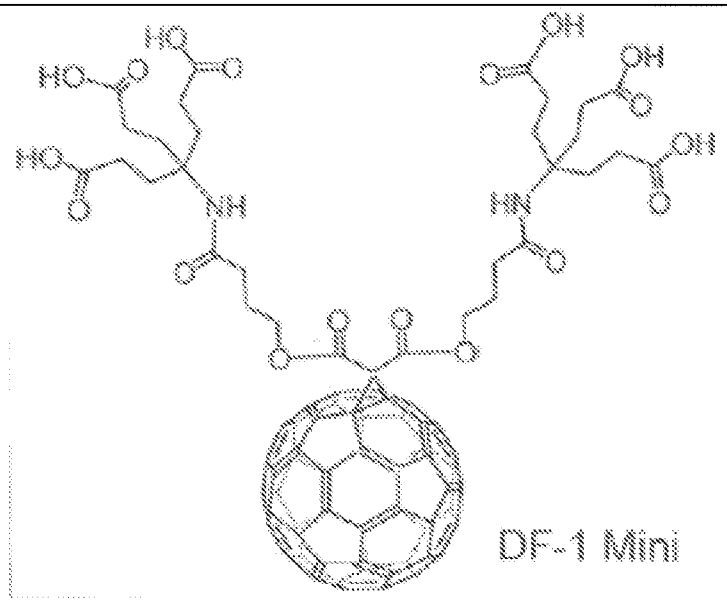
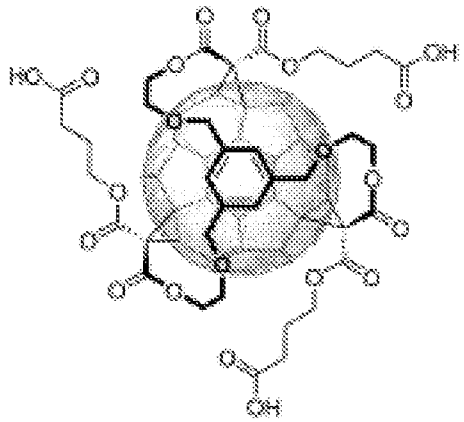
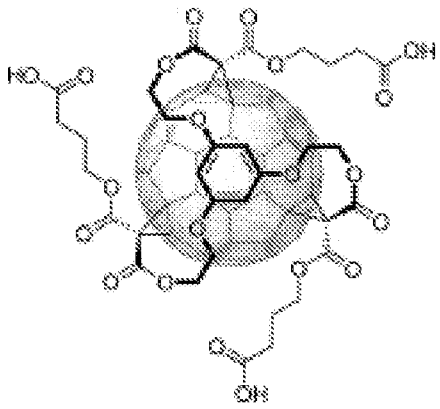
In one embodiment, the fullerene core or heterofullerene core is selected from the group consisting of C_{60}, C_{70}, and $C_{59}N$.

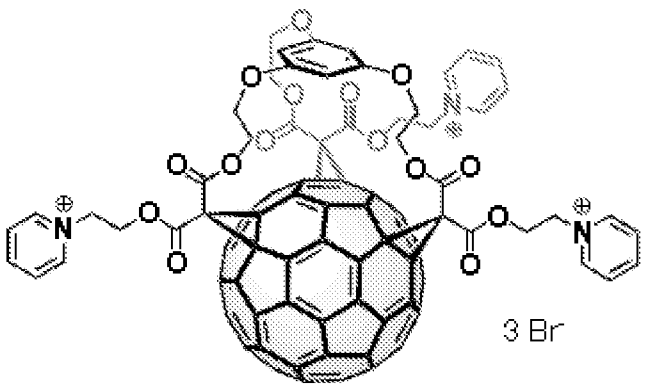
In a further embodiment, the substituted fullerene is selected from the group consisting of C3, FB115, PW75, DF-1, DF-1 Mini, FB02, FB03, FB10, cationic forms of the foregoing, anionic forms of the foregoing, metabolites and breakdown products of the foregoing, and mixtures thereof.

The structures of these substituted fullerenes are as follows:

C3	

FB115	
PW75	 PW 75
DF-1	

DF-1 Mini	
FB02	
FB03	

FB10	

In an even further embodiment, the substituted fullerene further comprises a functional moiety. The functional moiety can allow the substituted fullerene to have a different property or ability, such as preferentially association with a particular cell or tissue after administration of the substituted fullerene to a patient.

In one embodiment, the functional moiety is a targeting group, by which is meant a group that binds to a specific compound, and thus allows the substituted fullerene to be associated with the specific compound. In a further embodiment, the targeting group is biotin or a biotin-containing moiety, *i.e.*, a moiety which will bind to avidin or streptavidin.

In one embodiment, the functional moiety is docosahexaenoic acid (DHA).

In one embodiment, the functional moiety is polyethylene glycol (PEG).

In one embodiment, the functional moiety is selected from the group consisting of biotin, docosahexaenoic acid (DHA), polyethylene glycol (PEG), antibodies against amyloid plaque, antibodies against tau protein, antibodies against atherosclerotic plaque, antibodies against tumor antigens, antibodies against inflammatory cell antigens, antibodies against immune cell antigens, antibodies that bind to growth factor receptors, peptides that bind to amyloid plaque, peptides that bind to tau protein, peptides that bind to atherosclerotic plaque, peptides that bind to tumor antigens, peptides that bind to inflammatory cell antigens, peptides that bind to growth factor receptors, and peptides that bind to immune cell antigens.

Specifically, "antibody" herein refers to a moiety comprising an antigen-binding site. An “antigen,” as used herein, is a chemical compound or a portion of a chemical compound which can be recognized by a specific chemical reaction, a specific physical reaction, or both with another molecule. The antigen-recognition site of an antibody is an exemplary, but non-limiting, antigen-binding site. Examples of moieties comprising antigen-binding sites

include, but are not limited to, monoclonal antibodies, polyclonal antibodies, Fab fragments of monoclonal antibodies, Fab fragments of polyclonal antibodies, Fab_2 fragments of monoclonal antibodies, and Fab_2 fragments of polyclonal antibodies, among others. Single chain or multiple chain antigen-recognition sites can be used. Multiple chain antigen-recognition sites can be fused, joined by a linker, or unfused and unlinked.

The antibody can be selected from any known class of antibodies. Known classes of antibodies include, but are not necessarily limited to, IgG, IgM, IgA, IgD, and IgE. The various classes also can have subclasses. For example, known subclasses of the IgG class include, but are not necessarily limited to, IgG1, IgG2, IgG3, and IgG4. Other classes have subclasses that are routinely known by one of ordinary skill in the art.

The antibody can be derived from any species. "Derived from," in this context, can mean either prepared and extracted *in vivo* from an individual member of a species, or prepared by known biotechnological techniques from a nucleic acid molecule encoding, in whole or part, an antibody peptide comprising invariant regions which are substantially identical to antibodies prepared *in vivo* from an individual member of the species or an antibody peptide recognized by antisera specifically raised against antibodies from the species. Exemplary species include, but are not limited to, human, chimpanzee, baboon, other primate, mouse, rat, goat, sheep, and rabbit, among others known in the art. In one embodiment, the antibody is chimeric, *i.e.*, comprises a plurality of portions, wherein each portion is derived from a different species. A chimeric antibody, wherein one of the portions is derived from human, can be considered a humanized antibody.

Antibodies are available that recognize antigens associated with a wide variety of cell types, tissues, and organs, and a wide variety of medical conditions, in a wide variety of mammalian species. Exemplary medical conditions include, but are not limited to, cancers, such as lung cancer, oral cancer, skin cancer, stomach cancer, colon cancer, nervous system cancer, leukemia, breast cancer, cervical cancer, prostate cancer, and testicular cancer; arthritis; infections, such as bacterial, viral, fungal, or other microbial infections; and disorders of the skin, the eye, the vascular system, or other cell types, tissues, or organs; among others.

Exemplary antibodies include, but are not limited to, those derived from antibodies against vascular endothelial growth factor receptor (VEGF-r) (available from Imclone, New York, NY), antibodies against epidermal growth factor receptor (EGF-r) (available from

Abgenix, Fremont, CA), antibodies against polypeptides associated with lung cancers (available from Corixa Corporation, Seattle, WA), and antibodies against human tumor necrosis factor alpha (hTNF-α) (available from BASF A.G., Ludwigshafen, Germany), among others known in the art.

Antibodies can be prepared by various techniques known in the art. These techniques include, but are not limited to, the immunological technique described by Kohler and Milstein in Nature 256, 495-497 (1975) and Campbell in "Monoclonal Antibody Technology, The Production and Characterization of Rodent and Human Hybridomas" in Burdon et al., Eds., Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13, Elsevier Science Publishers, Amsterdam (1985); as well as by the recombinant DNA techniques described by Huse et al in Science 246, 1275-1281 (1989); among other techniques known to one of ordinary skill in the art.

In one embodiment, the antibody is derived from murine ZME-018, which recognizes the gp240 antigen present on more than 80% of melanoma biopsies and cell lines. The gp240 antigen can also be recognized by antibodies derived from SCFVMEL, an SCFV antibody; dSCFVMEL, a diabody antibody; and GD2, a chimeric antibody. In another embodiment, the antibody is derived from HuM195, a humanized antibody which recognizes CD-33, an antigen present on AMC and CML cells in the hematopoeic system. In a different embodiment, the antibody is derived from herceptin, a chimeric antibody which recognizes the HER2 antigen associated with certain breast, colon, and lung tumors. The HER2 antigen can also be recognized by Antibodies derived from the BACH 250 chimeric antibody, the ML 3-9 SCFV antibody, or the C 6.5 diabody antibody. In another embodiment, the antibody is derived from αMMPA, a chimeric antibody which recognizes the MMP9 antigen associated with certain lung tumors. In a different embodiment, the antibody is derived from Campath 1H, an antibody which recognizes the CD-52 antigen associated with leukemias. In a further embodiment, the antibody is derived from anti-TNF-r1, an antibody which recognizes the TNF-r1 antigen associated with leukemias. In yet a different embodiment, the antibody is derived from anti-CD-38, an antibody which recognizes the CD-38 antigen associated with leukemias. In still a further embodiment, the antibody is derived from Bexxar, an antibody which recognizes the CD-20 antigen associated with leukemias. In still an additional embodiment, the antibody is derived from VEGF121 or SuperGen, antibodies which recognize the VEGF Receptor 2 antigen associated with solid tumors.

In addition to the listed antibodies, the antibody can be constructed to recognize a target antigen associated with a solid tumor. For example, the antibody can be constructed to recognize HER2/neu, MUC-1, HMFG1, or EGFr, associated with breast tumors; MMP-9, HER2/neu, or NCAM, associated with lung tumors; HER2 or 171A, associated with colon tumors; gp240, gangliosides, or integrins, associated with melanomas; HER2 or CA-125, associated with ovarian tumors; or EGFr or tenascin, associated with brain tumors. This list of target antigens and tumor types is exemplary and not limiting.

In addition to the antigen-binding site, the antibody can comprise a linker. The linker can be any moiety covalently bound to the portion of the antibody containing the antigen-binding site and capable of associating with the substituted fullerene. In one embodiment, the association involves a specific physical interaction between the linker and the substituted fullerene. For example, the linker can be an antibody raised against the substituted fullerene to be used in the composition. In another embodiment, the association can be covalent. A covalent linker can be formed by, for example, (i) substituting the substituted fullerene with a sulfhydryl-containing (-SH) substituent; (ii) preparing an antibody with a sulfhydryl-containing linker; and (iii) reacting the antibody and the substituted fullerene to form a -S-S- bond between the antibody and the substituted fullerene. In another embodiment, the association can be non-covalent. Exemplary non-covalent associations include ionic and van der Waals associations.

In addition to the antibodies described above, any other compound that can recognize a specific antigen can be used as the antibody described herein. Such other compounds include antibody fragments and certain synthetic peptides that are known or are discovered to recognize specific antigens. Such other compounds can further comprise linkers, as described above.

Other than antibodies or antibody fragments, peptides that bind certain proteins or antigens can also be used. For example, a peptide comprising the substrate-binding domain of the serine/threonine kinase PKN can bind to tau protein, a protein in which pathologies can lead to neuronal degradation. Other peptides that bind certain proteins or antigens are known to the person of ordinary skill in the art.

Contrast agents have played an important role in medical imaging procedures to enhance the image contrast in images of a subject, using for example X-ray, magnetic resonance and ultrasound imaging. The resulting enhanced contrast enables different organs,

tissue types or body compartments to be more clearly observed or identified. In X-ray imaging the contrast agents function by modifying the X-ray absorption characteristics of the body sites in which they distribute. Commonly used magnetic resonance contrast agents generally function by modifying the density or the characteristic relaxation times, generally of water protons, from the resonance signals of which the images are generated. And, ultrasound contrast agents function by modifying the speed of sound or the density in the body sites into which they distribute.

While the phenomenon of nuclear magnetic resonance (hereinafter NMR) was discovered in 1945, it is only relatively recently that it has found application as a means of mapping the internal structure of the body as a result of the original suggestion of Lauterbur (Nature, 242, 190-191 (1973)). In 2003, Lauterbur and Mansfield received the Nobel Prize in physiology or medicine for their contributions to developing magnetic resonance imaging (hereinafter MRI) as a technique for 3-D imaging. MRI is a very powerful imaging tool that produces results analogous to X-ray images, but is advantageously non-invasive as it avoids the use of exposing the patient under study to harmful radiation. The fundamental lack of any known hazard associated with the level of the magnetic and radio-frequency (hereinafter RF) fields that are employed renders it possible to make repeated scans on vulnerable individuals. MRI encompasses the detection of certain atomic nuclei (those possessing magnetic dipole moments) utilizing magnetic fields and radio-frequency radiation. It is similar in some respects to X-ray computed tomography ("CT") in providing a cross-sectional display of the body organ anatomy with excellent resolution of soft tissue detail.

MRI works by exciting the molecules of a target object using a harmless pulse of RF energy to excite NMR active nuclei that have first been aligned using a strong external magnetic field and then measuring the nuclei's rate of return to an equilibrium state within the magnetic field following termination of the RF pulse. These nuclei, as they relax, subsequently emit RF energy at a sharp resonance frequency which depends on the applied magnetic field. The decay of the emitted radiation is characterized by two relaxation times, T_1 and T_2. T_1 is the spin-lattice relaxation time or longitudinal relaxation time, i.e., the time taken by the nuclei to return to equilibrium along the direction of the externally applied magnetic field. T_2 is the spin-spin relaxation time associated with the dephasing of the initially coherent precession of individual proton spins. These relaxation times have been established for various fluids, organs, and tissues in different species of mammals. For

protons and other suitable nuclei, these relaxation times are influenced by the environment of the nuclei (e.g., viscosity, temperature, and the like). These two relaxation phenomena are essentially mechanisms whereby the initially imparted RF energy is dissipated to the surrounding environment. Hence, the signal that is generated contains information on nuclear spin density, T_1 and T_2. The visually readable magnetic resonance images that are generated as output are the result of complex computer data reconstruction on the basis of this information.

Because successful imaging depends on the ability of the computer to recognize and differentiate between different types of tissue, it is routine to apply a contrast agent to the tissue prior to making the image. The contrast agent alters the response of the aligned protons or other NMR active nuclei to the RF signal. Good contrast agents interact differently with different types of tissue, with the result that the effect of the contrast agent is greater on certain body parts, thus making them easier to differentiate and image. The most common contrast agents involve the hydrogen atom, which has a nucleus consisting of a single unpaired proton, and therefore has the strongest magnetic dipole moment of any nucleus. Since hydrogen occurs in both water and lipids, it is abundant in the human body. Therefore, MRI is most commonly used to produce images based upon the distribution density of protons and/or the relaxation times of protons in organs and tissues.

Other atomic nuclei having a net magnetic dipole moment also exhibit a nuclear magnetic resonance (NMR) phenomenon which may be used in MRI applications. Such nuclei include ^{13}C (six protons and seven neutrons), ^{19}F (9 protons and 10 neutrons), ^{23}Na (11 protons and 12 neutrons), ^{15}N (7 protons and 8 neutrons), and ^{31}P (15 protons and 16 neutrons), etc.

Additionally, paramagnetic transition metal ions, metal complexes and chelates are NMR active and can be used in MRI. The use of paramagnetic metal ions, such as Mn (II), as contrast agents in MRI was first proposed by Lauterbur et al. in 1978. Since that time, a wide range of paramagnetic metal ion chelate complexes have been proposed. Metal ions that are reasonably stable and possess the highest magnetic moment, such as Mn2+, Fe3+, and Gd3+, are the most commonly employed, but any paramagnetic transition metal ion may also be suitable. More recently, the use of superparamagnetic particles as MRI contrast agents has been described in U.S. Pat. No. 4,863,715.

While metal ion contrast agents are often used in MRI, they are not suitable for all imaging applications. For example, they are not particularly useful in certain body areas such as the gastrointestinal (GI) tract. In addition, these contrast agents can be toxic and chemically reactive in vivo. Hence, the majority of contrast agent research has focused on developing non-toxic, stable chelates for binding these metal ions. Attempts have been made to achieve tissue-specific MRI contrast enhancement, to decrease toxicity, or to enhance stability and/or relaxivity by coupling of the paramagnetic chelates, or metal complexing groups, to various macromolecules or biomolecules such as polysaccharides, proteins, antibodies or liposomes. However, these metal chelates have not adequately solved the needs for non-toxic contrast agents for effective in vivo imaging.

Discovered in 1985, fullerenes are clusters of carbon with an even number of atoms forming cage-like structures. Because of the pattern formed by the linked carbon atoms, closed cage fullerenes have been given the informal name "buckyballs." The structures were named after Buckminster Fuller, the designer of the geodesic dome.

Fullerenes are notable for their hollow polyhedral shape and their stability. The most intensively studied such carbon molecule in this class is the C_{60} carbon cluster buckminsterfullerene in which all sixty atoms are equivalent and lie at the apices of a truncated icosahedron--the perfect soccer ball shape. C_{60} and its discovery are described extensively in the literature--see for example Kroto et al., Nature 318: 162 (1985); Kroto, Science 242: 1139 (1988); Curl and Smalley, Science 242: 1017-1022 (1988); Kroto, Pure and Applied Chem. 62: 407-415 (1990). Many other fullerenes having stable closed cage structures have been described, e.g., C_{28}, C_{32}, C_{50}, C_{70} (the most predominant after C_{60}), C_{82}, and the so-called "giant" fullerenes C_{240}, C_{540}, and C_{960} (see, e.g., Kroto (1990), supra).

Nuclear magnetic resonance (NMR) active (I=1/2) substituted fullerenes, such as substituted C_{60}, substituted C_{70}, and substituted heterofullerenes, such as substituted $C_{59}N$, can be used as magnetic resonance (MRI) contrast agents. By MRI agent it is meant an agent containing nuclei (MR imaging nuclei) capable of emitting magnetic resonance signals. Generally, such nuclei will be protons, such as water protons; however other non-zero nuclear spin nuclei ("non-zero spin isotopes") may be useful (e.g. ^{13}C nuclei, ^{19}F nuclei, ^{15}N nuclei, and ^{31}P nuclei) and in this event the MR signals from which the image is generated will be substantially only from the MR imaging agent (positive signal). Isotopically enriched ^{13}C fullerene contrast agents will typically have a stronger NMR signal compared to naturally

occurring fullerenes because, without the enrichment, the NMR signal is weak since the natural abundance of ^{13}C is only 1.1% and ^{13}C has a smaller gyromagnetic ratio, γ, than that of a proton (~1/4), leading to an inherently weaker NMR signal than the proton signal.

Procedures for producing fullerenes in macroscopic (multigram) quantities using electric-arc graphite decomposition are now well known and published in the literature (see Kratschmer et al., Nature 347-354 (1990); Kosh et al., J. Org. Chem. 56: 4543-4545 (1991); Scrivens et al. JACS 114:7917-7919 (1992); and Bhyrappa et al. JCS Chem. Comm. 936-937 (1992)), which are all herein incorporated by reference. ^{13}C-enriched fullerenes (or fullerene mixtures consisting primarily of C_{60} and C_{70}) are also commercially available (e.g., MER Corp., Tucson, Ariz.; Texas Fullerene Corp., Aldrich, Tex.; Strem Chemicals, Newburyport, Mass., etc.).

If enrichment of non-zero spin isotopes is desired, the fullerene contrast agents and DNP free radical sources can be prepared by enriching the ^{13}C abundance of the starting material by using any method well known to those in the art, including the electric-arc graphite decomposition method, to produce the fullerenes. A known method described by Holleman et al, Chem. Phys. Lett. 240:165-171 (1995) involves doping or sintering of ^{13}C-enriched graphite rods in a DC-arc-discharge procedure to create isotopically enriched ^{13}C fullerenes.

In another embodiment, the ^{13}C abundance of the fullerene contrast agent can be increased by incorporating ^{13}C into a substituent group of the substituted fullerene.

In another embodiment, the ^{15}N abundance of the fullerene contrast agent can be increased by incorporating ^{15}N into either a heterofullerene or a substituent group of a substituted fullerene.

Various embodiments for enrichment can be combined.

Concerning NMR spectra, the four-line infrared spectrum for C_{60}, as reported by Kratschmer et al. (Kratschmer & Lamb, 1990), supported the proposed truncated icosahedron structure. In addition, the ^{13}C NMR spectrum of the purified C_{60}, reported by Kroto et al. (Taylor, 1990). The NMR spectrum contained a single peak at δ142.7, as expected for the highly symmetrical truncated icosahedron structure in which all carbons are identical. This result eliminated planar graphite fragments and fullerenes of lower symmetry as possible structures for C_{60}. A sixty-membered polyalkyne ring would also be expected to exhibit one

^{13}C NMR signal but the observed chemical shift position (δ142.7) was inconsistent with this possibility. (Alkyne carbons generally resonate between δ50 and δ100.)

The ^{13}C NMR spectrum of purified C_{70} was also reported by Kroto and contained five peaks. The football-shaped C_{70} fullerene possesses five sets of inequivalent carbon atoms in a ratio of 10:10:20:20:10. This is precisely the ratio of the line intensities observed in the ^{13}C NMR spectrum.

There are major benefits in using non-zero spin isotope-enriched fullerenes for *in vitro* or *in vivo* MRI studies. Some of these advantages are analogous to the fullerene based X-ray contrast agents disclosed by Wilson et al. in U.S. Pat. No. 6,660,248, incorporated herein by reference. However, another significant benefit is that because non-zero spin isotope-enriched fullerenes are inherently magnetic, they do not require the presence of internal paramagnetic ions or external linkage to paramagnetic metal ions chelates or other type of magnetic targeting groups to achieve their relaxation ability. Besides, these compounds do not require the measurement of water proton relaxation measurements (negative signal) because the ^{13}C relaxation is directly measured (positive signal), allowing greater sensitivity and flexibility in MRI studies.

