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(54) Title: PREPARATION AND USE OF ALKYLATING AGENTS

(57) Abrégé/Abstract:
The present disclosure provides compositions and methods to rapidly and efficiently alkylate target compounds, which may be used for the synthesis or modification of radiopharmaceuticals.

ABSTRACT

The present disclosure provides compositions and methods to rapidly and efficiently alkylate target compounds, which may be used for the synthesis or modification of radiopharmaceuticals.

PREPARATION AND USE OF ALKYLATING AGENTS

1.

[0001]

2. FIELD

[0002] This invention relates, generally, to organic chemistry and more particularly to processes for synthesizing alkylating agents and methods of use.

3. BACKGROUND

[0003] Alkylation processes have been used to synthesize and modify myriad compounds (*see*, *e.g.*, U.S. Patent Nos. 6277982, 6278020, 6281380, 6281399, 6281405, 6288233, 6291716, 6291724, 6294499, 6303840, 6307048, 6313362, 6315964, 6339179, 6355839, 6376729, 6376730, 6387705, 6388157, 6392114, 6395871, 6395945, 6423871, 6429349, 6440886, 6448458, 6479721, 6486374, 6492571, 6500998, 6504071, 6512153, 6515169, 6525234, 6528316, 6541655, 6548113, 6552241, 6555722, 6642425, 6642426, 6664432, 6673977, 6677269, 6709638, 6747152, 6750354, 6759349). These processes typically require multiple steps of synthesis and purification of reaction intermediates and/or the removal of unwanted byproducts. Therefore, these processes can be unsuitable or of limited use for the synthesis or modification of radiolabeled compounds, especially when the radioisotope is relatively short lived and the radiolabeled compound is intended for use as a radiotracer or imaging agent. Thus, there is a need in the art for rapid and efficient alkylation processes that can be used for the synthesis or modification of radiolabeled compounds.

[0004] Therefore, the present disclosure provides compositions and methods to rapidly and efficiently alkylate target compounds, which may be used for the synthesis or modification of radiopharmaceuticals.

4. SUMMARY

[0005] The present disclosure provides methods of alkylating a target compound comprising one or more alkylation reactive groups. In some embodiments, the methods comprise synthesizing an alkylating agent. In some embodiments, the alkylating agent may comprise one or more leaving

groups and an alkylation moiety. In some optional embodiments, the alkylating agent may comprise a detectable moiety. In some embodiments, the alkylation moiety may comprises a detectable moiety. In some embodiments, the detectable moiety may be a radioisotope.

[0006] In some embodiments, an alkylating agent can be utilized in an alkylation reaction without an intervening purification step. Therefore, in some embodiments, an alkylating agent, once synthesized, can be used to directly alkylate a target compound. In some embodiments, the synthesis of an alkylating agent and alkylation of a target compound may occur in a single vessel.

[0007] In some embodiments, various aspects of the disclosed methods can be automated by a general purpose or special purpose device. In some embodiments, the device may comprise a processor or computer for storing data and/or executing computer program code instructions. Therefore, in some embodiments a computer readable memory to direct a computer to function in specified manner can be utilized. The computer or processor may control any one or more aspects of the disclosed methods.

[0008] In other aspects of the disclosure are provided alkylated and/or labeled target compounds. In some embodiments, the target compounds can be labeled with a radioactive detectable moiety. Therefore, in some embodiments the alkylated and/or labeled target compounds may be used a therapeutic, tracers, and or imaging agents either *in vitro* or *in vivo*. In some embodiments, an labeled target compound can be used as an imaging agent for positron emission tomography.

[0008a] In another aspect, the invention provides a method of alkylating a target compound comprising synthesizing an alkylating agent having the formula:

$$X\text{-}(CR^1R^2)_aCR^3R^4 - LG$$

wherein,

a is 0, 1, 2 or 3,

R^1, R^2, R^3 and R^4 are independently H, X or alkyl,

X is a halogen or a label, with the proviso that at least one X is a halogen,

LG is a leaving group; and

reacting said alkylating agent with a target compound comprising an alkylation reactive group under conditions suitable for the alkylation of said target compound, wherein said synthesizing of said alkylating agent occurs at a temperature from about 40°C to less than about 80°C and wherein said reacting is accomplished without purifying said alkylating agent after said synthesizing. In one embodiment, the synthesizing of the alkylating agent occurs at a temperature from about 40°C to about 50°C. In another embodiment, at least one X is [^{18}F]. In a further embodiment, the alkylating agent is chosen from the following:

$X\text{-}CH_2CH_2\text{-}LG$,

$X\text{-}CH_2\text{-}LG$,

$CH_3\text{-}CH(X)\text{-}CH_2\text{-}LG$,

$X\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}LG$

wherein,

X is [^{18}F]; and

LG is a sulfonate ester.