Moreover, the high symmetry of these compounds provides additional sensitivity by generation of a single frequency response from most of the ^{13}C atoms in the fullerene structure, which all have the same chemical shift for the C_{60}, C_{70} molecules etc. Other advantages include the biological compatibility, low toxicity, signal amplification through increased ^{13}C count, and long ^{13}C T_1 relaxation time for extended in vivo imaging studies.

More particularly, however, recent biological studies have shown that water solubilized fullerene molecules possess unique biodistributions, and therefore they may particularly represent novel utility and advantage as blood pool imaging agents for measuring blood flow and perfusion. The non-zero spin isotope-enriched fullerene may be carried to a desired tissue in the body as a result of its particular substituent groups. Additionally, fullerene-based agents can be targeted to specific tissues by appending tissue-targeting entities (i.e., small peptides or antibodies) to the fullerene core. See, Wilson et al. supra.

The pseudo-spherical shape is of special importance because agents with a reduced viscosity are produced, which increases the ease of injection into the body. Additionally, because C_{60}-based agents are larger than conventional contrast agents, such as iohexol, the diffusion rate through various tissues is slower. As mentioned above, this qualifies fullerene-

based contrast agents as a blood pool contrast agent. Further, another advantage for blood pool imaging and angiography studies is that fullerenes can be adjusted by the size needed, unlike small molecule based contrast agents. Therefore, this class of non-zero spin isotope-enriched fullerenes is substantially different from previously studied fullerene-derived MRI contrast agents and represent a unique class of MRI relaxation compounds.

However, fullerenes are not water soluble because of the hydrophobic carbon shell. Since fullerenes exhibit extended aromaticity, chemical modification of the fullerene structure is necessary to prepare compositions suitable for *in vivo* applications. In order to operate effectively within a living body, the paramagnetic fullerene shell can be rendered water-soluble by an appropriate derivation process. This can be performed by derivatizing the fullerene shell with targeting groups to impart water solubility and/or attaching the fullerene shell to a larger water-soluble molecule. The choice of functionalization method is extremely important for obtaining the desired bio-distribution, elimination pathways, or to reduce the toxicity of the compound. Some examples and potential uses of fullerenes in biology are given by Jenson et al. (1994). Several reactions for making fullerenes water soluble are described by Hirsch (1994) in his recent review of fullerene chemistry review. Suitable methods for solubilization include attachment of one or more carboxylic acid groups is conveniently performed through addition of malonic acid groups to a fullerene, as disclosed by Hirsch, et al., US 6,538,153; Hirsch, et al., US-2006-0047167-A1; and Bingel, US 5,739,376. The above patents and applications are hereby incorporated by reference.

However, the present invention is not limited to the literature methods and could include use of various other techniques without departing from the scope of the present invention.

Throughout this description, particular embodiments described herein may be described in terms of a particular acid, amide, ester, or salt conformation, but the skilled artisan will understand an embodiment can change among these and other conformations depending on the pH and other conditions of manufacture, storage, and use. All such conformations are within the scope of the appended claims. The conformational change between, *e.g.*, an acid and a salt is a routine change, whereas a structural change, such as the decarboxylation of an acid moiety to -H, is not a routine change.

A substituted fullerene can exist in one or more isomers. All structural formulas shown herein are not to be construed as limiting the structure to any particular isomer.

All possible isomers of the substituted fullerenes disclosed herein are within the scope of the present disclosure. For example, in $>CX^1X^2$, one group (X^1 or X^2) of each substituent points away from the fullerene core, and the other group points toward the fullerene core. Continuing the example, the central carbon of each substituent group (by which is meant the carbon with two bonds to the fullerene core, one bond to X^1, and one bond to X^2) is chiral when X^1 and X^2 are different.

It will also be apparent that substituted fullerenes having two or more substituent groups will have isomers resulting from the different possible sites of bonding of the substituent groups to the fullerene core.

In one embodiment, the substituted fullerene is a decarboxylation product of ($C_{60}(>C(COOH)_2)_3$) (C3). By "decarboxylation product of C3" is meant the product of a reaction wherein 0 or 1 carboxy (-COOH) groups are removed from each of the three malonate moieties ($>C(COOH)_2$) of C3 and replaced with -H, provided at least one of the malonate moieties has 1 carboxy group replaced with -H. This can be considered as the loss of CO_2 from a malonate moiety. Decarboxylation can be performed by heating C3 in acid, among other techniques.

During decarboxylation of C3, only CO_2 loss from C3 is observed; each malonate moiety retains at least one carboxyl; and the decarboxylation stops at loss of 3 CO_2 groups, one from each malonate moiety of C3. The skilled artisan having the benefit of the present disclosure will recognize that substituted fullerenes having 1, 2, 4, 5, or 6 malonate moieties would also undergo decarboxylation.

In C3, each malonate moiety has a carboxy group pointing to the outside (away from the fullerene), which we herein term *exo*, and a carboxy group pointing to the inside (toward the fullerene), which we herein term *endo*. Figure 1A presents a structural formula of C3.

Figure 2 shows C3 (in box **30**) and the products of subsequent loss via decarboxylation of one or two CO_2 groups, giving C3-penta-acids (in box **32**) and C3-tetra-acids (in box **34**). Decarboxylation is represented by the open arrows **31** and **33**; the isomers of C3, C3-penta-acid, and C3-tetra-acid are shown in box **30**, in box **32**, and in box **34**, respectively.

In the interest of precise nomenclature, we define the order of *exo* or *endo* by always naming the groups in a clockwise manner.

Figure 3 shows the products of subsequent loss via decarboxylation of a third CO_2 group from the C3-tetra-acids shown in box **34**, giving C3-tris-acids (box **42**). Decarboxylation is represented by the open arrow **41**; the isomers of C3-tetra-acid and C3-penta-acid are shown in box **34** and in box **42**, respectively. Isomers that differ only by rotation are connected by dashed lines **43**, **44**, and **45**.

Figure 4 shows the chirality of C3, both in a structural formula (mirror images **50a** and **50b**) and a schematic representation (mirror images **52a** and **52b**). Figure 5 shows the chirality of C3-penta-acids (mirror images **60a** and **60b;** mirror images **62a** and **62b**).

In another embodiment, the substituted fullerene comprises one of the structures **72**, **74**, **76**, **77**, or **78** shown in Figure 6.

In one embodiment, the substituted fullerene comprises C_{60} and 3 ($>CX^1X^2$) groups in the C3 orientation (e.g., the orientation of the substituents shown in structural formula **50a** in Fig. 4) or the D3 orientation (e.g., the orientation of the substituents shown in structural formula **50b** in Fig. 4). The D3 orientation is a mirror translation of the C3 orientation (e.g., structural formula **50b** in Fig. 4). The above description of C3-penta-acids, C3-tetra-acids, and C3-tris-acids also applies to D3 orientations of penta acids, tetra acids, and tris acids.

In one embodiment, as shown in Figure 10, the substituted fullerene comprises C_{60} and 2 ($>CX^1X^2$) groups in the trans-2 orientation **1206**, the trans-3 orientation **1207**, the e orientation **1208**, or the cis-2 orientation **1209**.

In another embodiment, also as shown in Figure 10, the substituted fullerene comprises C_{70} and 2 ($>CX^1X^2$) groups in the bis orientation **1210** or **1211**.

In another embodiment, the substituted fullerene has the structure shown in Figure 7B.

By adding one or more carboxylic acid groups, the substituted fullerene can be rendered water-soluble. In one embodiment, the substituted fullerene has a solubility in water of at least 1 mM. In a further embodiment, the substituted fullerene has a solubility in water of at least 50 mM.

Desirably, the contrast agent has a ^{13}C isotope abundance greater than its natural abundance. In one embodiment, the contrast agent has a ^{13}C isotope abundance of greater than about 1.1%. In a further embodiment, the contrast agent has a ^{13}C isotope abundance of greater than about 10%. In yet a further embodiment, the contrast agent has a ^{13}C isotope abundance of greater than about 30%.

The particular substituted fullerenes of at least some embodiments of the present invention possess a number of properties that make them uniquely and unpredictably suitable for use as contrast agents relative to the entire constellation of substituted fullerenes known in the art. One property is that at least some substituted fullerenes have much lower toxicity than many other substituted fullerenes. Another property is that at least some substituted fullerenes have much higher water solubility than many other substituted fullerenes. Still another property is that at least some substituted fullerenes have much higher lipid solubility than many other substituted fullerenes. Yet another property is that at least some substituted fullerenes have much higher CNS accessibility (i.e., can more readily cross the blood brain barrier) than many other substituted fullerenes. A further property is that at least some substituted fullerenes have much higher oral availability than many other substituted fullerenes. Still a further property is that at least some substituted fullerenes are much more amenable to precise stereolocalization of single or multiple targeting groups than many other substituted fullerenes, and as a result, their MRI signals can be more readily identified. Another property is that at least some substituted fullerenes tend to be deprotonated at physiological pH, and as a result, may be especially readily detectable in certain bodily environments.

Specifically regarding CNS accessibility, we have found that the substituted fullerene C3 readily crosses the blood brain barrier. Therefore, C3 can be used as a contrast agent for magnetic resonance imaging of central nervous system structures.

In addition to the substituted fullerene, the contrast agent may also comprise a sterile carrier. The carrier can be any aqueous solution in which the fullerene is soluble. In one embodiment, the aqueous solution is saline. The carrier can be sterilized by any appropriate technique known to the person of ordinary skill in the art.

In one embodiment, the present invention relates to a method of magnetic resonance imaging a sample, comprising:

(i) administering to the sample a plurality of substituted fullerenes; and

(ii) detecting magnetic resonance signals from the sample.

In one embodiment, the sample can be a tissue or organ of a mammal, such as a human being, and the method performed *in vivo*. In another embodiment, the sample can be cells or other material from a plant or animal donor and the method performed *in vitro*. In

one embodiment, the substituted fullerenes can be enriched in a paramagnetic isotope, such as ^{13}C or ^{15}N, among others.

Turning to particular embodiments of the method, in one embodiment, the present invention relates to a method of magnetic resonance imaging a tissue or organ of a mammal, comprising:

(i) administering to the mammal a plurality of non-zero spin isotope-enriched substituted fullerenes; and

(ii) detecting magnetic resonance signals from the tissue or organ of the mammal.

In a further embodiment, the water soluble fullerenes are enhanced for imaging studies using the method of dynamic nuclear polarization (DNP). The ^{13}C signal of a fullerene, even an isotopically enriched fullerene, produces an inherently weak signal in direct ^{13}C spectroscopy and imaging. However, the sensitivity of the MRI signal is enhanced several fold (about 10^3) by using DNP, also called the "Overhauser effect." This technique has been described in complete detail by Ardenkjaer-Larson et al, US 6466814, and the main embodiments of the technique are highlighted in this disclosure. In this method, the enhancement arises from the enhanced polarization of nuclear spins due to the transfer of the larger electron spin polarization through microwave radiation at or near the electron paramagentic resonance frequency. Thus, *ex-vivo* polarization can be achieved by using a polarizing paramagnetic species such as $MnCl_2$ (Mn^{2+}), $FeCl_3$ (Fe3+) or organic radicals or hyperpolarizable noble gases such as ^3He and ^{129}Xe (OMRI agents) in the vicinity of the fullerenes.

In one embodiment, the water soluble non-zero spin isotope-enriched fullerenes are enhanced for imaging studies using the method of Parahydrogen and Synthesis Allow Dramatically Enhanced Nuclear Alignment (PASADENA). PASADENA has been described by Chekmenev, *et al.*, "PASADENA Hyperpolarization of Succinic Acid for MRI and NMR Spectroscopy," *J. Am. Chem. Soc.* (2008 Mar 12).

In one embodiment, the substituted fullerene is enriched in ^{13}C. In one embodiment, the substituted fullerene contains at least one ^{13}C per molecule (e.g. 1/60, or ~1.7%). This embodiment also includes molecules with 2 ^{13}C per molecule (e.g. 2/60, or ~3.3%), 3 ^{13}C per molecule e.g. 3/60, or ~5.0%), 4 ^{13}C per molecule e.g. 4/60, or ~6.6%), 5 ^{13}C per molecule (e.g. 5/60, or ~8.3%), 6 ^{13}C per molecule (e.g. 6/60, or ~10.0%), 7 ^{13}C per molecule (e.g. 7/60, or ~11.6%), 8 ^{13}C per molecule (e.g. 8/60, or ~13.3%), 9 ^{13}C per molecule (e.g. 9/60, or

~15%), and/or 10 ^{13}C per molecule (e.g. 10/60, or ~16.6%). This embodiment also includes molecules with from about 1-10 ^{13}C per molecule, 10-20 ^{13}C per molecule, 20-30 ^{13}C per molecule, 30-40 ^{13}C per molecule, 40-50 ^{13}C per molecule, and/or 50-60 ^{13}C per molecule, and/or increments therein.

The technique of *ex vivo* DNP is suited to fullerenes because it has a long T_1 relaxation time (carbon-spin lattice relation time, which is held to range from ~2-100 s depending upon the temperature and viscosity). Thus, owing to this high T_1 relaxation time, once the fullerene is polarized, it will remain so for a sufficiently long time to allow the imaging procedure to be carried out in a fairly comfortable time span.

In one embodiment, any method of the present invention can further comprise hyperpolarizing the plurality of substituted fullerenes, to yield a plurality of hyperpolarized substituted fullerenes, prior to the administering step.

Hyperpolarization may be carried out by one of four possible mechanisms: (1) the Overhauser effect, also known as DNP, (2) the solid effect, (3) the thermal mixing effect (see A. Abragam and M. Goldman, Nuclear Magnetism: Order and Disorder, Oxford University Press, 1982), and (4) PASADENA, as described above. By hyperpolarization, it is meant that the sample is polarized to a level over that found at room temperature and 1 T, such as polarized to a polarization degree in excess of 0.1%, for example in excess of 1%, for further example in excess of 10%. The Overhauser effect is one useful technique. It is envisaged that, in one embodiment, the level of polarization achieved should be sufficient to allow the hyperpolarized solution of the fullerenes to achieve a diagnostically effective contrast enhancement in the sample to which it is subsequently administered in whatever form. In general, it is desirable to achieve a level of polarization which is at least a factor of 2 or more above the field in which MRI is performed, such as a factor of 10 or more, for example a factor of 100 or more and for further example a factor of 1000 or more, e.g. 50,000.

In another embodiment, hyperpolarization of the MR imaging nuclei is effected by a DNP free radical source. In this embodiment, step (i) of the method comprises: (a) bringing an DNP free radical source and the fullerene into contact in a uniform magnetic field (the primary magnetic field B_o); (b) exposing said DNP free radical source to a first radiation of a frequency selected to excite electron spin transitions in said DNP free radical source; and (c) dissolving in a physiologically tolerable solvent said fullerenes. In one embodiment, the DNP free radical source and fullerene are present as a composition during polarization.

For the purposes of administration, the fullerenes can be administered in the absence of the whole of, or substantially the whole of, the DNP free radical source. In one embodiment, at least 80% of the DNP free radical source is removed, at least 85% of the DNP free radical source is removed, such as 90% or more, for example 95% or more, for further example 99% or more. In general, it is desirable to remove as much DNP free radical source as possible prior to administration to improve physiological tolerability and to increase T_1. The DNP free radical source for use in the method can be one which can be conveniently and rapidly separated from the polarized fullerene MR imaging agent using known techniques.

However, where the DNP free radical source is non-toxic, the separation step may be omitted. A solid (e.g. frozen) composition comprising a DNP free radical source and fullerene agent which has been subjected to polarization may be rapidly dissolved in saline (e.g. warm saline) and the mixture injected shortly thereafter.

Unless the hyperpolarized agent is stored (and/or transported) at low temperature and in an applied field as described above, since imaging is desirably carried out within the time that the hyperpolarized solution of the fullerene agent remains significantly polarized, it is desirable for administration of the polarized fullerene MRI agent to be effected rapidly and for the MR measurement to follow shortly thereafter.

It is envisaged that in one embodiment, use may be made of any known DNP free radical source capable of polarizing a fullerene agent to an extent such that a diagnostically effective contrast enhancement, in the sample to which the fullerene agent is administered, is achieved.

In a particular embodiment, paramagnetic metal complexes are used. For example, these metal ions are chromium (III), manganese (II), manganese (III), iron (III), praseodymium (III), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), terbium (III), dysprosium (III), holmium (III), or erbium (III).

Where the DNP free radical source is a paramagnetic free radical, the radical may be conveniently prepared in situ from a stable radical precursor by a conventional physical or chemical radical generation step shortly before polarization, or alternatively by the use of ionizing radiation. This is particularly important where the radical has a short half-life. In these cases, the radical will normally be non-reusable and may conveniently be discarded

once the separation step has been completed. Exemplary paramagnetic free radicals include TRITYL radical and TEMPO radical.

In one embodiment, a chosen DNP free radical source will exhibit a long half-life (such as at least one hour), long relaxation times (T_{1e} and T_{2e}), high relaxivity, and a small number of ESR transition lines. Thus the paramagnetic oxygen-based, sulphur-based or carbon-based organic free radicals or magnetic particles referred to in WO-A-88/10419, WO-A-90/00904, WO-A-91/12024, WO-A-93/02711 or WO-A-96/39367 would also be suitable DNP free radical source.

In another embodiment, DNP free radical sources include hyperpolarizable gases. By hyperpolarizable gas, it is meant a gas with a nonzero spin angular momentum capable of undergoing an electron transition to an excited electron state and thereafter of decaying back to the ground state. Depending on the transition that is optically pumped and the helicity of the light a positive or negative spin hyperpolarisation may be achieved (up to 100%). Examples of gases suitable for use include the noble gases He (e.g., ^{3}He or ^{4}He), Ne, Ar, Kr and Xe (e.g. ^{129}Xe), such as He, Ne or Xe, particularly He, more particularly ^{3}He. Alkali metal vapors may also be used, e.g., Na, K, Rb, or Cs vapors. Mixtures of the gases may also be used. In one embodiment, the hyperolarizable gas may be used in liquid form.

In the separation step, it is desirable to remove substantially the whole of the DNP free radical source from the composition (or at least to reduce it to physiologically tolerable levels) as rapidly as possible. Many physical and chemical separation or extraction techniques are known in the art and may be employed to effect rapid and efficient separation of the DNP free radical source and fullerene agent. In one embodiment, the separation technique is one which can be performed rapidly, such as in less than one second. In this respect, magnetic particles (e.g., superparamagnetic particles) may be advantageously used as the DNP free radical source as it will be possible to make use of the inherent magnetic properties of the particles to achieve rapid separation by known techniques. Similarly, where the DNP free radical source or the particle is bound to a solid bead, it may be conveniently separated from the liquid (i.e., if the solid bead is magnetic by an appropriately applied magnetic field).

For ease of separation of the DNP free radical source and the fullerene agent, in one embodiment the combination of the two is a heterogeneous system, e.g., a two phase liquid, a solid in liquid suspension or a relatively high surface area solid substrate within a liquid, e.g.,

a solid in the form of beads, fibers or sheets disposed within a liquid phase fullerene agent. In all cases, the diffusion distance between the fullerene agent and DNP free radical source must be small enough to achieve an effective Overhauser enhancement. Certain DNP free radical source are inherently particulate in nature, e.g., the paramagnetic particles and superparamagnetic agents referred to above. Others may be immobilized on, absorbed in or coupled to a solid substrate or support (e.g., an organic polymer or inorganic matrix such as a zeolite or a silicon material) by conventional means. Strong covalent binding between DNP free radical source and solid substrate or support will, in general, limit the effectiveness of the agent in achieving the desired Overhauser effect and so it is desirable that the binding, if any, between the DNP free radical source and the solid support or substrate is weak so that the DNP free radical source is still capable of free rotation. The DNP free radical source may be bound to a water insoluble substrate/support prior to the polarization or the DNP free radical source may be attached/bound to the substrate/support after polarization. The DNP free radical source may then be separated from the fullerene agent, e.g., by filtration before administration. The DNP free radical source may also be bound to a water soluble macromolecule and the DNP free radical source-macromolecule may be separated from the fullerene agent before administration.

Where the combination of an DNP free radical source and fullerene agent is a heterogeneous system, it will be possible to use the different physical properties of the phases to carry out separation by conventional techniques. For example, where one phase is aqueous and the other non-aqueous (solid or liquid) it may be possible to simply decant one phase from the other. Alternatively, where the DNP free radical source is a solid or solid substrate (e.g., a bead) suspended in a liquid fullerene agent the solid may be separated from the liquid by conventional means, e.g., filtration, gravimetric, chromatographic or centrifugal means. It is also envisaged that the DNP free radical source may comprise lipophilic moieties and so be separated from the fullerene by passage over or through a fixed lipophilic medium or the DNP free radical source may be chemically bound to a lipophilic solid bead. The fullerene agent may also be in a solid (e.g., frozen) state during polarization and in close contact with a solid DNP free radical source. After polarization it may be dissolved in heated water or saline or melted and removed or separated from the DNP free radical source where the latter may be toxic and cannot be administered.

In one embodiment, the administration route for the polarized MRI agent is parenteral, e.g., by bolus injection, by intravenous, intraarterial or peroral injection. The injection time should be equivalent to 5 T_1 or less, such as 3 T_1 or less, for example T_1 or less, and for another example 0.1 T_1 or less. The lungs may be imaged by spray, e.g., by aerosol spray. Parenteral compositions may be injected directly or mixed with a large volume parenteral composition for systemic administration. Formulations for enteral administration may vary widely, as is well-known in the art. In general, such formulations include a diagnostically effective amount of the carbon cluster derivatives. Such enteral compositions may optionally include buffers, surfactants, thixotropic agents, and the like. Compositions for oral administration may also contain flavoring agents and other ingredients for enhancing their organoleptic qualities.

The diagnostic compositions are administered in doses effective to achieve the desired enhancement of the NMR image. Such doses may vary widely, depending upon the percentage of ^{13}C enrichment, the organs or tissues which are the subject of the imaging procedure, the NMR imaging equipment being used, etc. The diagnostic compositions are used in a conventional manner in magnetic resonance procedures. Compositions may be administered in a sufficient amount to provide adequate visualization, to a warm-blooded mammal either systemically or locally to an organ or tissues to be imaged, and the mammal then subjected to the MRI procedure. The compositions enhance the magnetic resonance images obtained by these procedures.

Another embodiment encompasses any method that would polarize the free radical agents described herein over thermal equilibrium (e.g., storing the compound at low temperature and high field).

In another embodiment, the general protocol comprises polarizing and solublizing the molecule in a magnet, where the radical is filtered out, and a quality control (temperature, pH, polarization) is made quickly followed by intravascular injection.

In another embodiment, the present invention relates to a method of magnetic resonance imaging a tissue or organ of a mammal, comprising:

(i) administering to the mammal a plurality of substituted fullerenes; and

(ii) detecting magnetic resonance signals from the tissue or organ of the mammal.

This method is substantially the same as the method described above, but differs in that the substituted fullerenes are not non-zero spin isotope-enriched. They may have a ^{13}C content equal to or less than the natural abundance of the ^{13}C isotope. The substituted fullerenes may, but need not, be hyperpolarized as described above.

The following examples are included to demonstrate particular embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1

Study of longitudinal relaxation times (T_1) of ^{13}C on 25% labeled fullerene

Figure 11 shows a ^{13}C spectrum of a fullerene sample acquired with 1 scan at 4.7T MR scanner, B_1=50 kHz, and HHLW=5 Hz.

T_1 for the ~144 ppm resonance was determined by inversion recovery method, and T_1= 65±10 seconds. The spectra shown in Figure 12 illustrate the inversion on the NMR resonance with variable inversion delay.

Example 2

Hyperpolarization studies with DNP apparatus

Both ^{13}C enriched native fullerene and a dendrimer derivatized fullerene (PW75) were subjected to hyperpolarization using HyperSense DNP (Oxford Biotools, Oxford, UK). Hyperpolarization data (Figure 13) showed that unlabelled PW75 can be readily hyperpolarized and the hyperpolarized signal was long lasting because of the relatively long T_1 times which can be utilized for biomedical imaging for over 5 minutes (5 times T_1, corresponding to about 325 seconds).

Hyperpolarized ^{13}C fullerene provided a unique, single shot ^{13}C NMR spectrum using HyperSense DNP (Figure 13). This clearly showed two peaks indicating that all fullerene carbons have the same chemical shift and that the expected contamination from C_{70} always

present in C_{60} is readily observable (but only after hyperpolarization). Expanded C_{70} hyperpolarized spectra is shown in the inset.

Unlabelled dendrimer (the derivatizing reagent minus fullerene) yielded a fine hyperpolarized signal, easily assigned to known portions of the chain and fullerene binding site (Figure 14).

Unlabelled, derivatized fullerene PW75 was readily hyperpolarized. The spectrum was distinct from that of the dendrimer itself (Figure 15). Key features of this reagent include readily assigned carbon resonances of all carbons of the molecule. It appears the carbonyl at the junction with fullerene was hyperpolarized. It appears that the carbons in the free 'tail' of the dendrimer can also be hyperpolarized. In an expanded ^{13}C spectrum of the hyperpolarized derivatized fullerene (PW75), individual assignments can be made (Figure 15, inset).

Conclusions

A: Fullerenes are readily hyperpolarizable.

B: Derivatized fullerenes (PW75) can be hyperpolarized in aqueous medium.

C: Single scan NMR spectra can be acquired readily from ^{13}C labeled and unlabelled fullerene derivatives. In fact, ^{13}C enrichment of fullerenes is not necessary to their detection and analysis by DNP (this can potentially save time and money).

D: Finger-printing of derivatized fullerenes is possible from the analysis of their single shot DNP hyperpolarized ^{13}C spectra.

E: T_1 relaxation times of fullerenes are more than 1 minute.

F: Hyperpolarized signal from fullerene will last over 5 x T_1 => 5 minutes, enabling real-time ^{13}C MRI, MRA or spectroscopy *in vivo*. Dendrimer-derivatized fullerene (PW75) is expected to be suitable as an *in vivo* hyperpolarized ^{13}C contrast agent.

Example 3

Modeling and hyperpolarization studies with non-derivatized unlabelled and ^{13}C labelled fullerenes to determine their potential. Also, initial hyperpolarization of derivatized fullerenes was attempted, followed by a longer program of *in vitro* and *in vivo* validation.

Modeling: Longitudinal relaxation times (T_1) of more than 1 minute in both fullerene derivatives, FB03 and PW75, are suitable for a long-lived contrast reagent. The structure of FB03 is shown in Figure 16.

Hyperpolarization can be achieved at three separate positions of the PW75/FB03 molecular moieties; all of which may provide sound options for real time ultrafast spectroscopy and imaging these molecules in biomedical systems.

On the fullerene end: The longitudinal relaxation times (T_1) of ^{13}C on fullerene are on the order of minutes due to lack of availability of the relaxation pathways in fullerene systems. Hyperpolarizing fullerene ^{13}C nuclei provided an effective imaging and spectroscopic time window of over 5 minutes (5 x T_1).

On the fullerene-dendrimer junction: The carbonyl carbons sitting on the top of fullerene sphere are outstanding target points of hyperpolarization given that these two points can be isotopically labelled by ^{13}C. The longitudinal relaxation times (T_1) of ^{13}C nuclei here are on the order of 50 seconds.

On the dendrimer terminus: There are numerous carbonyl carbons present in the loose end of the dendrimer moiety. Any of these carbonyl carbons can be isotopically labeled and are excellent targets for hyperpolarization. Because of through space interactions in these carbonyl functionalities, the estimated longitudinal relaxation times (T_1) of ^{13}C here are on the order of 40 seconds.

Conclusion: 1. All fullerene carbons possess long T_1's suitable for MR imaging and spectroscopy.

We expect strategic placement of double bond adjacent to a labeled carbonyl carbon in the dendrimer chain would make these molecules amenable for PASADENA hyperpolarization, as well.

Hyperpolarization studies with Oxford Biotools, HyperSense DNP apparatus: Comparison between PW75 and FB03

Hyperpolarized NMR spectroscopy provided a unique, single shot ^{13}C NMR spectrum using HyperSense DNP.

Unlabelled, derivatized fullerene PW75 and FB03 were readily hyperpolarized. The spectra were distinct from that of the dendrimer itself. The carbon resonances of all carbons

of the fullerene were readily assigned in both cases from a single shot hyperpolarized spectra. Furthermore, the carbonyl at the junction with fullerene can be hyperpolarized as well as the carbons in the free 'tail' of the dendrimer. The spectrum of FB03 was better resolved and possibly easier to interpret owing to the presence of C3 axis of symmetry in this molecule. Also, FB03 was polarized much more readily in solid state as compared to PW75. (Figures 17-18).

Conclusions (Hyperpolarization studies):

A: Single scan NMR spectra can be acquired readily from unlabelled fullerene derivatives. In fact, ^{13}C enrichment of fullerenes is not necessary to their detection and analysis by DNP.

B: Finger-printing of derivatized fullerenes is possible from the analysis of their single shot DNP hyperpolarized ^{13}C spectra.

C: FB03 is polarized in a shorter time and yields better resolved and interpretable ^{13}C NMR spectra than PW75.

D: Hyperpolarized signal from fullerene will last over 5 x T_1 => 5 minutes, enabling real-time ^{13}C MRI, MRA or spectroscopy *in vivo*. Both PW75 and FB03 are expected to be suitable as an *in vivo* hyperpolarized ^{13}C contrast agents.

Example 4

The hyperpolarization potential of ^{13}C labeled PW75 was experimentally verified and the hyperpolarized data were analyzed. The longitudinal relaxation time (T_1) was measured and the window of time to perform imaging and spectroscopy experiments with this agent was estimated.

Theory and Modeling of ^{13}C PW75:

To get a comprehensive understanding of the hyperpolarization of ^{13}C PW75, it is imperative to understand the types of carbons nuclei in this molecule. There are four types of carbons on this molecule of interest. (Figure 20)

Pure aliphatic carbon chain (>CH2); Carbonyl carbons (>C=O) as well as aliphatic carbons (>CH2 & quaternary C) on the dendrimeric chain; Aromatic carbons from fullerene

moiety; and Carbonyl as well as the quaternary carbon at the dendrimer and fullerene junction

Hyperpolarization can only be achieved at three separate positions of the ^{13}C PW75 molecular moiety; all of allow real time ultrafast spectroscopy and imaging these molecules in biomedical systems.

On the fullerene end: The longitudinal relaxation times (T_1) of ^{13}C on fullerene are very long in the order of minutes due to lack of availability of the relaxation pathways in fullerene systems. Hyperpolarizing fullerene ^{13}C nuclei will provide an effective imaging and spectroscopic time window of over 5 minutes (5 x T_1).

On the fullerene-dendrimer junction: The two carbonyl carbons sitting on the top of fullerene sphere are outstanding target points of hyperpolarization given that these two points can be isotopically labeled by ^{13}C. The longitudinal relaxation times (T_1) of ^{13}C nuclei here is on the order of 50 seconds.

On the dendrimer terminus: There are numerous carbonyl carbons present in the loose end of the dendrimer moiety. Any of these carbonyl carbons can be isotopically labeled and are excellent targets for hyperpolarization. Because of through space interactions in these carbonyl functionalities, the estimated longitudinal relaxation times (T_1) of ^{13}C here is on the order of 40 seconds. Furthermore, the quaternary carbons may also be hyperpolarized because these too have relatively long relaxation times.

Hyperpolarization studies with Oxford Instruments, DNP HyperSense polarizer:

A study built on the earlier findings indicated that ^{13}C enriched native fullerene and a dendrimer derivatized fullerene were readily hyperpolarized by Dynamic Nuclear Polarization. .

Preliminary hyperpolarization data showed that ^{13}C PW75 can be readily hyperpolarized within hours by Dynamic Nuclear Polarization in aqueous solution. The enhanced signal from hyperpolarization is very long lasting (>5 minutes), desirable for *in vivo* trials.

2 mg of the molecule was weighed out for DNP trial using FINLAND radical initiator. A point to note here that the molecule is only sparingly soluble in water at a physiological pH of 7.4 (less than 1 mg was dissolved in 400 microliters of purified water and pH adjusted). The exact concentration of ^{13}C PW75 in the final solution was not determined. Unlike unlabeled PW75 which takes over 10 hr to build up polarization, the growth of solid-state polarization of ^{13}C PW75 in the DNP matrix was rapid and >5% polarization was achieved in 90 minutes. Due to time constraint at the polarizer, the dissolution process commenced after 90 minutes of polarization and a single shot hyperpolarized ^{13}C NMR spectrum was acquired on a JEOL 300MHz NMR spectrometer. The T_1 of the fullerene signal was determined to be 75 ± 10 seconds and the hyperpolarized signal was long lasting because of the relatively long T_1 times which can be utilized for biomedical imaging for over 6 minutes (5 X T_1 corresponding to about 385 seconds or over 6 minutes).

The assignment of the single scan hyperpolarized spectrum is given in Figure 21. All types of carbon nuclei were visible in this enhanced single shot ^{13}C NMR spectrum of ^{13}C PW75. The aliphatic >CH2 carbons resonances are around 50 ppm. As observed in the earlier studies with unlabeled PW75 and DF1, the ^{13}C singlet of the C60 fullerene at ~142 ppm underwent peak splitting into at least 16 different resonances between 140-145 ppm (Figure 22). Though not to be bound by theory, we submit this is because the symmetry of the C60 moiety is broken by substitution by the dendrimer chain. A point to emphasize here is that the symmetry breakage is local and does not extend to the entire fullerene moiety but only up to the first "sumanene" type fragment (Figure 23) of the C60 moiety. All the fullerene resonances can be potentially assigned and fingerprinting of different fullerene derivatives can be achieved with single scan ^{13}C NMR spectrum of their aromatic region with hyperpolarization. The quaternary carbons of the dendrimer which are attached to the electronegative nitrogen (N) were shifted upfield around 70 ppm. The carbons of the fullerene-dendrimer junction (one quaternary and two attached with the fullerene) have quasi-aromatic character and are shifted even more upfield, possibly appearing between 125-130 ppm. Note that there are three distinct resonances, corresponding to the strained cyclopropene ring. Though not to be bound by theory, we believe the peak at 125 ppm corresponded to the quaternary carbon of the junction. All the carbonyl carbons from the

dendrimer as well as from the fullerene-dendrimer junction resonated around 190 ppm. The two types of carbonyl carbons were not distinguishable in the hyperpolarized spectrum.

Comparison with the hyperpolarized spectrum of unlabeled PW75:

Comparison with the unlabeled spectrum of PW75 revealed some interesting findings. It took much longer to hyperpolarize the unlabeled type (>10 hrs) as compared to 90 mins in ^{13}C PW75 via Dynamic Nuclear Polarization. Furthermore, the signal enhancement was considerably greater in ^{13}C PW75 because of the isotopic labeling; as a result a significantly smaller concentration was needed to achieve a high quality reproducible ^{13}C NMR spectrum by hyperpolarization. This is desirable because a lower concentration of ^{13}C PW75 (in the range of 10 mM) would be expected to have a reduced risk of any biochemical toxicity.

Conclusions:

A: ^{13}C PW75 can be readily hyperpolarized in aqueous solution at physiological pH to a high degree of spin polarization.

B: Finger-printing of derivatized fullerenes is possible from the analysis of their aromatic regions in the single scan DNP hyperpolarized ^{13}C NMR spectra.

C: The longitudinal relaxation time (T_1) of ^{13}C PW75 is greater than 1 minute (~75 seconds).

D: The hyperpolarized signal from ^{13}C PW75 lasts over 6 minutes which enables real-time ^{13}C MRI, MRA or spectroscopy *in vivo*. ^{13}C PW75 is suitable as an *in vivo* hyperpolarized ^{13}C contrast agents.

Example 5

Figures 24-28 show additional MRI spectra of various fullerenes.

Figure 24 shows the ^{13}C NMR spectrum of fullerene. The parameters were 2.40 mg fullerene, 1.52 mg 1,3-bisdiphenylene-2-phenyallyl (BDPA), 200 µl toluene-d8; Polarization time 14 h; Dissolution in toluene; Single scan, 30° pulse.

Figure 25 shows the ^{13}C NMR spectrum of PW75. The parameters were 1.98 mg derivatized fullerene PW75, 2.95 mg FINLAND, MeOD-d4:DMSO-d6; Polarization time 8 h 2 m; Dissolution in methanol; Single scan, 90° pulse.

Figure 26 shows the ^{13}C NMR spectrum of dendrimer. The parameters were 6.76 mg dendrimer, 3.15 mg FINLAND, DMSO-d6:glycol; Polarization time 16 h 24 m; Dissolution in 2ml DMSO, 2ml methanol; Single scan, 90° pulse

Figure 27 shows the ^{13}C NMR spectrum of C_{60}. 1.14 mg C_{60}, 1.56 mg BDPA, toluene; Polarization time 3 hours; Probe = 10 mm, 60° pulse.

Figure 28 shows the ^{13}C NMR spectrum of C_{70}. 2.01 mg C_{70}, 1.51 mg BDPA, toluene; Polarization time 2h 57m; Probe = 10 mm; 45° pulse.

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of particular embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

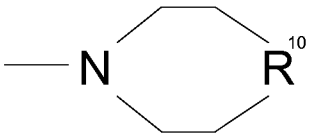
WHAT IS CLAIMED IS:

1. A contrast agent for enhancing contrast in *in vivo* magnetic resonance imaging, comprising a water-soluble, non-zero spin isotope-enriched substituted fullerene having a fullerene core or heterofullerene core and at least one substituent group bonded to at least one carbon of the fullerene core, wherein the substituent group is selected from the group consisting of (i) m ($>CX^1X^2$) groups bonded to the fullerene core, wherein: (i-a) m is an integer from 1 to 6, inclusive, (i-b) each X^1 and X^2 is independently selected from –H; -COOH; $-CONH_2$; -CONHR'; $-CONR'_2$; -COOR'; -CHO; $-(CH_2)_dOR^{11}$; a peptidyl moiety; -R; -RCOOH; $-RCONH_2$; -RCONHR'; $-RCONR'_2$; -RCOOR'; -RCHO; $-R(CH_2)_dOR^{11}$; a heterocyclic moiety; a branched moiety comprising one or more terminal -OH, $-NH_2$, triazole, tetrazole, or sugar groups; or a salt thereof, wherein each R is a hydrocarbon moiety having from 1 to about 6 carbon atoms and each R' is independently a hydrocarbon moiety having from 1 to about 6 carbon atoms, an aryl-containing moiety having from 6 to about 18 carbon atoms, a hydrocarbon moiety having from 1 to about 6 carbon atoms and a terminal carboxylic acid or alcohol, or an aryl-containing moiety having from 6 to about 18 carbon atoms and a terminal carboxylic acid or alcohol, and d is an integer from 0 to about 20, and each R^{11} is independently –H, a charged moiety, or a polar moiety;

(ii) p $-X^3$ groups bonded to the fullerene core, wherein (ii-a) p is an integer from 1 to 18, inclusive; and (ii-b) each $-X^3$ is independently selected from:

$-N^+(R^2)(R^3)(R^4)$, wherein R^2, R^3, and R^4 are independently -H or $-(CH_2)_d-CH_3$, wherein d is an integer from 0 to about 20;

$-N^+(R^2)(R^3)(R^8)$, wherein R^2 and R^3 are independently -H or $-(CH_2)_d-CH_3$, wherein d is an integer from 0 to about 20, and each R^8 is independently $-(CH_2)_f-SO_3^-$, $-(CH_2)_f-PO_4^-$, or $-(CH_2)_f-COO^-$, wherein f is an integer from 1 to about 20;



, wherein each R^{10} is independently >O, $>C(R^2)(R^3)$, $>CHN^+(R^2)(R^3)(R^4)$, or $>CHN^+(R^2)(R^3)(R^8)$;

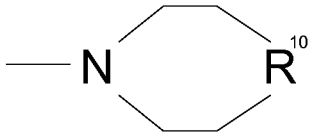
$-C(R^5)(R^6)(R^7)$, wherein R^5, R^6, and R^7 are independently -COOH, -H, -CH(=O), $-CH_2OH$, or a peptidyl moiety;

$-C(R^2)(R^3)(R^8)$,

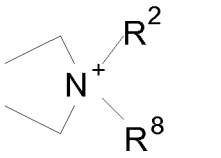
$-(CH_2)_e$-COOH, wherein e is an integer from 1 to about 6

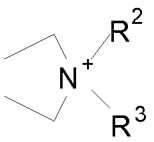
$-(CH_2)_e$-$CONH_2$, or

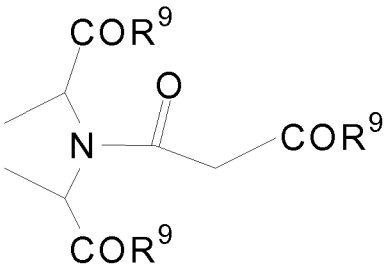
$-(CH_2)_e$-COOR';

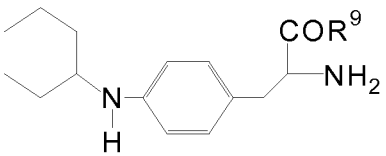
wherein when the $-X^3$ group is selected from , the substituted fullerene can further comprise from 1 to 6 >O groups;

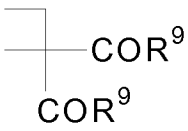
(iii) q $-X^4$- groups bonded to the fullerene core, wherein (iii-a) q is an integer from 1 to 6, inclusive; and (iii-b) each $-X^4$- group is independently:

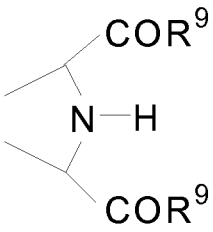
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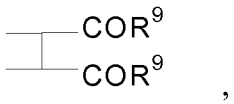
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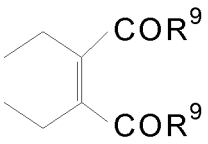
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or ; and

(iv), r dendrons bonded to the fullerene core and s nondendrons bonded to the fullerene core, wherein: (iv-a) r is an integer from 1 to 6, inclusive; (iv-b) s is an integer from 0 to 18, inclusive; (iv-b) each dendron has at least one protic group which imparts water solubility, and (iv-d) each nondendron independently comprises at least one drug, amino acid, peptide, nucleotide, vitamin, or organic moiety.

2. The contrast agent of claim 1, wherein the substituted fullerene is selected from the group consisting of C3, FB115, PW75, DF-1, DF-1 Mini, FB02, FB03, FB10, cationic forms of the foregoing, anionic forms of the foregoing, metabolites and breakdown products of the foregoing, and mixtures thereof.

3. The contrast agent of claim 1, wherein the substituted fullerene has a solubility in water of at least 1 mM.

4. The contrast agent of claim 1, wherein the substituted fullerene has a ^{13}C isotope abundance of greater than about 1.1%.

5. The contrast agent of claim 4, wherein the substituted fullerene has a ^{13}C isotope abundance of greater than about 10%.

6. The contrast agent of claim 5, wherein the substituted fullerene has a ^{13}C isotope abundance of greater than about 30%.

7. The contrast agent of claim 1, further comprising a functional moiety selected from the group consisting of biotin, docosahexaenoic acid (DHA), polyethylene glycol (PEG), antibodies against amyloid plaque, antibodies against tau protein, antibodies against

atherosclerotic plaque, antibodies against tumor antigens, antibodies against inflammatory cell antigens, antibodies against immune cell antigens, antibodies that bind to growth factor receptors, peptides that bind to amyloid plaque, peptides that bind to tau protein, peptides that bind to atherosclerotic plaque, peptides that bind to tumor antigens, peptides that bind to inflammatory cell antigens, peptides that bind to growth factor receptors, and peptides that bind to immune cell antigens.