[0008b] In another embodiment, a precursor of the alkylating agent has the formula $LG\text{-}(CR^1R^2)_aCR^3R^4\text{-}LG$ or $LG\text{-}(CH_2)_2\text{-}LG$. In a further embodiment, LG is tosylate, mesylate or triflate ester. In another embodiment, the alkylation reactive group comprises an alkyl, substituted alkyl, alcohol, carboxyl acid, saturated cycloalkyl, unsaturated cycloalkyl, aryl, substituted aryl, saturated heterocycle, unsaturated heterocycle, sulfhydryl, amine, N, O, S or C. In another embodiment, the target compound is morphine, heroin, pethine, tamoxifen, codeine, nicotine, thioproperazine, diazepam, caffeine, flunitrazepam, hexamethonium, methiodide, 3-quinuclidinyl benzilate (MQNB), neostigmine, N-methyl-4-phenyl-pyridinium (MPP), N-methylspiperone (NMS), tyrosine, spiperone, spiroperidol glucose, lactic acid, hexobarbital, thymidine, iodoantipyrine, antipyrine coenzyme Q or dimethylethanolamine. In another embodiment, the alkylated target compound is

[^{18}F] fluoromethyl-MQNB, [^{18}F]N-fluoromethyl-MPP, [^{18}F]N-fluoromethylspiperone (FNMS), [^{18}F]fluoroethylspiperone, [^{18}F]fluoromethyl-neostigmine, [^{18}F]fluoromethyl-tyrosine, [^{18}F]dimethyl-N-fluoromethylethanolamine or [^{18}F]N,N-dimethyl-N-fluoroethylethanolamine.

[0008c] In another embodiment, when the target compound is dimethylethanolamine, the alkylating agent has the formula:

$$^{18}F\text{-}(CH_2)_a\text{-}LG$$

wherein,

a is 1 or 2, and

LG is a leaving group.

[0008d] In another aspect, the invention provides a method of synthesizing an ^{18}F-labeled target compound comprising synthesizing an alkylating agent having the formula:

$$X\text{-}(CR^1R^2)_aCR^3R^4 - LG$$

wherein,

a is 0, 1, 2 or 3,

R^1, R^2, R^3 and R^4 are independently H, X or alkyl,

X is a halogen or a label, with the proviso that at least one X is [^{18}F],

LG is a leaving group; and

contacting the alkylating agent with a target compound comprising an alkylation reactive group under conditions suitable for the alkylation of said target compound, wherein the target compound is other than [^{18}F]fluorocholine (FCH), [^{18}F]flouroethylcholine (FECh) or [^{18}F]fluorodeoxyglucose, and wherein the synthesizing of the alkylating agent occurs at a temperature from about 40°C to less than about 80°C and wherein said reacting is accomplished without purifying said alkylating agent after said synthesizing.

[0008e] In a further aspect, the invention provides a computer readable memory to direct a computer to function in a specified manner, comprising executable instructions to direct a computer that controls a synthesis module to synthesize in a reaction vessel an alkylating agent having the formula:

$$X\text{-}(CR^1R^2)_aCR^3R^4 - LG$$

as described above, using ^{18}F ions in a fluoridation reaction; and executable instructions directing said computer that controls said synthesis module to directly react in said reaction vessel said alkylating agent with a target compound comprising an alkylation reactive group under conditions suitable to alkylate said target compound. In one embodiment, the computer readable memory further comprises executable instructions directing said computer that controls said synthesis module to bind the alkylated target compound to a first solid support, wherein said reaction vessel and said first solid support are in fluidic communication. In another embodiment, the computer readable memory further comprises executable instructions directing said computer that controls said synthesis module to elute said alkylated target compound from said first solid support. In another embodiment, the computer readable memory further comprises executable instructions directing said computer that controls said synthesis module to bind the eluted alkylated target compound to a second solid support, wherein said first and second solid supports are in fluidic communication, and to elute said alkylated target compound from said second solid support.