8. A method of magnetic resonance imaging a sample, comprising:

(a) administering to the mammal a plurality of substituted fullerenes; and

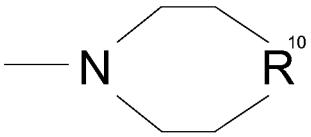
(b) detecting magnetic resonance signals from the sample,

wherein the substituted fullerenes have a fullerene core and at least one substituent group bonded to at least one carbon of the fullerene core, wherein the substituent group is selected from the group consisting of (i) m ($>CX^1X^2$) groups bonded to the fullerene core, wherein: (i-a) m is an integer from 1 to 6, inclusive, (i-b) each X^1 and X^2 is independently selected from –H; -COOH; $-CONH_2$; -CONHR'; $-CONR'_2$; -COOR'; -CHO; $-(CH_2)_dOR^{11}$; a peptidyl moiety; -R; -RCOOH; $-RCONH_2$; -RCONHR'; $-RCONR'_2$; -RCOOR'; -RCHO; $-R(CH_2)_dOR^{11}$; a heterocyclic moiety; a branched moiety comprising one or more terminal -OH, $-NH_2$, triazole, tetrazole, or sugar groups; or a salt thereof, wherein each R is a hydrocarbon moiety having from 1 to about 6 carbon atoms and each R' is independently a hydrocarbon moiety having from 1 to about 6 carbon atoms, an aryl-containing moiety having from 6 to about 18 carbon atoms, a hydrocarbon moiety having from 1 to about 6 carbon atoms and a terminal carboxylic acid or alcohol, or an aryl-containing moiety having from 6 to about 18 carbon atoms and a terminal carboxylic acid or alcohol, and d is an integer from 0 to about 20, and each R^{11} is independently –H, a charged moiety, or a polar moiety;

(ii) p $-X^3$ groups bonded to the fullerene core, wherein (ii-a) p is an integer from 1 to 18, inclusive; and (ii-b) each $-X^3$ is independently selected from:

$-N^+(R^2)(R^3)(R^4)$, wherein R^2, R^3, and R^4 are independently -H or $-(CH_2)_d-CH_3$, wherein d is an integer from 0 to about 20;

$-N^+(R^2)(R^3)(R^8)$, wherein R^2 and R^3 are independently -H or $-(CH_2)_d-CH_3$, wherein d is an integer from 0 to about 20, and each R^8 is independently $-(CH_2)_f-SO_3^-$, $-(CH_2)_f-PO_4^-$, or $-(CH_2)_f-COO^-$, wherein f is an integer from 1 to about 20;



, wherein each R^{10} is independently >O, >C(R^2)(R^3), >CHN$^+$(R^2)(R^3)(R^4), or >CHN$^+$(R^2)(R^3)(R^8);

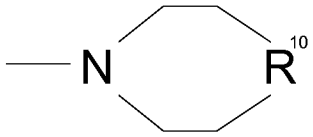
-C(R^5)(R^6)(R^7), wherein R^5, R^6, and R^7 are independently -COOH, -H, -CH(=O), -CH_2OH, or a peptidyl moiety;

-C(R^2)(R^3)(R^8),

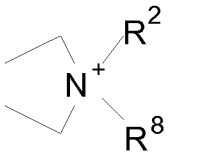
-$(CH_2)_e$-COOH, wherein e is an integer from 1 to about 6

-$(CH_2)_e$-$CONH_2$, or

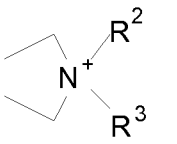
-$(CH_2)_e$-COOR';

wherein when the –X^3 group is selected from , the substituted fullerene can further comprise from 1 to 6 >O groups;

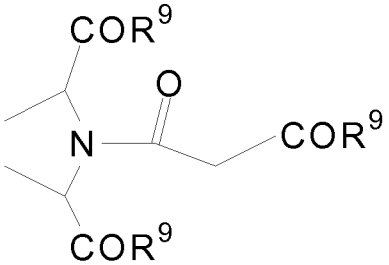
(iii) q -X^4- groups bonded to the fullerene core, wherein (iii-a) q is an integer from 1 to 6, inclusive; and (iii-b) each -X^4- group is independently:



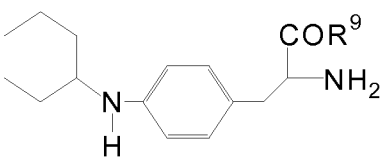
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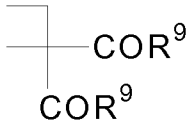
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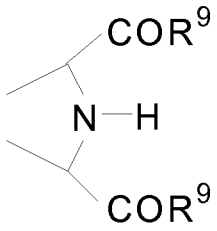
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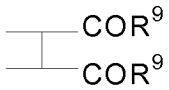
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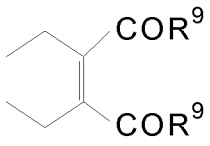
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or 

; and

(iv), r dendrons bonded to the fullerene core and s nondendrons bonded to the fullerene core, wherein: (iv-a) r is an integer from 1 to 6, inclusive; (iv-b) s is an integer from 0 to 18, inclusive; (iv-b) each dendron has at least one protic group which imparts water solubility, and (iv-d) each nondendron independently comprises at least one drug, amino acid, peptide, nucleotide, vitamin, or organic moiety.

9. The method of claim 8, wherein the substituted fullerene is selected from the group consisting of C3, FB115, PW75, DF-1, DF-1 Mini, FB02, FB03, FB10, cationic forms of the foregoing, anionic forms of the foregoing, metabolites and breakdown products of the foregoing, and mixtures thereof.

10. The method of claim 8, wherein the substituted fullerene further comprises a functional moiety selected from the group consisting of biotin, docosahexaenoic acid (DHA), polyethylene glycol (PEG), antibodies against amyloid plaque, antibodies against tau protein, antibodies against atherosclerotic plaque, antibodies against tumor antigens, antibodies against inflammatory cell antigens, antibodies against immune cell antigens, antibodies that bind to growth factor receptors, peptides that bind to amyloid plaque, peptides that bind to tau protein, peptides that bind to atherosclerotic plaque, peptides that bind to tumor antigens,

peptides that bind to inflammatory cell antigens, peptides that bind to growth factor receptors, and peptides that bind to immune cell antigens.

11. The method of claim 8, wherein the substituted fullerene is enriched in one or more paramagnetic nuclei.

12. The method of claim 8, wherein the substituted fullerene has a ^{13}C isotope abundance of greater than about 1.1%.

13. The method of claim 12, wherein the substituted fullerene has a ^{13}C isotope abundance of greater than about 10%.

14. The method of claim 13, wherein the substituted fullerene has a ^{13}C isotope abundance of greater than about 30%.

15. The method of claim 8, further comprising (c) generating a signals visualization selected from the group consisting of an image, a set of dynamic flow data, a set of diffusion data, and a set of perfusion data from the detected magnetic resonance signals.

16. The method of claim 8, further comprising hyperpolarizing the plurality of substituted fullerenes, to yield a plurality of hyperpolarized substituted fullerenes, prior to the administering step.

17. The method of claim 16, wherein hyperpolarization is effected by a polarizing agent.

18. The method of claim 17, wherein the polarizing agent is selected from the group consisting of paramagnetic metals, paramagnetic free radicals, and hyperpolarized gases.

19. The method of claim 18, wherein the paramagnetic metal is selected from the group consisting of chromium (III), manganese (II), manganese (III), iron (III),

praseodymium (III), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), terbium (III), dysprosium (III), holmium (III), and erbium (III).

20. The method of claim 18, wherein the hyperpolarized gas is selected from the group consisting of ^{129}Xe, ^{3}He, and ^{4}He.

21. The method of claim 8, wherein the sample is a cell or tissue of a mammal and the method is performed *in vivo*.

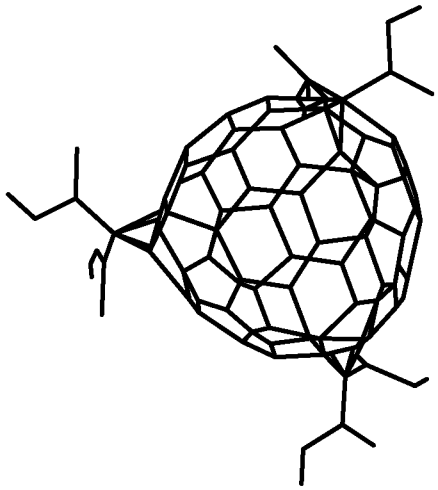


FIG. 1A

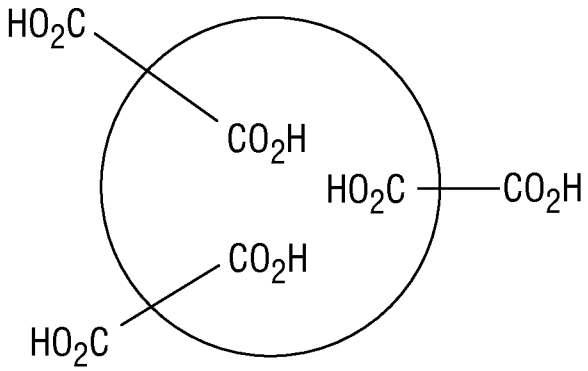


FIG. 1B

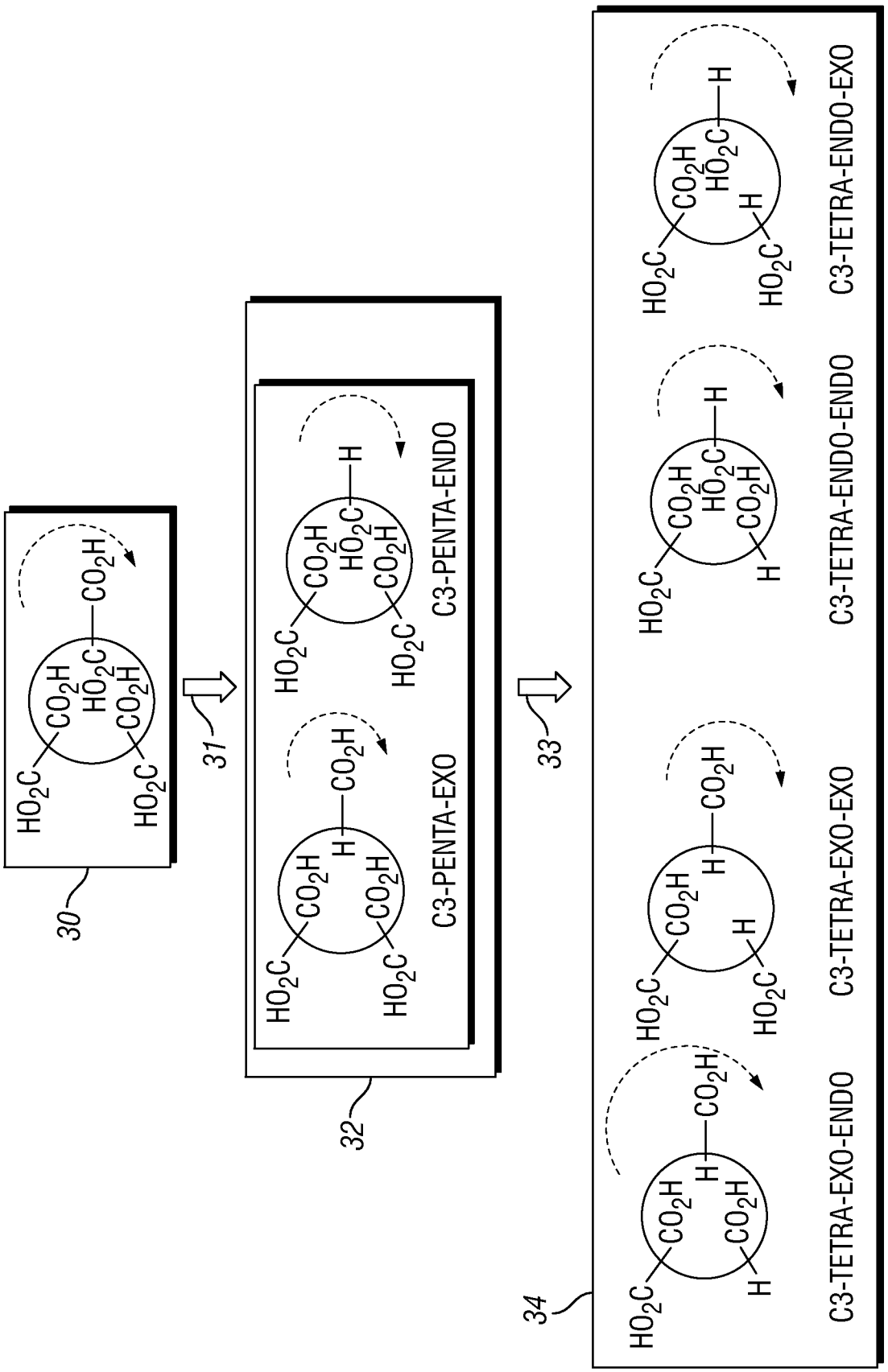

30
31
C3-PENTA-ENDO
C3-PENTA-EXO
32
33
C3-TETRA-ENDO-EXO
C3-TETRA-ENDO-ENDO
C3-TETRA-EXO-EXO
C3-TETRA-EXO-ENDO
34

FIG. 2

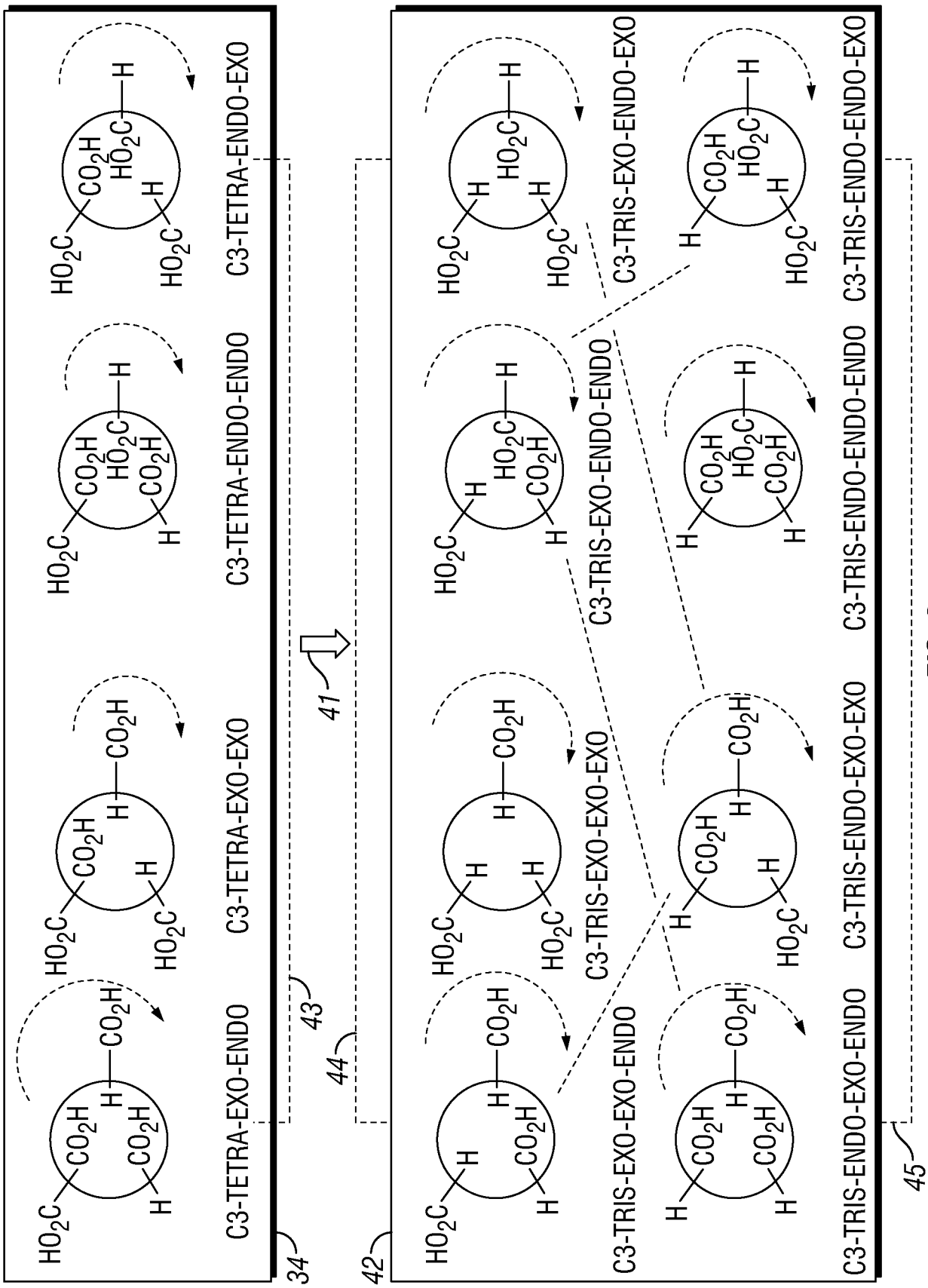

C3-TETRA-EXO-ENDO
C3-TETRA-ENDO-ENDO
C3-TETRA-EXO-EXO
C3-TETRA-ENDO-EXO
C3-TRIS-EXO-EXO-ENDO
C3-TRIS-EXO-ENDO-ENDO
C3-TRIS-EXO-EXO-EXO
C3-TRIS-EXO-ENDO-EXO
C3-TRIS-ENDO-EXO-ENDO
C3-TRIS-ENDO-ENDO-ENDO
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C3-TRIS-ENDO-ENDO-EXO
34
41
42
43
44
45

FIG. 3

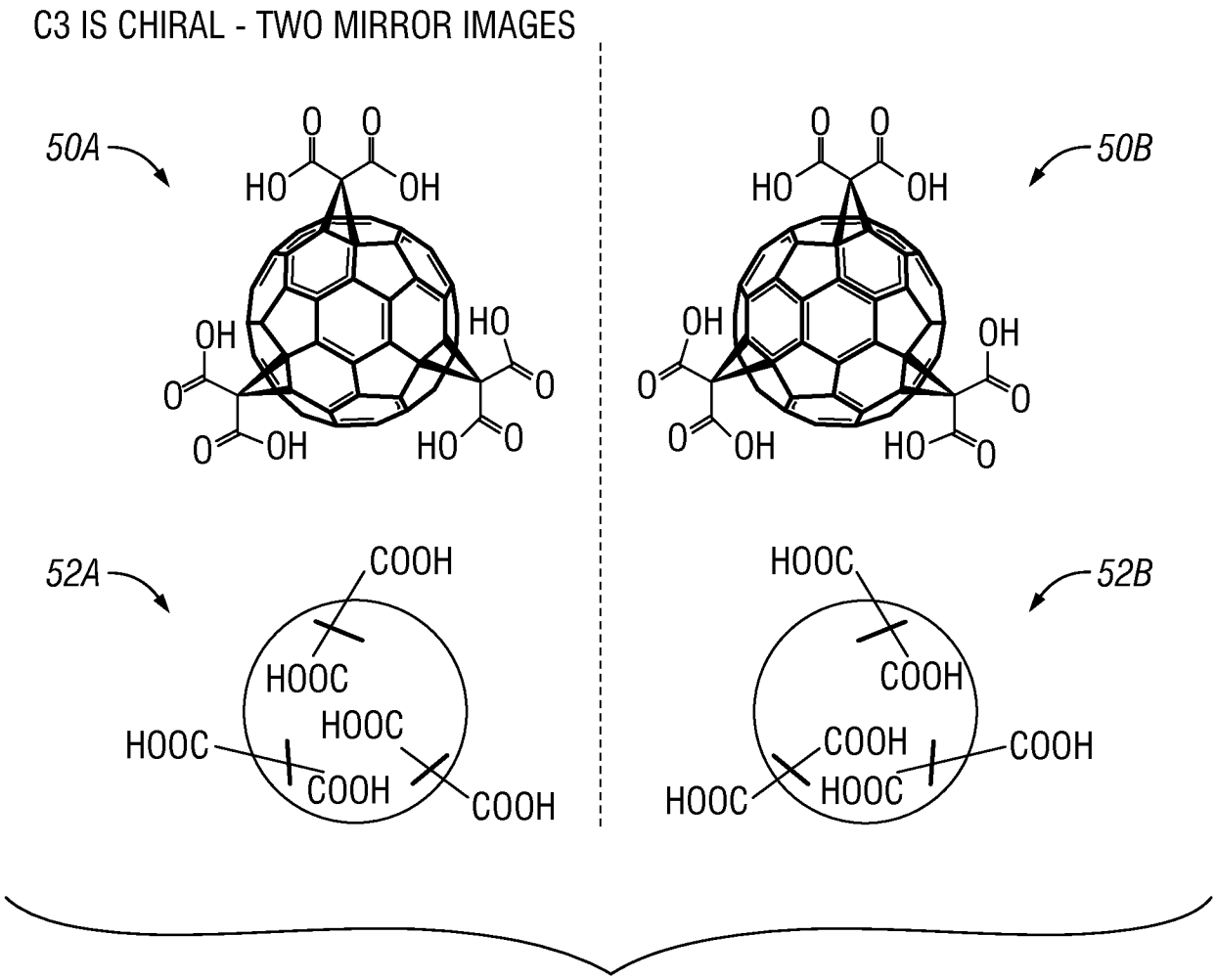

C3 IS CHIRAL - TWO MIRROR IMAGES
50A
50B
52A
52B
HO
OH
O
HOOC
COOH

FIG. 4

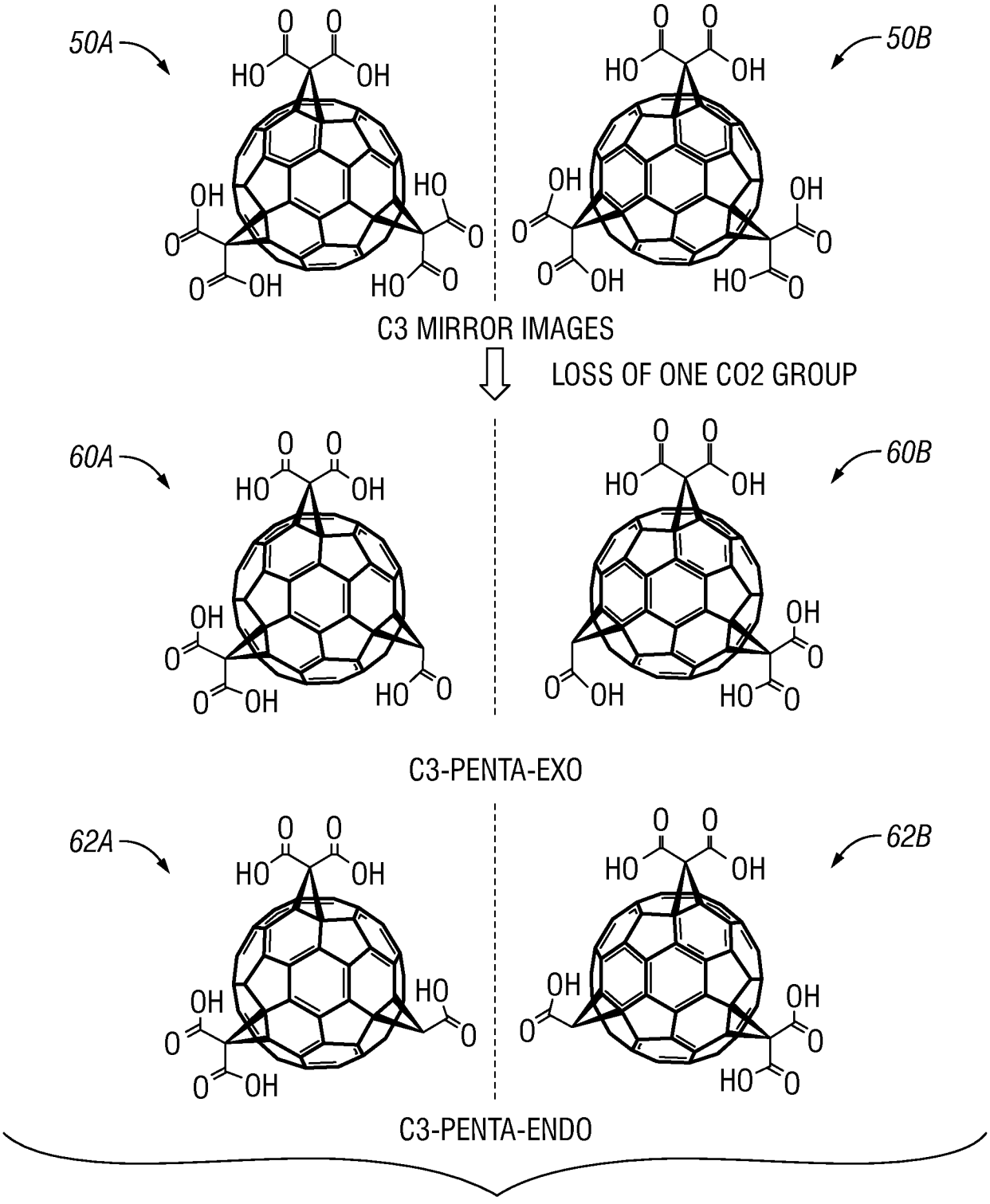

50A
50B
C3 MIRROR IMAGES
LOSS OF ONE CO2 GROUP
60A
60B
C3-PENTA-EXO
62A
62B
C3-PENTA-ENDO

FIG. 5

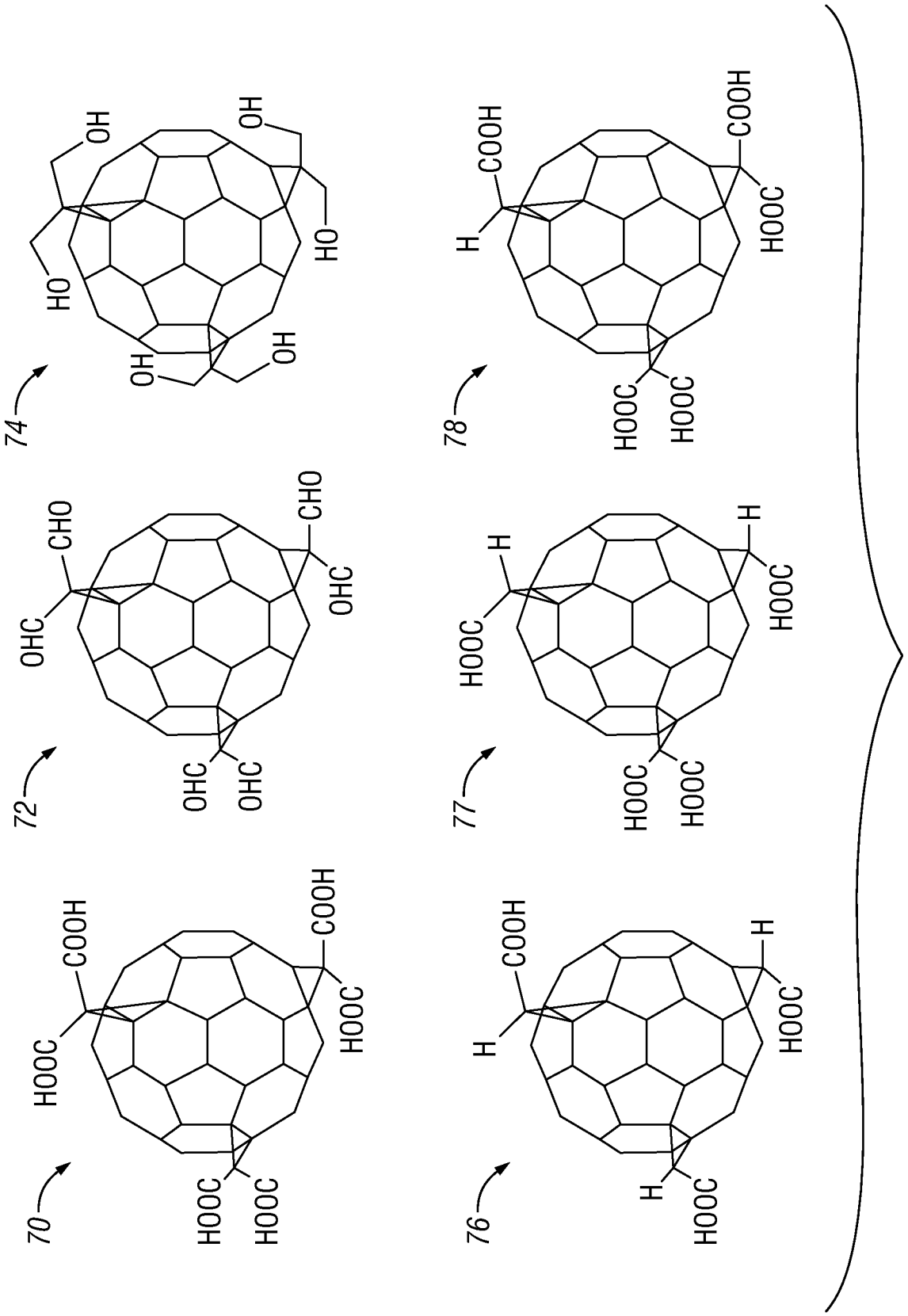

70
HOOC
COOH
HOOC
HOOC
HOOC
COOH
72
OHC
CHO
OHC
OHC
OHC
CHO
74
HO
OH
HO
OH
OH
OH
76
H
COOH
H
HOOC
H
HOOC
77
HOOC
H
HOOC
HOOC
HOOC
H
78
H
COOH
HOOC
HOOC
HOOC
COOH

FIG. 6

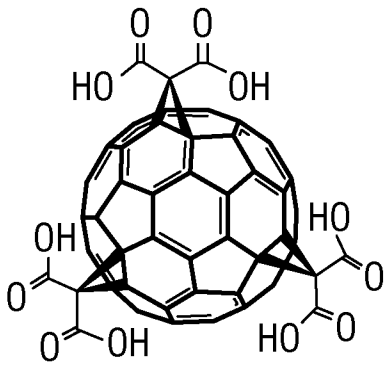


FIG. 7A

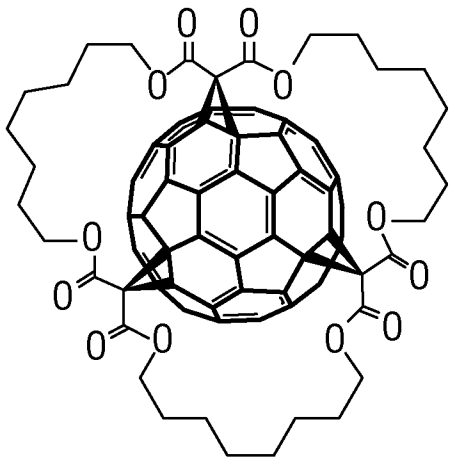


FIG. 7B

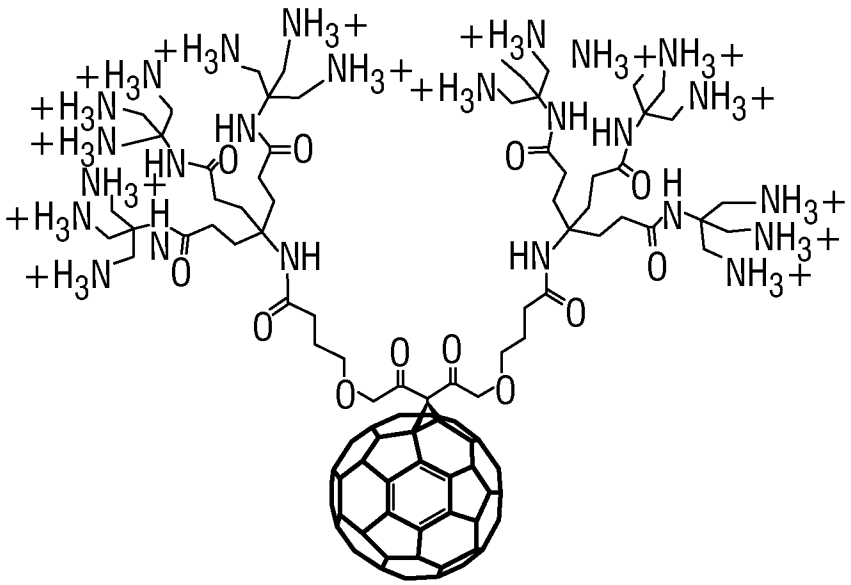


FIG. 8A

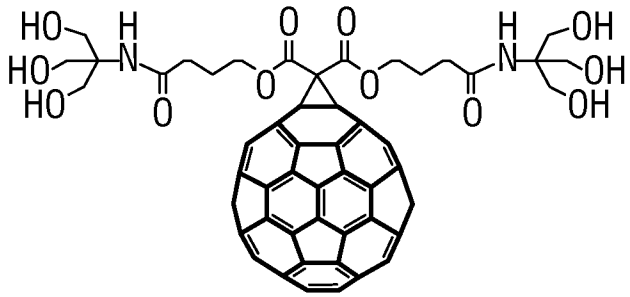


FIG. 8B

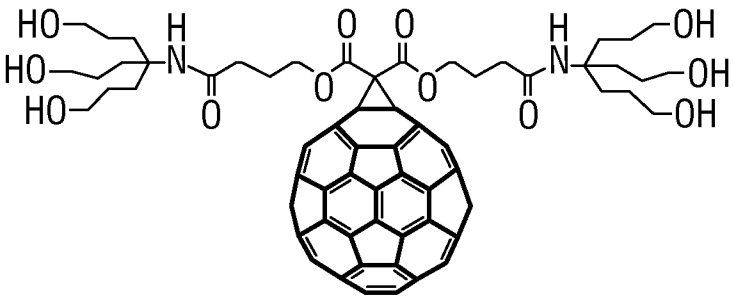


FIG. 8C

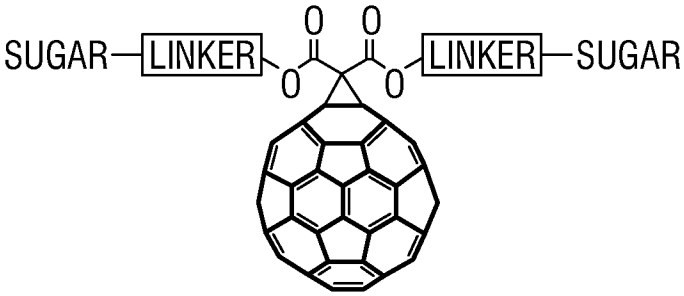


FIG. 8D

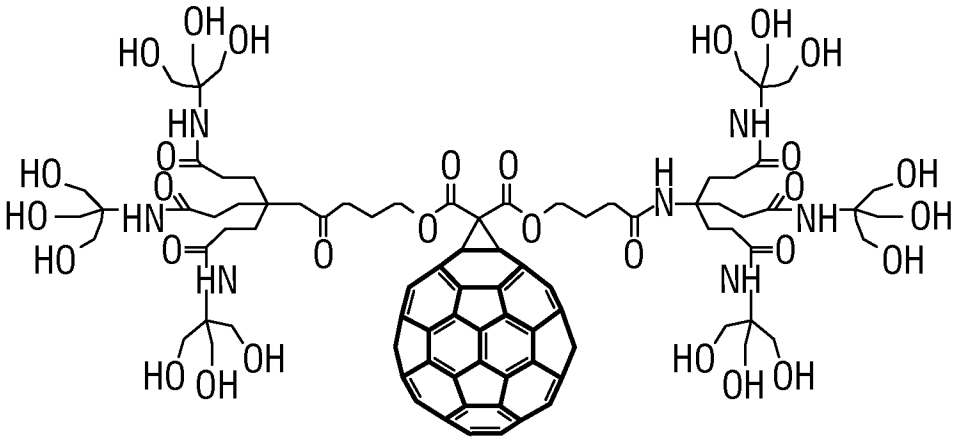


FIG. 8E

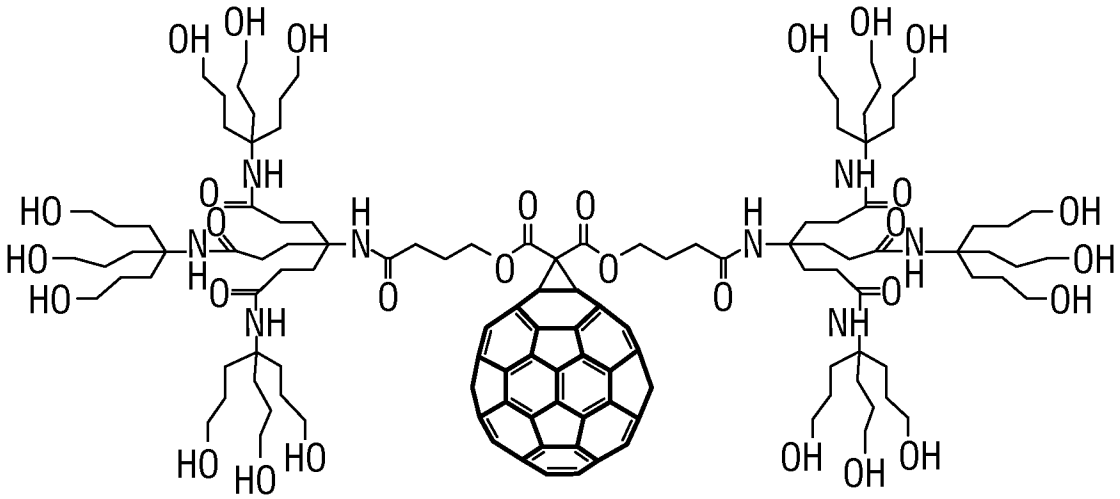


FIG. 8F

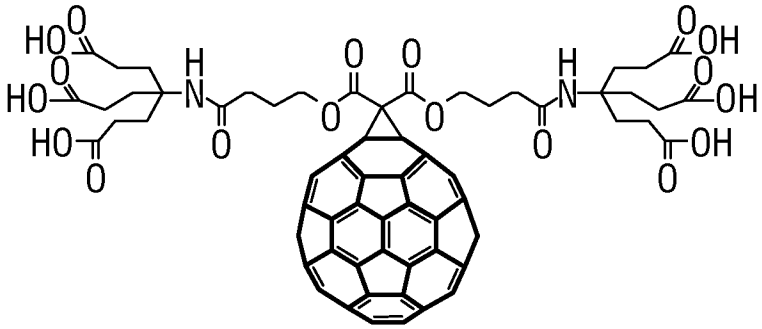


FIG. 8G

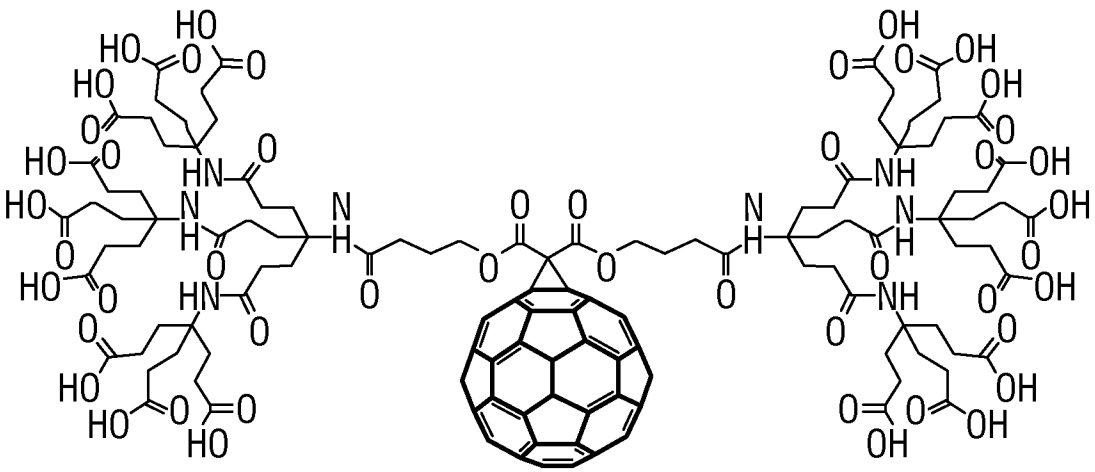


FIG. 9

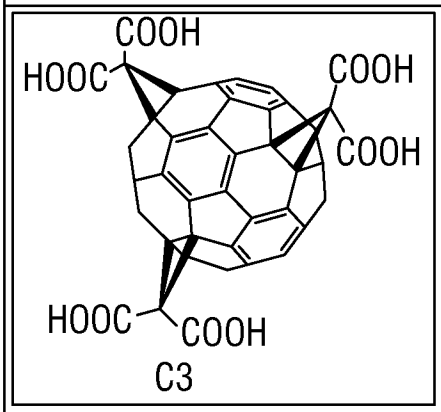
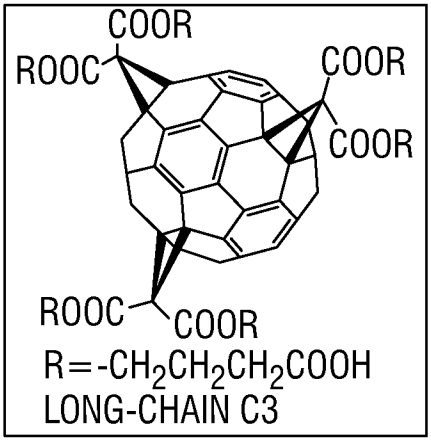
COMPOUND	REF. NO.	IC_{50} (μM)
TROLOX (COMPARATIVE)	110	440
	111	174
	1201	42

FIG. 10A

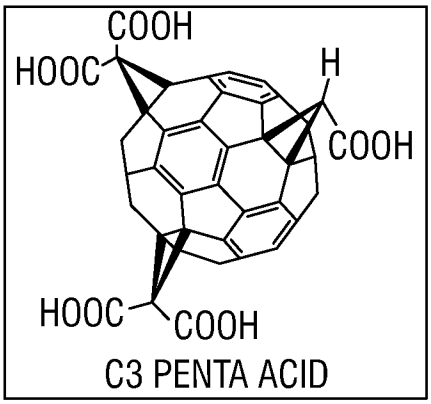
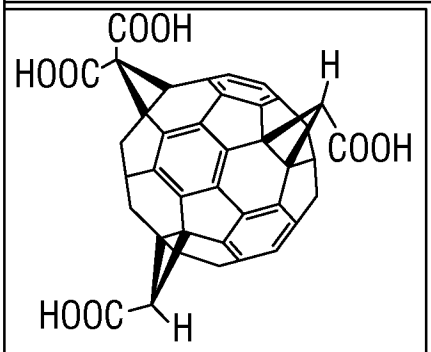
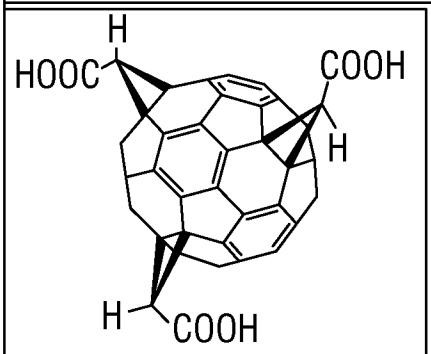
 C3 PENTA ACID	1202	62
 C3 TETRA ACID	1203	48
 C3 TRIS ACID	1204	44

FIG. 10B

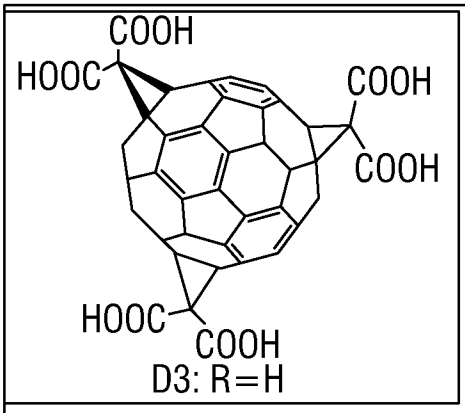
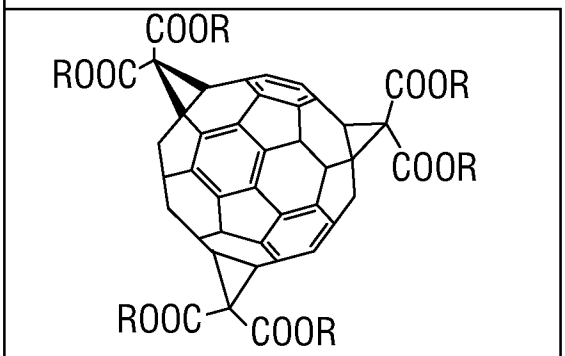
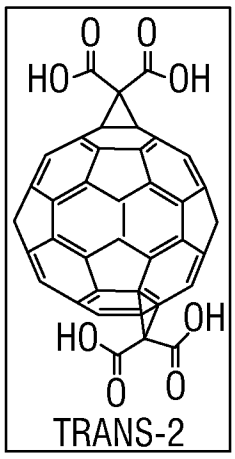
 D3: R=H	112	965
 LONG-CHAIN D3: R=$CH_2CH_2CH_2COOH$	1205	264
 TRANS-2	1206	90

FIG. 10C

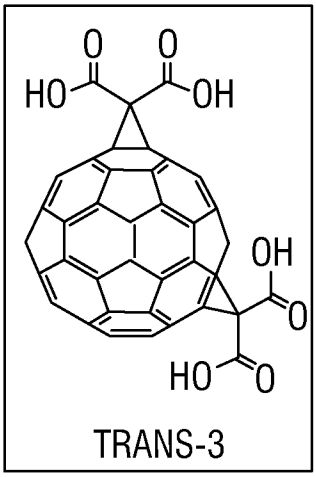
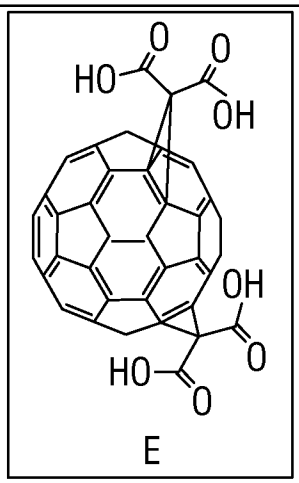
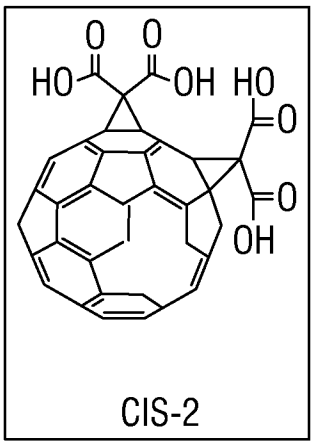
 TRANS-3	1207	55
 E	1208	32
 CIS-2	1209	23