[0008f] In another aspect, the invention provides a system comprising a synthesis module and a computer comprising (i) at least one processor, (ii) a memory and (iii) at least one program, wherein the at least one program is stored in the memory and executable by the at least one processor, the at least one program comprising instructions to control said synthesis module to synthesize in a reaction vessel in said synthesis module an alkylating agent, and executable instructions directing said computer to control said synthesis module to directly react in said reaction vessel said alkylating agent with a target compound comprising an alkylating rective group under conditions suitable to alkylate said target compound. In one embodiment, the reaction vessel is associated with a thermal control module. In another embodiment, the system further comprises means for directing the mixing of the reactants in the reaction vessel. In one embodiment, the means for directing the mixing of the reactants in the reaction vessel comprises an inert gas feed. In another embodiment, the inert gas feed comprises an argon feed. In one embodiment, the system further comprises means for purifying the alkylated compound. In a further embodiment, the means for purifying the alkylated compound comprises one or more solid supports. In another embodiment, the one or more solid supports comprises on or more of a silica column or an ion-exchanger.

[0012] FIG. 4 depicts the structure of an [^{18}F]fluoroalkylated quaternary amine, 3-quinuclidinyl benzilate (MQNB), according to one embodiment.

[0013] FIG. 5 depicts the structure of an [^{18}F]fluoroalkylated quaternary amine, neostigmine, an acetylcholinesterase inhibitor, according to one embodiment.

[0014] FIG. 6 depicts the structure of an [^{18}F]fluoroalkylated quaternary amine, *N*-methyl-4-phenyl-pyridinium (MPP), a neurotoxin, according to one embodiment.

[0015] FIG. 7 depicts the O-fluoroalkylation of tyrosine (Tyr) using [^{18}F]fluoromethyl tosylate in dimethylsulfoxide (DMSO), according to one embodiment.

[0016] FIG. 8 depicts the O-fluoroalkylation of a carboxylic acid, benzoic acid, according to one embodiment.

[0017] FIG. 9 depicts the S-fluoroalkylation of benzyl mercaptan (α-toluenethiol), according to one embodiment.

[0018] FIG. 10 provides a schematic illustrating a system suitable for automated synthesis of an alkylating agent, the alkylation of a target compound (T_c) and the purification of an alkylated target compound, according to one embodiment.

[0019] FIG. 11 provides a flow diagram of the synthesis of an radioalkylated target compound, according to one embodiment. The numbers to the left of the diagram indicate the approximate time point of the exemplified synthesis.

[0020] FIG. 12 depicts a structure of a polymer resin containing a covalently attached quaternary ammonium salt, polystyrene 4-dialkylaminopyridinium, according to one embodiment, , wherein R^{11} and R^{12} are alkyl groups.

[0021] FIG. 13. depicts the [^{18}F]fluoroalkylation of cysteine to produce [^{18}F]-labeled methionine and the linkage of the [^{18}F]-labeled methionine to the amino terminus of a peptide according to one embodiment.

[0022] FIG. 14 shows an HPLC tracing of [^{18}F]FECh using a radioactivity detector (mV vs. min.), according to one embodiment. The tracing shows a major radioactive peak, [^{18}F]FECh.

[0023] FIG. 15 shows an HPLC tracing of [^{18}F]FECh using an ultraviolet (UV) detector (Absorbance Units (AU) vs. time), according to one embodiment. The tracing shown a major peak detectable by UV absorbance, dimethylethanolamine. [^{18}F]FECh is not detectable by UV absorbance.

[0024] FIG. 16 shows an HPLC tracing of [^{18}F]FCH using a radioactivity detector (mV vs. min.), according to one embodiment. The tracing shows a major radioactive peak, [^{18}F]FCH;

[0025] FIG. 17 shows an HPLC tracing of [^{18}F]FCH using a radioactivity detector (mV vs. min.), according to one embodiment. The tracing shown a major peak detectable by UV absorbance, dimethylethanolamine. [^{18}F]FCH is not detectable by UV absorbance.

6. DETAILED DESCRIPTION

[0026] The present disclosure is directed to the discovery of methods of synthesizing an alkylating agent and alkylating a target compound. In some embodiments, the alkylating agent can be labeled. In some embodiments, the label can be transferred to a target compound in an alkylation reaction. In some embodiments, an alkylated target compound finds use as a tracer molecule, imaging agent or therapeutic. Thus, in some embodiments, a labeled alkylated target compound can be a radiopharmaceutical that can be used to determine the metabolic or physiologic status of a cell or tissue *in vivo* or *in vitro*. Therefore, in some embodiments the present disclosure provides methods of detecting or monitoring a radioactive compound in a tissue or cell. In some embodiments, the detected or monitored radioactive compound can be used to assess the metabolic or physiologic status of a cell or tissue.