FIG. 10D

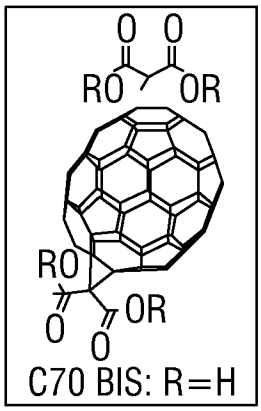
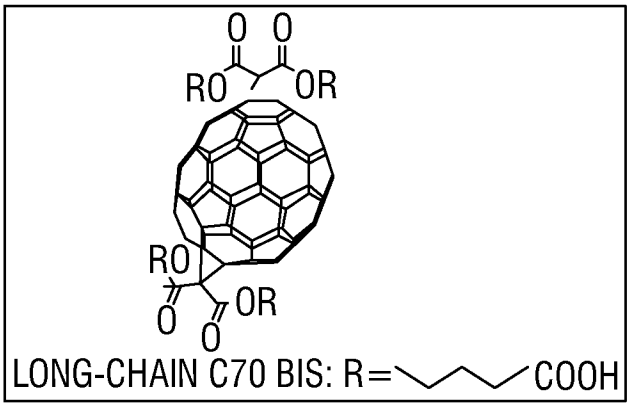
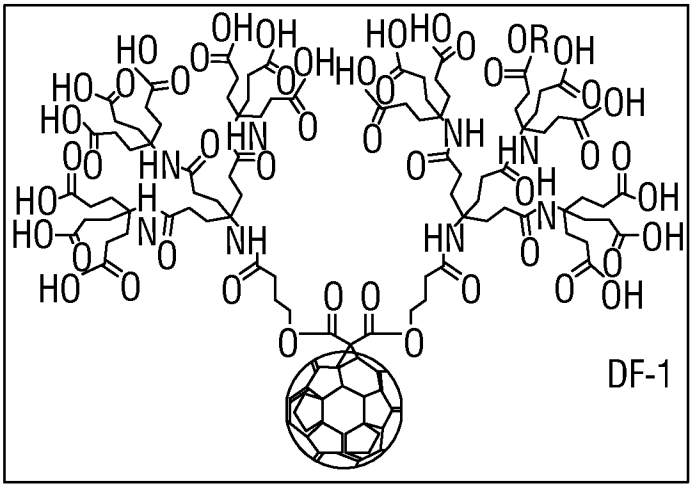
	1210	22
	1211	11
	113	102

FIG. 10E

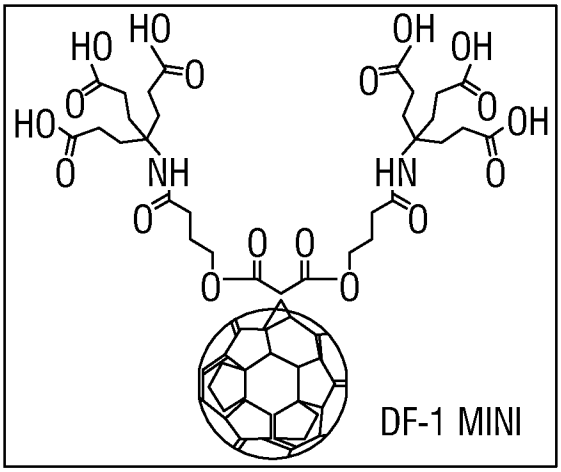
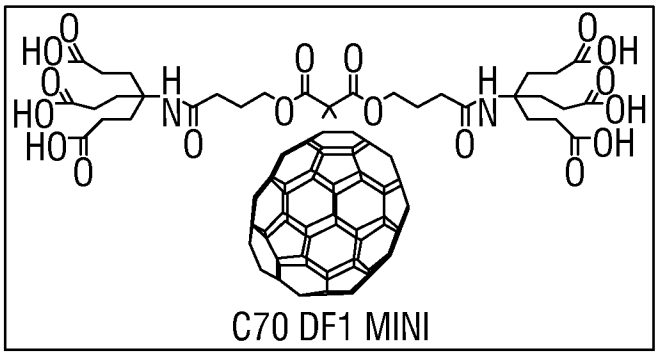
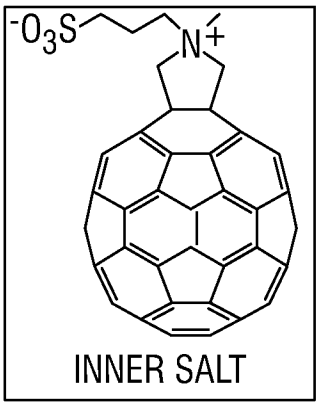
 DF-1 MINI	1212	12
 C70 DF1 MINI	1213	6
 INNER SALT	1214	92

FIG. 10F

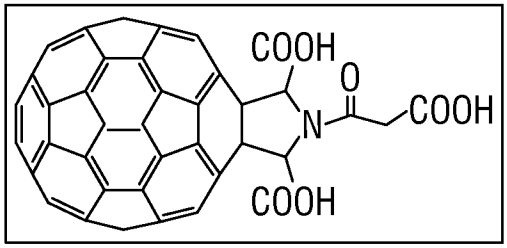
	1215	17
H0@C3	1216	38
$C_{60}(OH)_{12}$	114	189

FIG. 10G

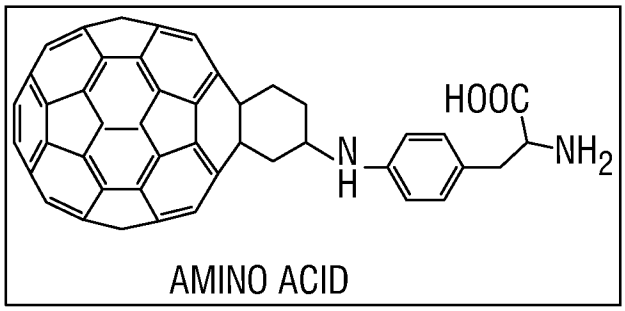
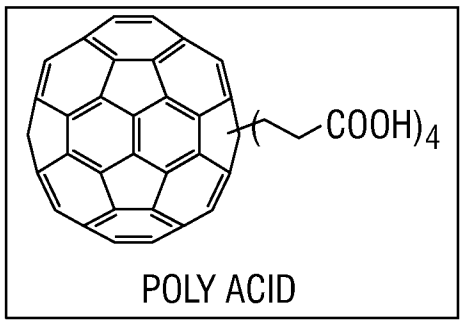
	1217	56
	1218	43

FIG. 10H

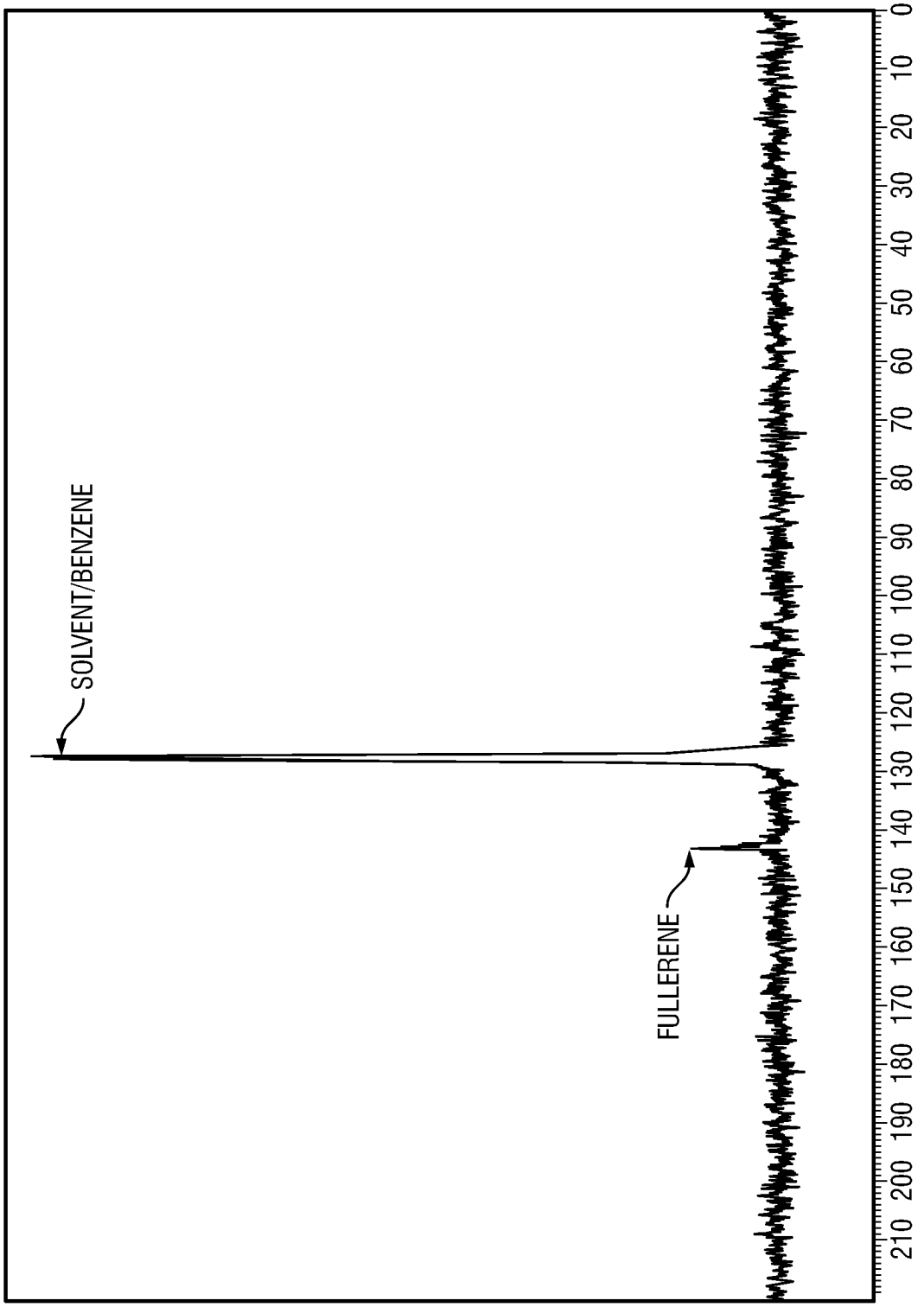

SOLVENT/BENZENE
FULLERENE
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
ppm

FIG. 11

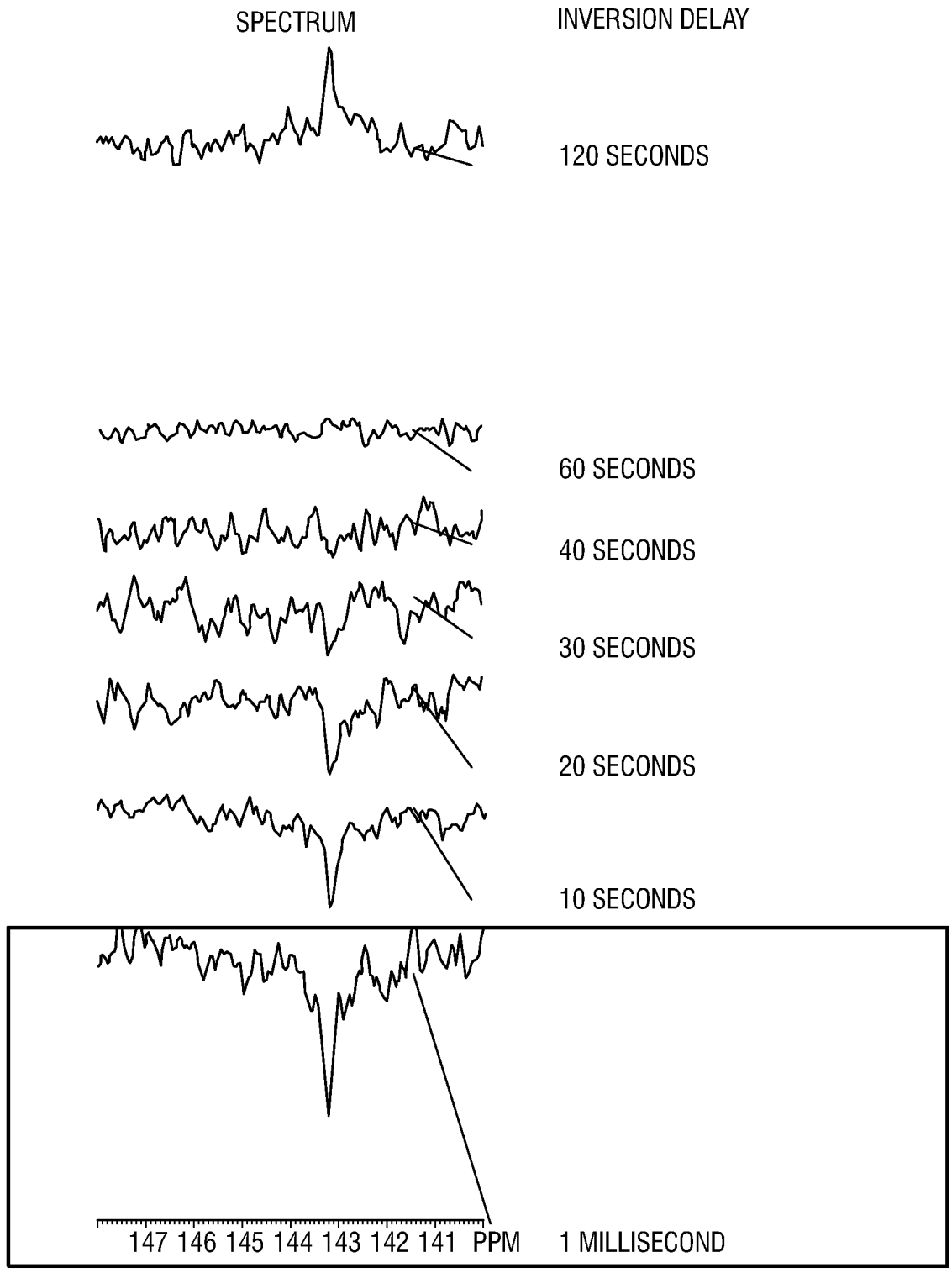

SPECTRUM
INVERSION DELAY
120 SECONDS
60 SECONDS
40 SECONDS
30 SECONDS
20 SECONDS
10 SECONDS
147 146 145 144 143 142 141 PPM
1 MILLISECOND

FIG. 12

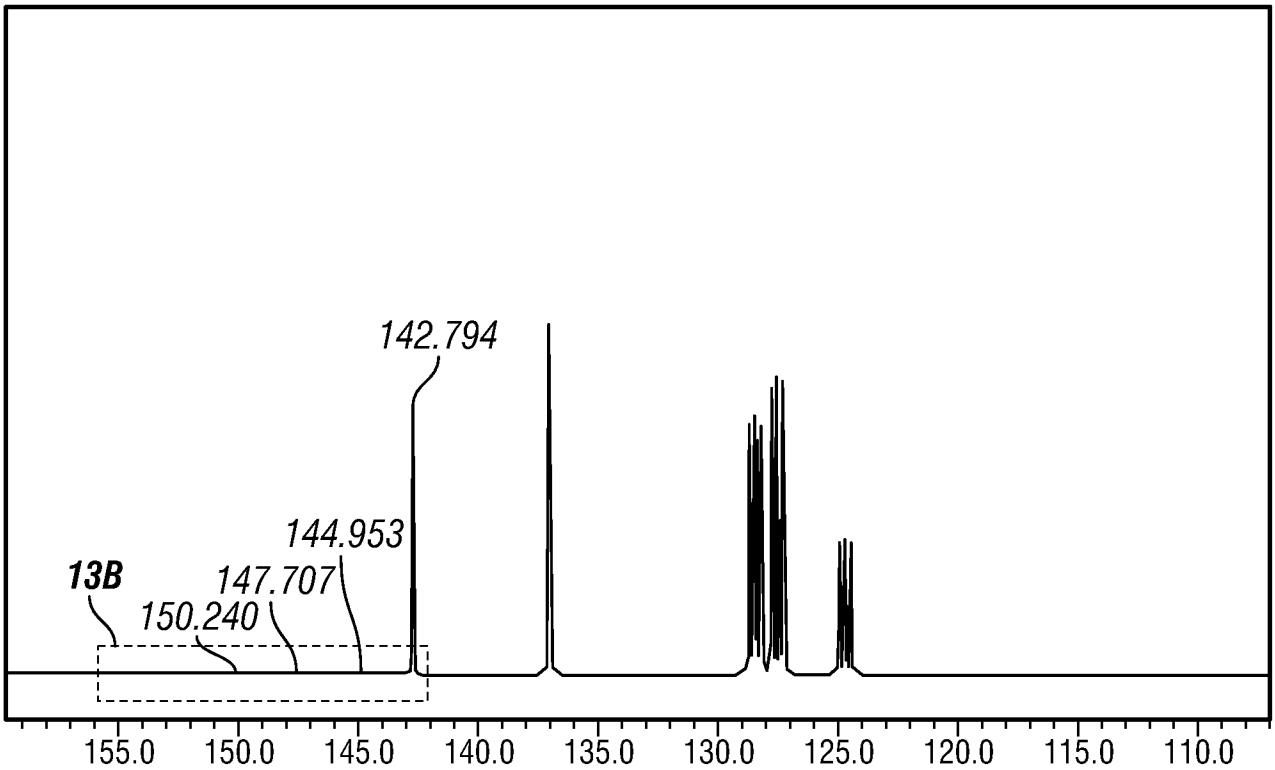


FIG. 13A

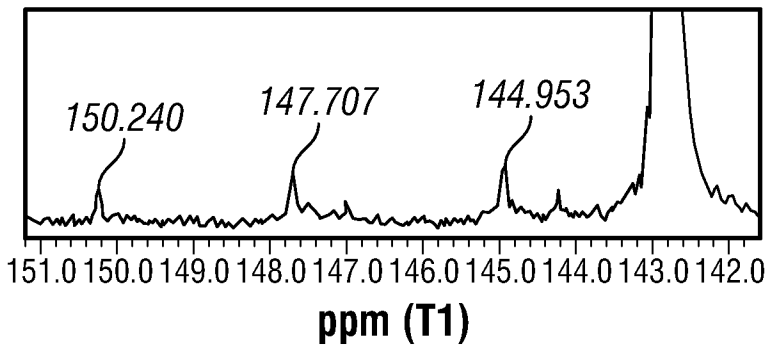


FIG. 13B

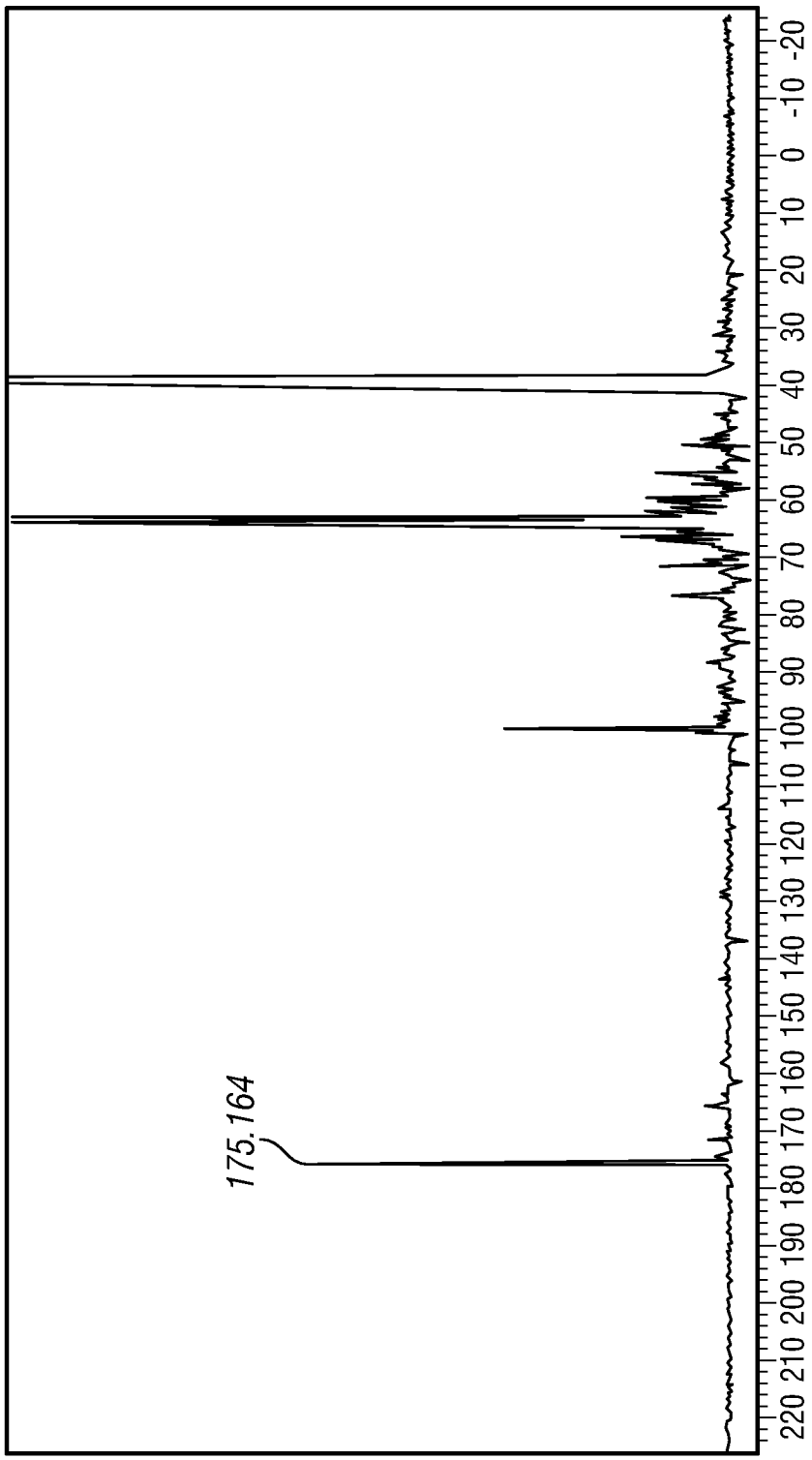

175.164
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
ppm (T1)