[0027] In some embodiments, the disclosed methods comprise synthesizing an alkylating agent and the alkylation of a target compound in a single reaction vessel. In some embodiments, the synthesis and alkylation reactions can be a coupled procedure without an intervening purification step. In some embodiments, the methods further comprise isolating or purifying an alkylated target compound.

[0028] In some embodiments, one or more aspects of the disclosed methods can be automated. Thus, in various embodiments, the disclosure provides one or more automated modules each capable of performing and/or providing reaction conditions suitable for one or more steps of the disclosed methods. In some embodiments, one or more modules can be controlled by a device having a processor for storing data and/or commands.

[0029] "Alkylating agent", "alkylation agent" and grammatical equivalents herein refer to compounds having a moiety suitable for forming an alkyl group that can be transferred to an alkylation reactive center or group of another compound, *e.g.*, a target compound, in an alkylation reaction. By "alkylation reaction", "alkylating", "alkylation" and grammatical equivalents herein are meant a reaction in which an alkyl group is transferred to an alkylation reactive center or group of a compound by substitution and/or addition. Thus, in some embodiments an alkyl group can be transferred to an alkylation reactive center of a target compound to form an alkylated target compound. By "alkylation reactive center", alkylation reactive group" and grammatical equivalents herein are meant at least one atom of a compound that reacts with an alkylating agent in a process in which an alkyl group can be transferred to the compound from the alkylating agent. In some embodiments, an "alkyl group" and grammatical equivalents herein refers to any of a series of univalent groups of the general formula $C_nH_{(2n+1)}$. Thus, in various exemplary embodiments an alkyl group can be methyl, ethyl, propyl, isopropyl, C_2H_3 and the like, and as further described below. In some embodiments, an alkyl group may further comprise a detectable moiety.

[0030] In various exemplary embodiments, an alkylation reaction may comprise a nucleophilic attack of an alkylation reactive center of a target compound to an electron deficient region of an alkylating agent according to an SN_1 or SN_2 mechanism, as known in the art (*see, e.g.,* Jerry March, *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*. 293-871 (4^{th} ed. John Wiley & Sons 1992)). Therefore, the skilled artisan will appreciate that in some embodiments, an alkylating agent further comprises one or more leaving groups (LGs). By "leaving group" and grammatical equivalents herein are meant an atom or molecule that detaches from a molecule, *e.g.*, an organic molecule. In some embodiments, the residual part can be the alkyl group which becomes covalently bonded to a target compound. Accordingly, in various

exemplary embodiments, a leaving group can be an atom or group, charged or uncharged, that becomes detached from an atom or molecule in what is considered the residual or main part of the substrate in a specified reaction. The ability of a leaving group to leave the alkylating agent can be a function of the leaving group's lability. Thus, a leaving group can affect the intrinsic reactivity of the alkylating agent in an alkylation reaction. In some embodiments, the lower the pKa of the conjugate acid of the leaving group, the better the leaving group, because, in some embodiments, the leaving group can more easily stabilize the developing negative charge that can occur in an alkylation reaction. Therefore, in some embodiments, a leaving group can be an electronegative atom or molecule. Examples of leaving groups include, but are not limited to, acetate (AcO), *p*-nitrobenzoate (PNBO), sulfonates (*e.g.*, methanesulfonate (Mesylate: MsO), *p*-toluenesulfonate (tosylate: TsO), *p*-bromobenzenesulfonate (Brosylate: BsO), *p* -nitrobenzenesulfonate (Nosylate: NsO), fluoromethanesulfonate, difluoromethanesulfonate, trifluoromethanesulfonate (Triflate: TfO) and ethanesulfonate), NH_3, halide esters, halogen ions (*e.g.*, I^-, Br^-, Cl^-) and H_2O. Therefore, in various exemplary embodiments, alkylation agents include but are not limited to $(TsO)_2CH_2$, $(TsO)CH_3$, $(TsO)_2C_2H_5$, $(TsO)_2C_3H_7$, $(MsO)C_2H_5$, $(I)_2CH_2$, $(I)CH_3$, $(I)_2C_2H_5$, $(I)_2C_3H_7$, $(I)C_2H_5$, $(Br)_2CH_2$, $(Br)CH_3$, $(Br)_2C_2H_5$, $(Br)_2C_3H_7$, $(Br)C_2H_5$, and the like.

[0031] In some embodiments, an alkylating agent comprises one or more labels. In some embodiments, the a group or moiety suitable for transfer to a target compound (*i.e.*, an alkylation moiety) comprises one or more labels. By "label", "detectable moiety", and grammatical equivalents herein are meant any distinguishing feature of a molecule or compound suitable for detecting or monitoring. Accordingly, a label may be an inherent feature of a compound and/or a moiety attached either covalently or non-covalently to a compound. Labels are well-known in the art and are chosen at the discretion of the practitioner based on the method of synthesizing, using and/or detecting a compound comprising the label. The skilled artisan will appreciate that in some embodiments a label does not substantially interfere or inhibit a function or an activity of the compound comprising the label. Therefore, in some embodiments a label of an alkylating agent may not substantially interfere with the alkylation of another compound, *e.g.*, a target compound, by the alkylating agent. Determining the degree or extent to which a label may

interfere with an activity or function of a compound to which it can be attached is within the abilities of the skilled artisan.

[0032] In various exemplary embodiments, labels include but are not limited to, a fluorophore (*e.g.*, Richard P. Haugland, *Handbook of Fluorescent Probes and Research Chemicals Sixth Edition* (Michelle T.Z. Spence, ed., Molecular Probes, Inc. 1996));

a ligand (*e.g.*, a hapten, biotin), a mobility modifier (*e.g.*, U.S. Pat. Nos. 5470705, 5514543, 6395486), an encoded microbead (*e.g*, U.S. Pat. Nos. 6630307, 6500622, 6274323), an enzymatic label, organic or inorganic compounds, a radiolabel (*e.g.*, ^{18}F, ^{11}C, ^{14}C, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{76}Br), or combinations thereof. In some embodiments, a radiolabel preferably has a high specific activity, such as, at least about 600 mCi/mmol or higher and/or a short positron range, such as, from about 2 mm to about 5 mm. In some embodiments, the position range can be less than about 2 mm. Accordingly, in some embodiments, a radiolabel can be a suitable positron emitter for radioimaging techniques including but not limited to positron emission tomography (PET).

[0033] In some embodiments, an alkylating agent has the structure of Formula I and comprises an alkylation moiety, a leaving group and a label:

$$X(CR^1R^2)_aCR^3R^4\text{–}LG \qquad (I)$$

[0034] wherein,

a is an integer from 0 to 3;

R^1, R^2, R^3, and R^4 are independently H, X, or alkyl;

X is a H, a halogen, or a label; and

LG is a leaving group.

[0035] In some embodiments:

a is 1;

R^1, R^2, R^3, and R^4 are H;

X is a ^{18}F, ^{123}I, ^{124}I, ^{125}I, ^{131}I or ^{76}Br; and

LG is a sulfonate ester.

[0036] In some embodiments:

a is 0;

R^3 and R^4 are H;

X is [^{18}F]; and

LG is tosylate, mesylate or triflate.

[0037] In some embodiments:

a is 0;

R^3 and R^4 are H or X;

X is [^{18}F]; and

LG is tosylate, mesylate or triflate.

[0038] Thus, in various exemplary embodiments, alkylating agents comprising radiolabels include but are not limited to haloalkylsulfonates, such as, [^{18}F]fluoroalkylsulfonates (*e.g.*, [^{18}F]fluoroethane sulfonates (*e.g.*, [^{18}F]fluoroethane tosylate, [^{18}F]fluoroethane mesyltate, [^{18}F]fluoroethane triflate), [^{18}F]fluoromethane sulfonates (*e.g.*, [^{18}F]fluoromethane tosylate, [^{18}F]fluoromethane mesylate, [^{18}F]fluoromethane triflate)), [^{76}B]bromoalkylsulfonates (*e.g.*, [^{76}B]bromoethane sulfonates (*e.g.*, [^{76}B]bromoethane tosylate, [^{76}B]bromoethane mesyltate, [^{76}B]bromoethane triflate), [^{76}B]bromomethane sulfonates (*e.g.*, [^{76}B]bromomethane tosylate, [^{76}B]bromomethane mesylate, [^{76}B]bromomethane triflate)), [^{125}I]iodoalkylsulfonates (*e.g.*, [^{125}I]iodoethane sulfonates (*e.g.*, [^{125}I]iodoethane tosylate, [^{125}I]iodoethane mesyltate, [^{125}I]iodoethane triflate), [^{125}I]iodomethane sulfonates (*e.g.*, [^{125}I]iodomethane tosylate, [^{125}I]iodomethane mesylate, [^{125}I]iodomethane triflate)), and the like.