FIG. 14

FIG. 15A

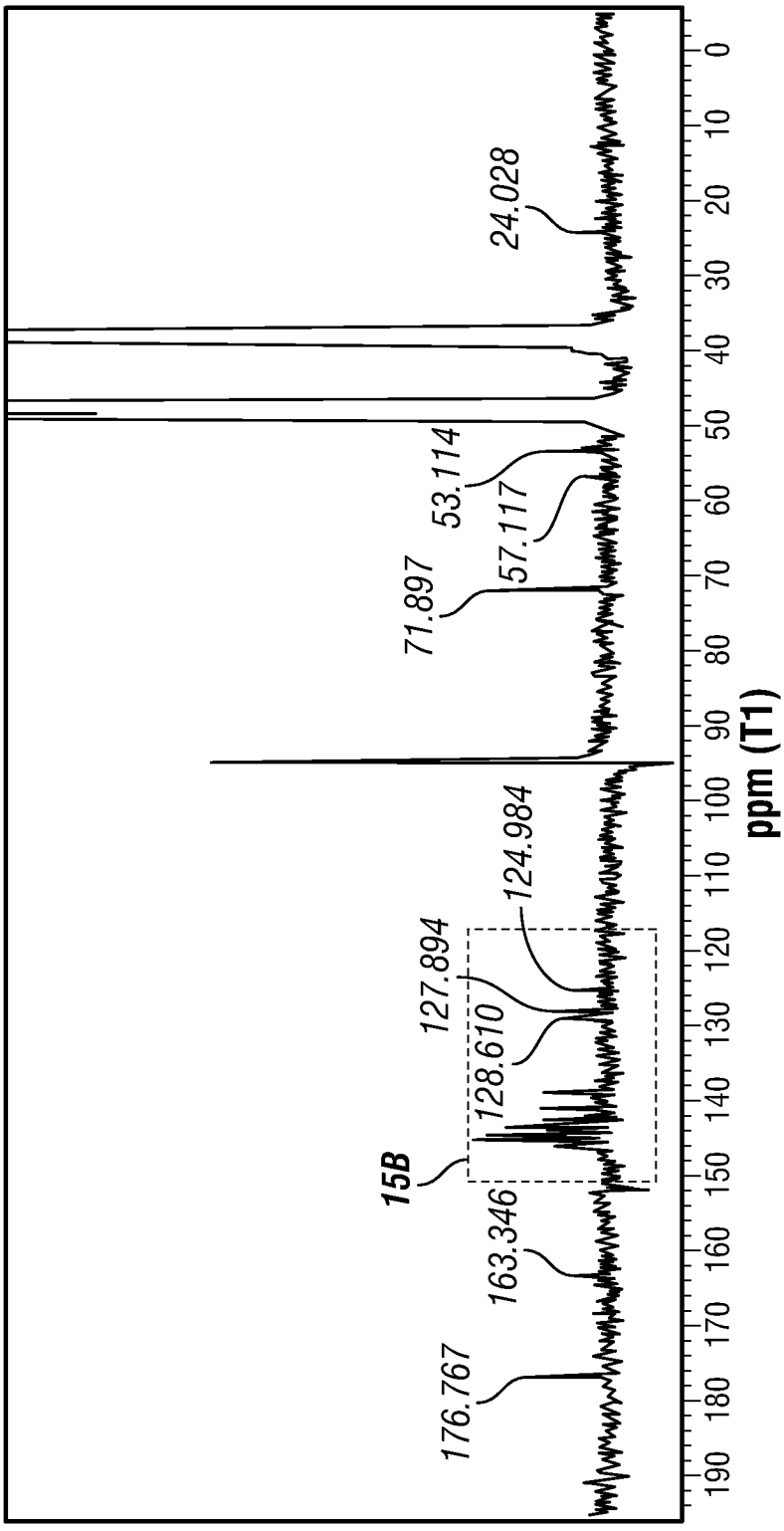
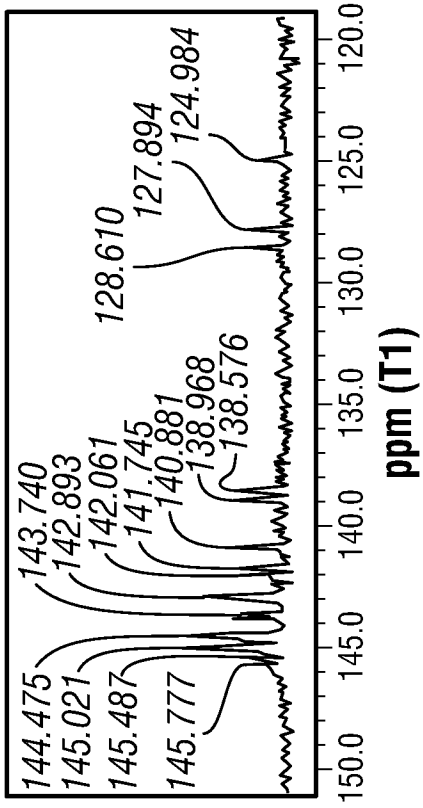


FIG. 15B



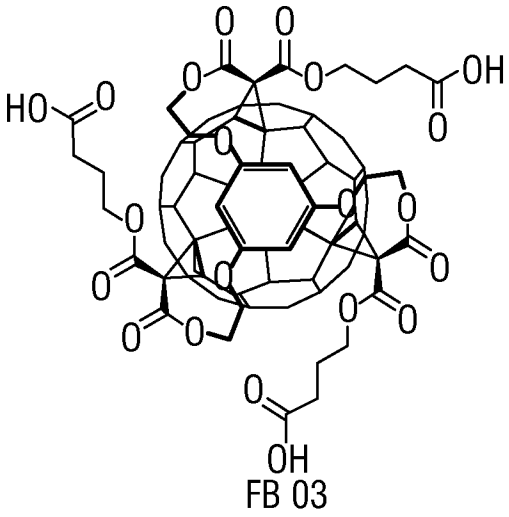


FIG. 16

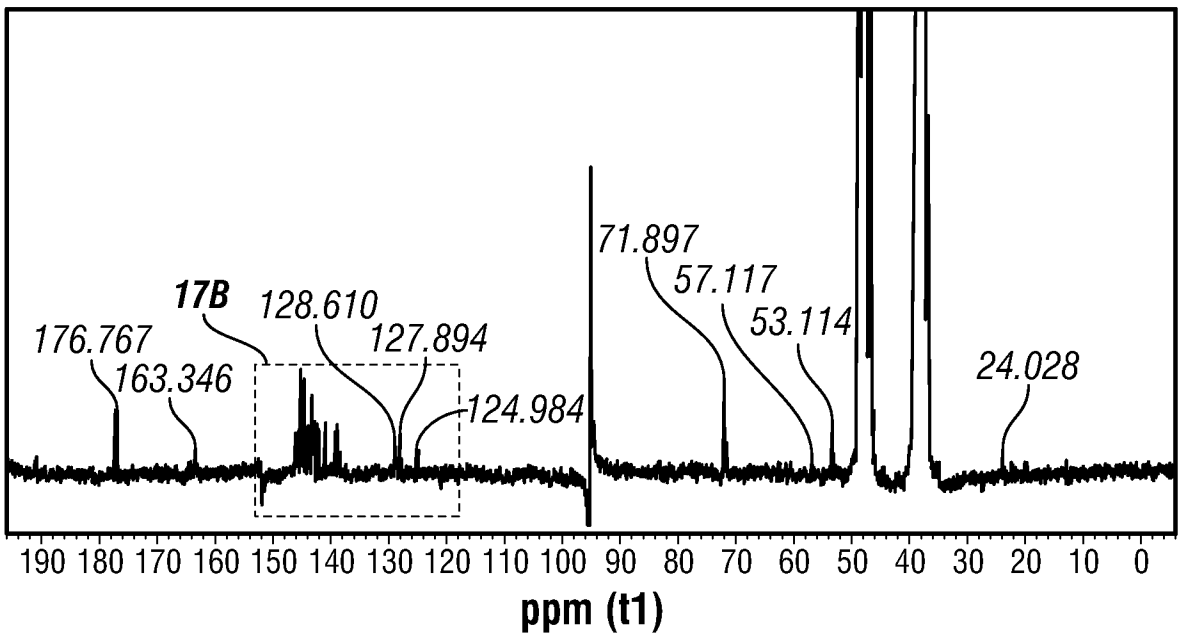


FIG. 17A

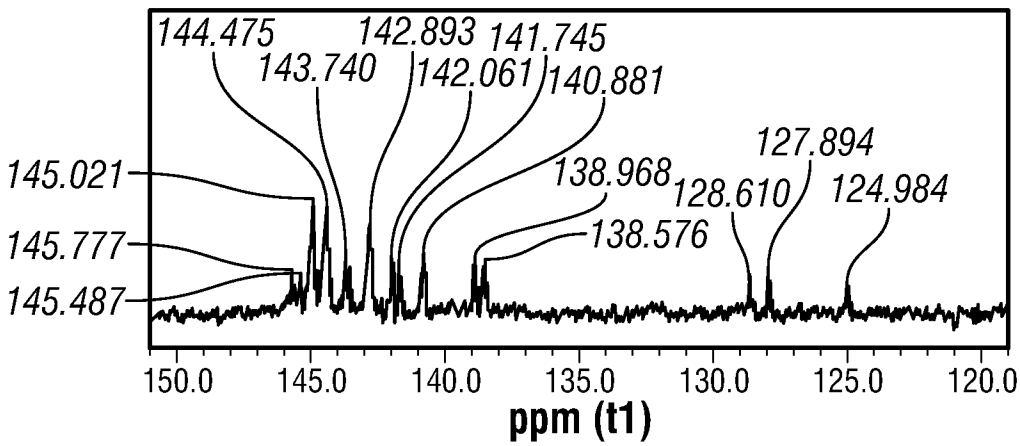


FIG. 17B

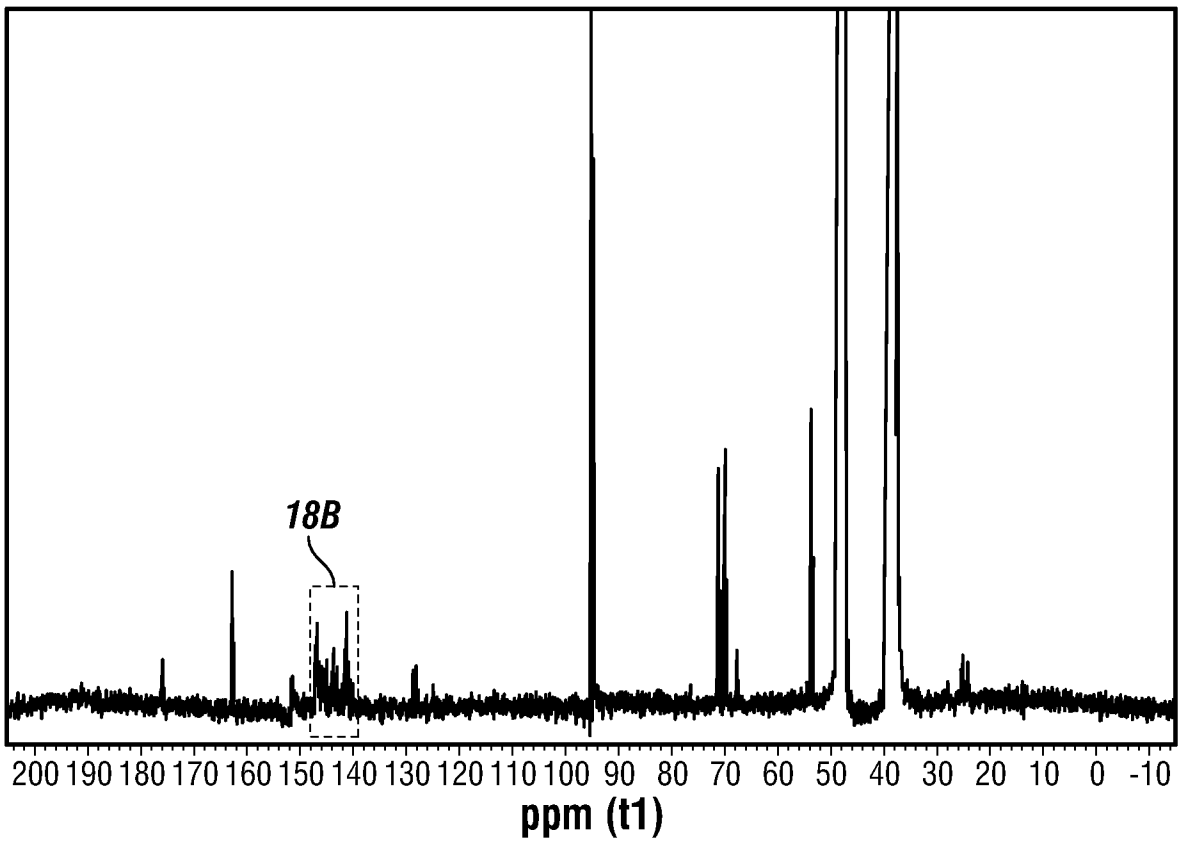


FIG. 18A

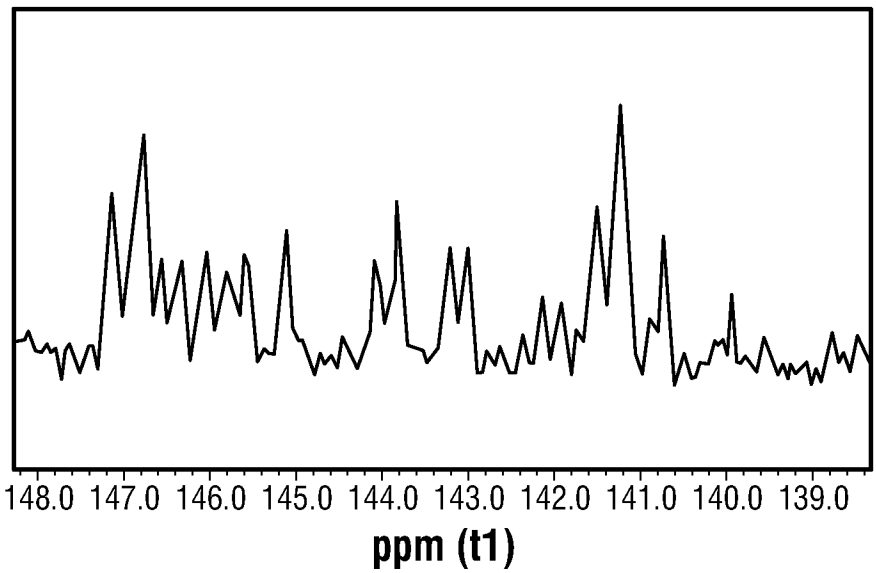
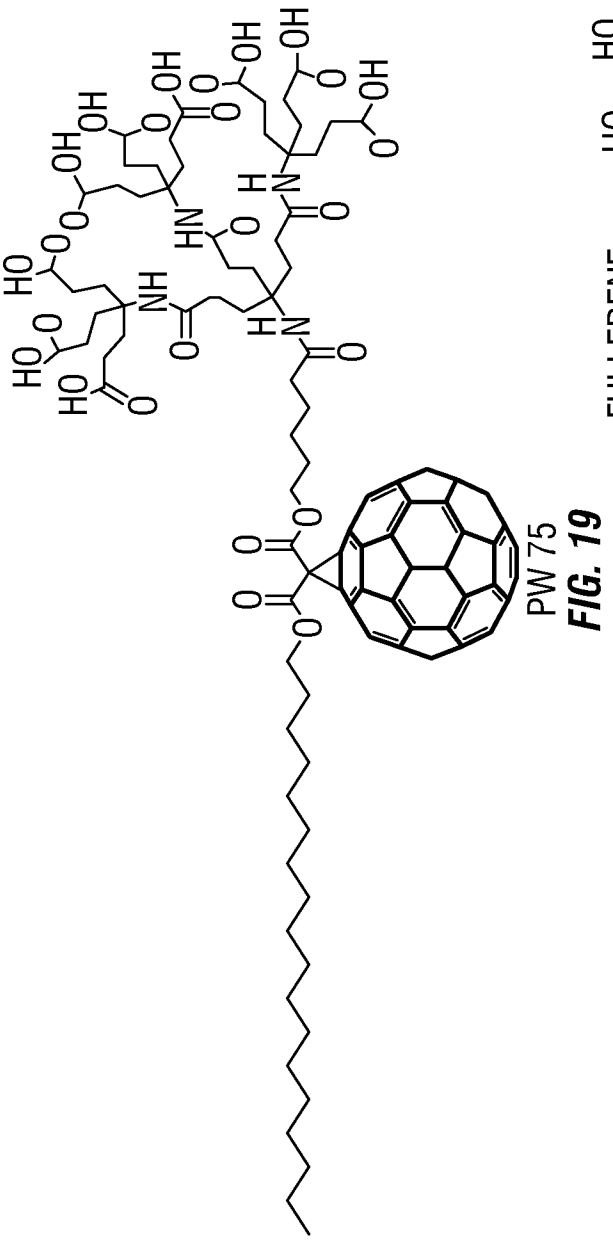


FIG. 18B



PW 75

FIG. 19

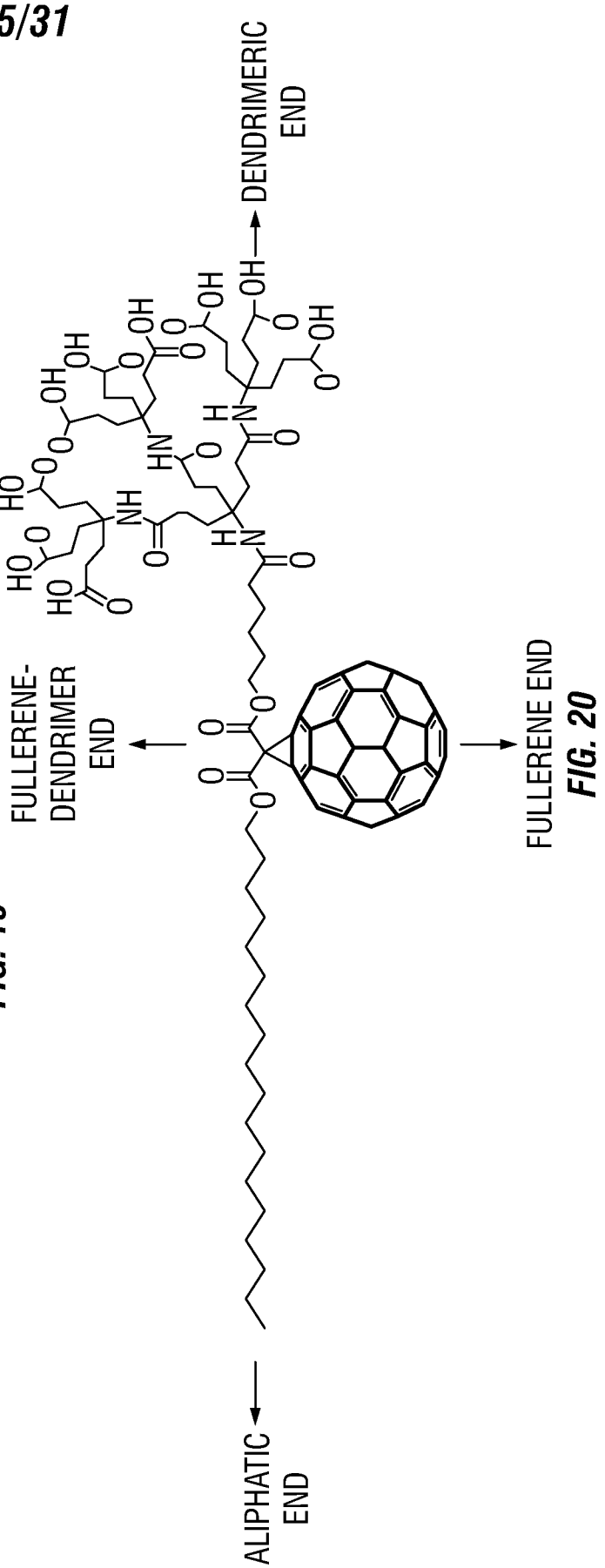


FIG. 20

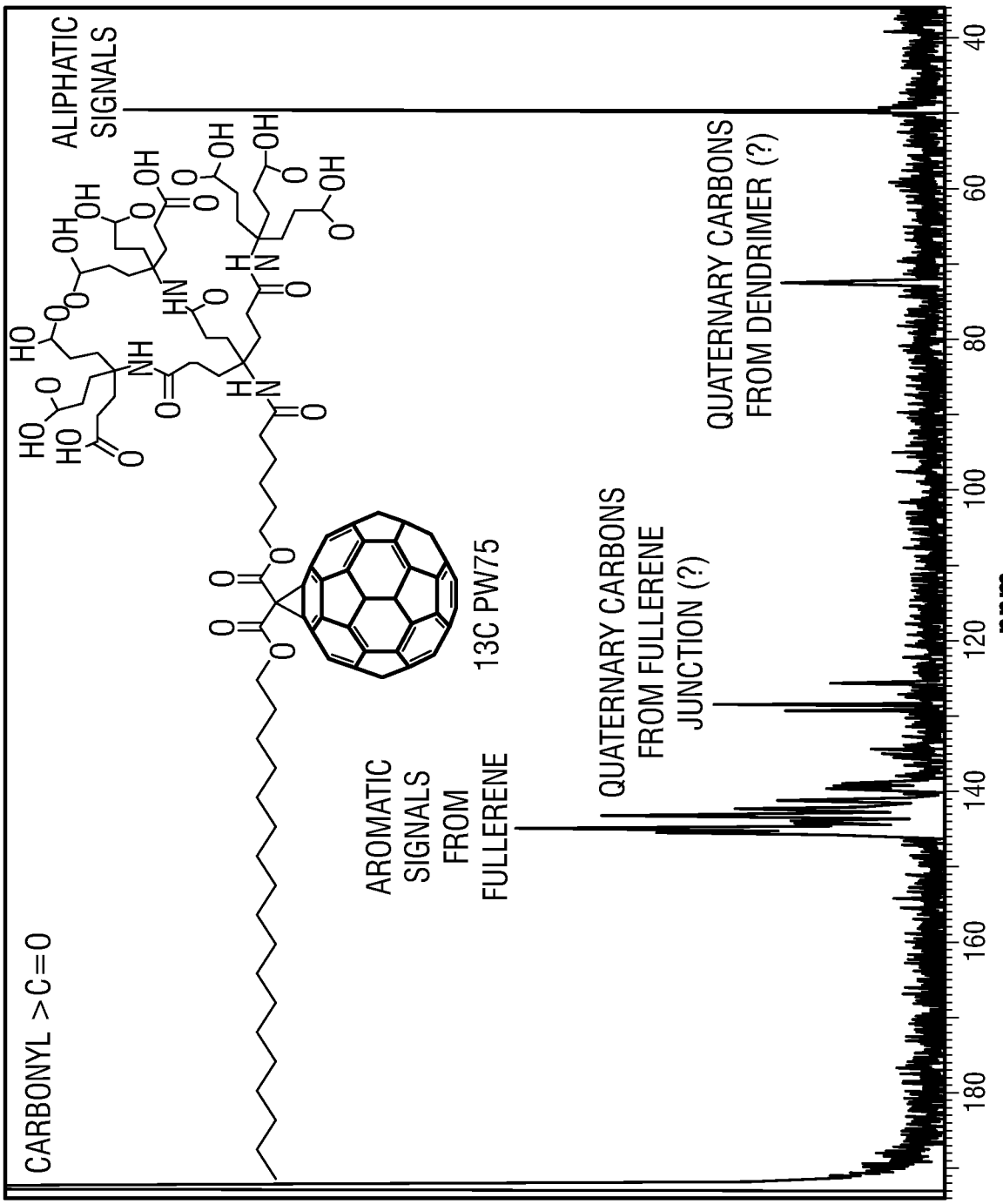

CARBONYL >C=O
AROMATIC SIGNALS FROM FULLERENE
13C PW75
QUATERNARY CARBONS FROM FULLERENE JUNCTION (?)
QUATERNARY CARBONS FROM DENDRIMER (?)
ALIPHATIC SIGNALS
ppm
180
160
140
120
100
80
60
40