[0039] In various exemplary embodiments, alkylating agents find use in methods of alkylating or labeling a target compound. "Target compound" and grammatical equivalents herein refer to a compound comprising one or more alkylation reactive centers and, accordingly, can be alkylated by any one or more alkylating agents, either alone or in any combination, by the methods disclosed herein. In some embodiments, two or more target compounds may be alkylated by any one or more alkylating agents either simultaneously and/or sequentially. Determining the suitability of one or more target compounds or one or more alkylation reactive centers for

alkylation by any one or more alkylating agents either simultaneously or sequentially is within the abilities of the skilled artisan. In some embodiments, a target compound comprises a label, as described above. Accordingly, in some embodiments, a label does not substantially interfere with or inhibit the alkylation and/or use of the alkylated target compound.

[0040] In some embodiments, the alkylation reactive center or group of a target compound can be a suitable nucleophile for an alkylation reaction. Examples of alkylation reactive centers include but are not limited to a N, O, S, P, C, aldehydes, aliphatic carbons, alkanes, alkenes, alkynes, alcohols (-OH), amines (*e.g.*, primary, secondary, tertiary and quaternary amines), aromatic compounds (*e.g.*, benzene, phenol), carboxylic acids (-COOH), esters, diazonium ions, dithianes, enamines, enolates, heterocycles, hydrazones, imines, ketones, nitriles, oxazines, oxazolines, selenoxides, sulfones, sulfonates, and cyclic, linear, and branched, and substituted and unsubstituted derivatives thereof. *Dictionary of Chemical Terms* (Parker, *et al.* eds., McGraw-Hill Book Co.), *Encyclopedia of Chemistry* (Considine, *et al.* eds., 4th ed., Van Nostrand Reinhold Co.), March, *Advanced Organic Chemistry*). In a some embodiments, an alkylation reactive group can be an alkyl, substituted alkyl, saturated and unsaturated cycloalkyl, aryl, substituted aryl, sulhydryl (-SH), amino, and saturated and unsaturated heterocycles comprising, for example, one or more of nitrogen, oxygen, sulfur, and combinations thereof.

[0041] By "alkyl group" herein is meant a saturated or unsaturated, branched, straight-chain or cyclic monovalent hydrocarbon group derived by the removal of one hydrogen atom or leaving group from a single carbon atom of a parent alkane, alkene or alkyne. If branched, it may be branched at one or more positions and unless specified at any position. Typical alkyl groups include, but are not limited to, methyl; ethyls such as ethanyl, ethenyl, ethynyl; propyls such as propan-1-yl, propan-2-yl, cyclopropan-1-yl, prop-1-en-1-yl, prop-1-en-2-yl, prop-2-en-1-yl (allyl), cycloprop-1-en-1-yl; cycloprop-2-en-1-yl, prop-1-yn-1-yl , prop-2-yn-1-yl, *etc.*; butyls such as butan-1-yl, butan-2-yl, 2-methyl-propan-1-yl, 2-methyl-propan-2-yl, cyclobutan-1-yl, but-1-en-1-yl, but-1-en-2-yl, 2-methyl-prop-1-en-1-yl, but-2-en-1-yl , but-2-en-2-yl, buta-1,3-dien-1-yl, buta-1,3-dien-2-yl, cyclobut-1-en-1-yl, cyclobut-1-en-3-yl, cyclobuta-1,3-dien-1-yl, but-1-yn-1-yl, but-1-yn-3-yl, but-3-yn-1-yl, *etc.*, and the like.

[0042] Thus, in some embodiments, "alkyl groups" refers to groups having any degree or level of saturation, *i.e.*, groups having exclusively single carbon-carbon bonds, groups having one or more double carbon-carbon bonds, groups having one or more triple carbon-carbon bonds and groups having mixtures of single, double and triple carbon-carbon bonds. Where a specific level of saturation is intended, the expressions "alkanyl," "alkenyl," and "alkynyl" are used. In various exemplary embodiments, an alkyl group comprises from about 1 to about 20 carbon atoms (C_1-C_{20} alkyl), from about 1 to about 15 carbon atoms (C_1-C_{15} alkyl), from about 1 to about 10 carbon atoms (C_1-C_{10} alkyl), or from about 1 to about 6 carbon atoms (C_1-C_6 alkyl), or from about 1 to about 3 carbon atoms (C_1-C_3).

[0043] The term "substituted alkyl group" refers to an alkyl group as defined above in which one or more of the hydrogen atoms can be replaced by a substituent group, for example, a halide (*e.g.* F, Br, I, Cl), alkyl, amine, aromatic compound, aryl, hydroxyl, carbonyl, diazo, imines, nitrile, nitro, sulhydryl and sulfonyl.

[0044] The term "cycloalkyl group" refers to an alkyl group as defined above having cyclic structure having up to about 20 carbon atoms (C_{20}). In various exemplary embodiments a cycloalky can be mono- or polycyclic, *e.g.*, bicyclic. A cycloalkyl group may optionally contain one or more carbon-carbon double bounds provided that the group is not aromatic. Therefore, in various exemplary embodiments a cycloalky group can be saturated or unsaturated.

[0045] As used herein "aryl group" refers to an aromatic monocyclic or polycyclic hydrocarbon group containing about 6 to about 20 carbon atoms (C_6-C_{20} aryl), from about 6 to about 15 carbon atoms (C_6-C_{15} aryl) or from about 6 to about 10 carbon atoms (C_6-C_{10} aryl) and any carbocylic ketone or thioketone derivative thereof, wherein the carbon atom with the free valence is a member of an aromatic ring, (*e.g.*, aryl includes phenyl, naphthyl, anthracenyl, phenanthrenyl, 1,2,3,4-tetrahydro-5-naphthyl, 1-oxo-1,2-dihydro-5-naphthyl, 1-thioxo-1,2-dihydro-5-naphthyl, *etc.*).

[0046] In some embodiments, an aryl can be a heteroaryl. "Heteroaryl group" refers to aromatic monocyclic or polycyclic hydrocarbon group containing overall from 6 to 20 atoms, wherein at

least 1 to about 5 of the indicated carbon atoms are replaced by a heteroatom chosen from N, O, S, P or As, wherein the atom with the free valence is a member of an aromatic ring, and any heterocyclic ketone and thioketone derivative thereof (*e.g.*, thienyl, furyl, pyrrolyl, pyrimidinyl, isoxazolyl, oxaxolyl, indolyl, benzo[b]thienyl, isobenzofuranyl, purinyl, isoquinolyl, pterdinyl, pyrimidinyl, imidazolyl, pyridyl, pyrazolyl, pyrazinyl, 4-oxo-1,2-dihydro-1-naphthyl, 4-thioxo-1,2-dihydro-1-naphthyl, *etc.*). Thus, hetero(C_6)aryl includes the groups pyridyl, pyrimidinyl, and the like.

[0047] In some embodiments, an aryl can be a subtituted aryl. "Substituted aryl group" refers to an aryl group as defined above in which one or more of the hydrogen atoms can be replaced by a substituent group, for example, a halide (*e.g.* F, Br, I, Cl), alkyl, amine, aromatic compound, aryl, hydroxyl, carbonyl, diazo, imines, nitrile, nitro, sulhydryl and sulfonyl.

[0048] As used herein, "heterocycle" refers to ring composed of atoms of more than one kind. Therefore, in some embodiments a heterocycle includes but is not limited to a carbon ring having at least one other kind of atom in the ring. In various exemplary embodiments a heterocycle can be saturated or unsaturated. In some embodiments, heteroatoms can be attached to the ring structure. In some embodiments, a heterocycle can have up to 20 atoms in one or more rings. Therefore, in various exemplary embodiments a heterocycle can mono- or polycyclic, *e.g.*, bicyclic.

[0049] Accordingly, in various exemplary embodiments, a target compound may comprise an organic compound, an inorganic compound, a naturally occurring compound (*e.g.*, isolated from nature (*e.g.*, polypeptide, a nucleic acid, a hormone, a cytokine, an antibody and the like), a non-naturally occurring compound (*e.g.*, synthetic or not known to occur in nature, *e.g.*, peptide nucleic acids (PNA)), a pharmaceutical (*e.g.*, an antiviral, a pro-drug) and/or combinations thereof. In some embodiments, a target compound can be a biological compound. By "biological compound", "biochemical compound" and grammatical equivalents herein are meant a compound having at least one biological or biochemical activity *in vivo* (*e.g.*, in a subject) or *in vitro* (*e.g.*, in tissue culture or in an assay). Therefore, in various exemplary embodiments a biologically active compound may specifically or non-specifically bind to another molecule and/or cell and/or enter