FIG. 21A

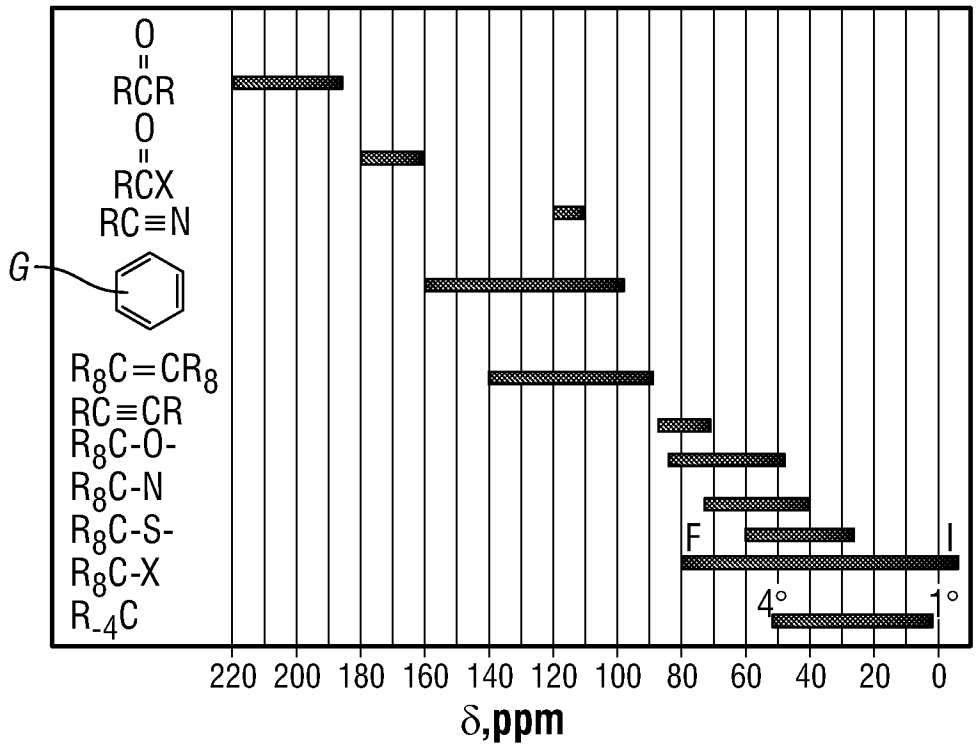


FIG. 21B

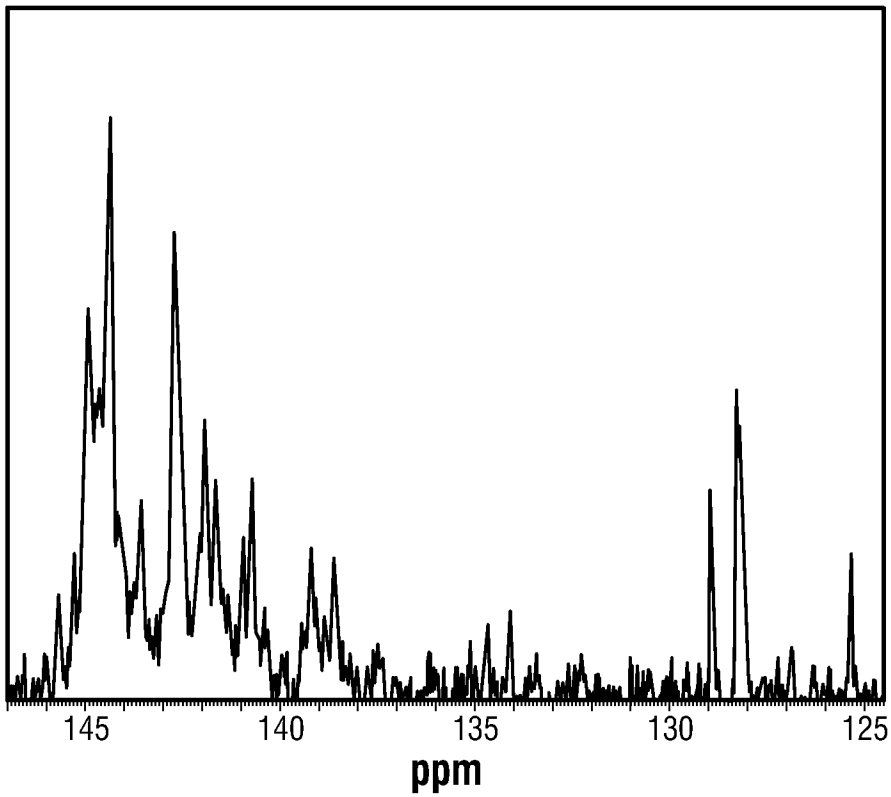


FIG. 22

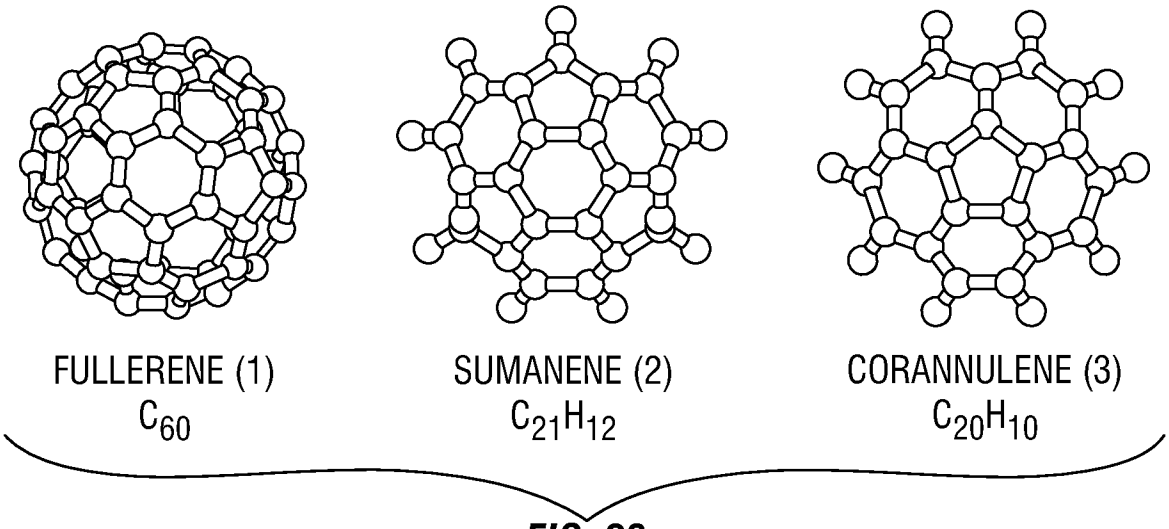


FIG. 23

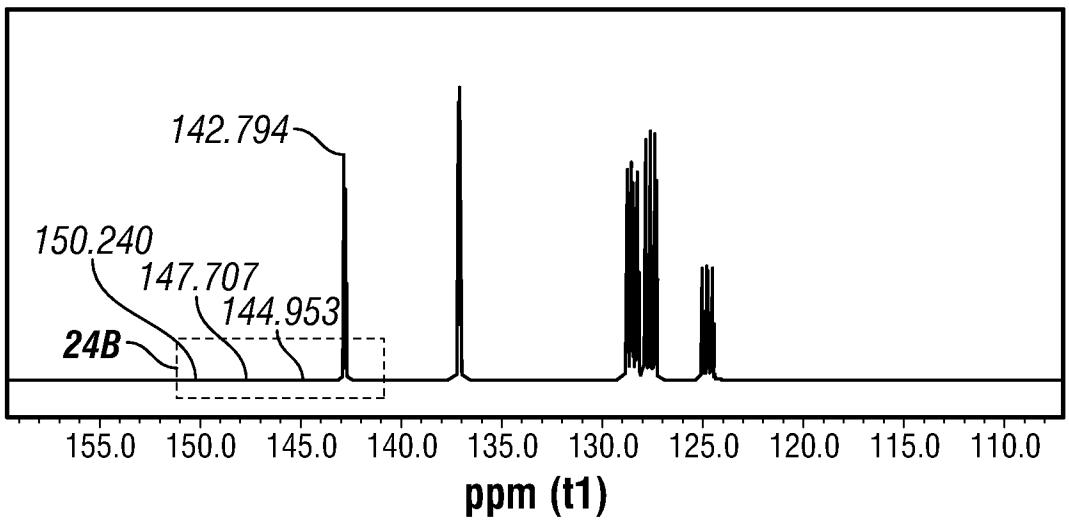


FIG. 24A

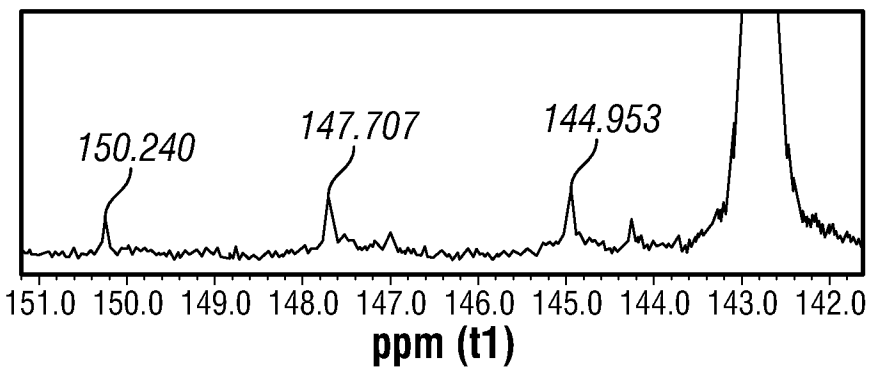


FIG. 24B

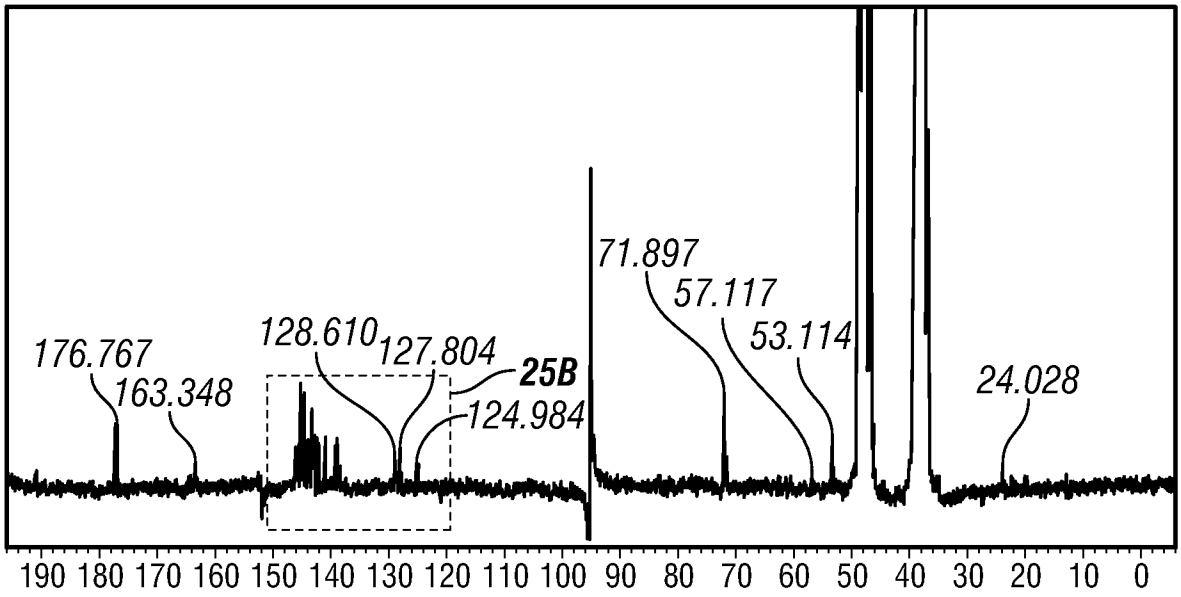


FIG. 25A

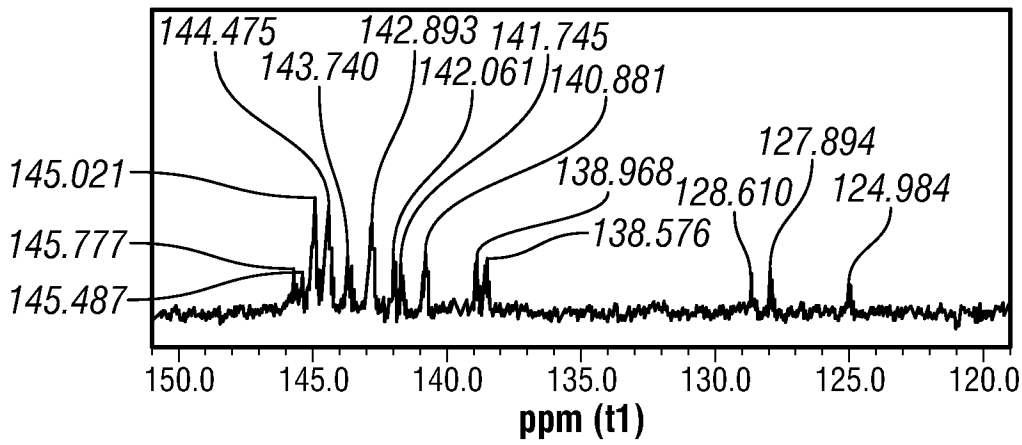


FIG. 25B

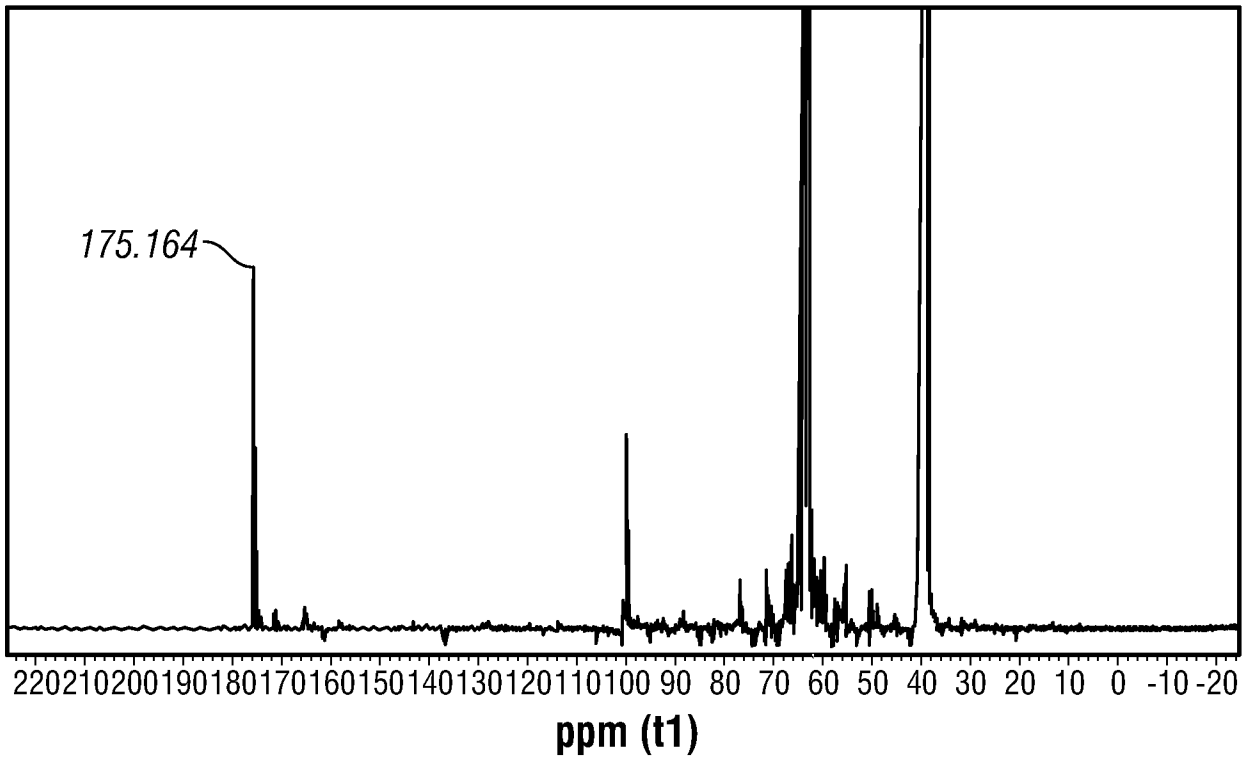

175.164
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
ppm (t1)

FIG. 26

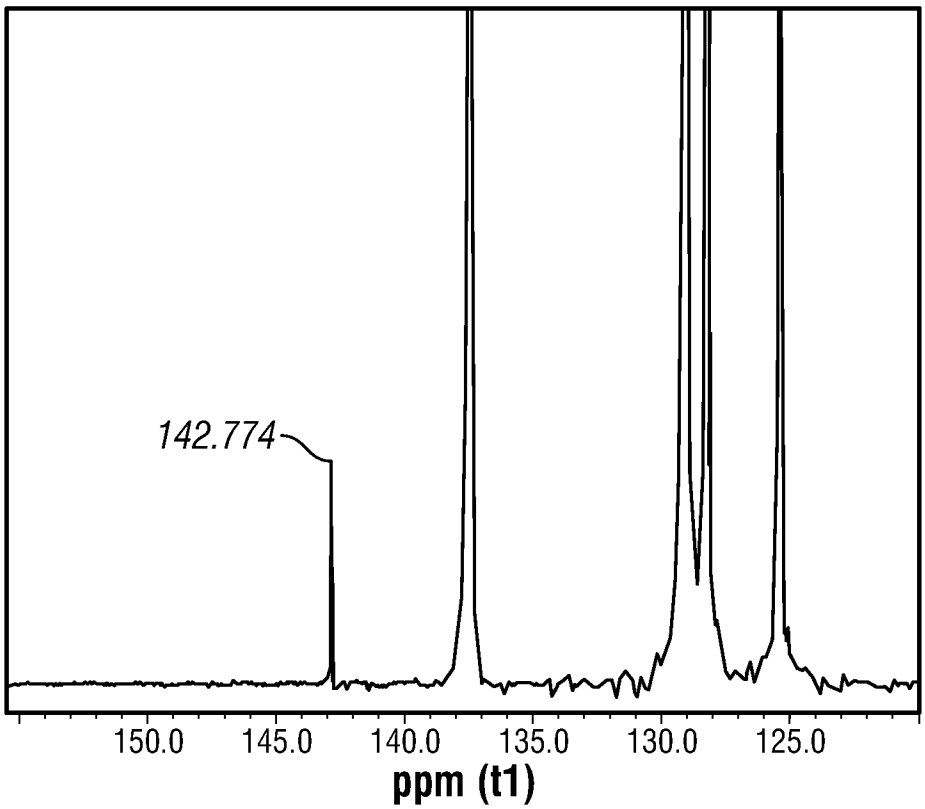

142.774
150.0 145.0 140.0 135.0 130.0 125.0
ppm (t1)

FIG. 27

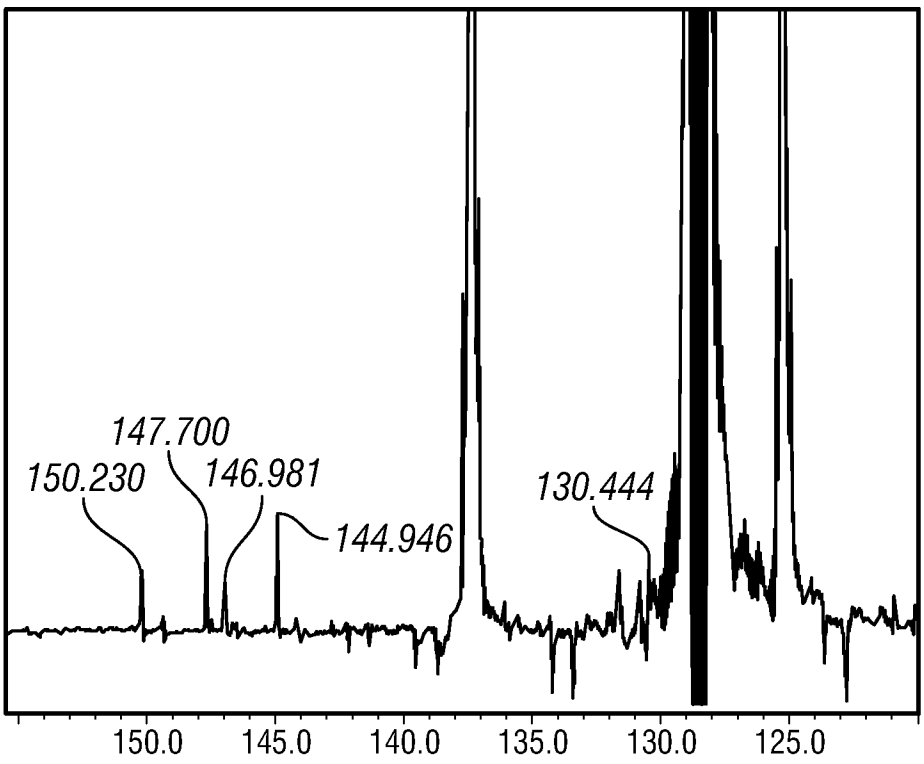

147.700
150.230
146.981
144.946
130.444
150.0
145.0
140.0
135.0
130.0
125.0
ppm (t1) Cross Section #2 (row): 0.00 ppm

FIG. 28