a cell of interest. In some embodiments, a cell may be a prokaryotic cell, a eukaryotic cell (*e.g.*, a tumor cell), a corpuscle, an anucleate cell, an enucleate cell and the like. In various exemplary embodiments, binding can be to a molecule (*e.g.*, an antibody or binding fragment thereof), a class of molecules (*e.g.*, MHC Class II molecules), a specific receptor or class of receptors and the like. In some embodiments, a biologically active compound preferably traverses one or cellular membrane(s) and/or cellular walls. In some embodiments, a biologically active compound may accumulate in a cellular organelle or region (*e.g.*, cytoplasm, mitochondria, endoplasmic reticulum, one or more surfaces of a membrane *etc.*). A biologically active compound may enter a cell by various mechanisms, such as, active or passive mechanisms. These mechanisms include but are not limited to, diffusion and endocytic mechanisms, *e.g.*, receptor-mediated endocytosis, and active and passive transport. In some embodiments, a biologically active compound is taken up by a cell of interest at a rate or magnitude that substantially exceeds other cells. Thus, the present disclosure contemplates biologically active compounds that enter a cell of interest at a rate that exceeds other cells and biologically active compounds that accumulate in a cell of interest at a final concentration that exceeds the concentration in other cells. For example, in some embodiments, a biological compound may preferentially enter a tumor cell in comparison to a non-tumor cell.

[0050] In some embodiments, upon entry into a cell, a biologically active compound can be metabolized by the cell, whereby the hydrophobicity of the biologically active compound can be altered. In some embodiments, the hydrophobicity of the biologically active compound, once modified, is decreased to facilitate retention of the compound in a cell. The hydrophobicity of the biologically active compound may be decreased by a number of cellular processes, such as, the addition of a polar (*e.g.*, hydroxyl) or charged group (*e.g.*, phosphate, carboxylate), by oxidation (*e.g.*, conversion of an hydroxyl group to a carbonyl), or by the removal of a hydrophobic group (*e.g.*, alkyl). The method by which the hydrophobicity of a biologically active compound can be decreased is within the abilities of the skilled artisan and can be based on the biologically active compound and the metabolic process of the cell of interest.

[0051] Examples of biologically active compounds include but are not limited to choline, dimethylethanolamine (DeGrado, *et al.* (2001) *J. Nucl. Med.* (42:1805-1814); U.S. Pat. No.

6630125; Hara, *et al.* (2002) *J. Nucl. Med.* 43(2):187-199; Hara, *et al.* Japanese Patent Office, Patent Journal (A), Kokai Patent Application No. HEI 9[1997]-48747), morphine, heroin, pethine, tamoxifen, codeine, nicotine, thioproperazine, diazepam, caffeine, flunitrazepam, hexamethonium, methiodide, quinuclidinyl benzilate, glucose, deoxyglucose, lactic acid, hexobarbital, thymidine, iodoantipyrine, antipyrine, coenzyme Q, adenosylmethionine, phenylamines (Huang, *et al.* (2002) *Nuc. Med. Biol.* 29(741-751), CP-126,998 (Musachio, *et al.* (2002) *Nucl. Med. Biol.* 29:547-552), cocaine derivatives (Schönbächler, *et al.* (2002) *Nucl Med. Biol.* 29:19-27), proteins and peptides (U.S. Pat. No. 6358489), neurokinin-1 receptor antagonists (U.S. Pat Nos. 6241964, 6187284), spiroperidols (U.S. Inv. Reg. H1209, U.S. Pat. No. 4871527), fatty acids (U.S. Pat No. 6362352), platelet GPIIb/IIIa receptor antagonists (U.S. Pat. Nos. 5879657, 6022523), fluoromisonidazole (U.S. Pat. No. 5886190), tamoxifen derivatives (U.S. Pat. No. 5219548), opioid ligands (U.S. Pat. No. 4775759; Ravert, *et al.* (2002) *Nucl. Med. Biol.* 29:47-53; Ogawa, *et al.* (2001) *Nucl. Med. Biol.* 28:941-947), non-steroidal aromatic compounds (U.S. Pat. No. 6019957), amino acid analogs (U.S. Pat. Nos. 5187776, 5808146), MQNB, neostigmine, MPP, NMS, amino acids (*e.g.*, tyrosine (*e.g.*, -OH), serine (*e.g.*, -OH), threonine (*e.g.*, -OH), cysteine (*e.g.*, thio), aspartic acid (*e.g.*, -COOH), glutamic acid (*e.g.*, -COOH), carboxylic acids (*e.g.*, benzoic acid and amino acids), spiperone, spiroperidol.

[0052] An alkylating agent can be synthesized from a disubstituted alkyl precursor. Therefore, in some embodiments, a precursor of an alkylating agent may have the structure of Formula II:

$$LG^1\text{–}(CR^1R^2)_aCR^3R^4\text{–}LG^2 \qquad \text{(II)}$$

[0053] wherein,

a is 0 to 2;

R^1, R^2, R^3, and R^4 are independently H, a label, CH_3, C_2H_3; and

LG^1 and LG^2 are leaving groups.

[0054] In some embodiments:

a is 0 to 1;

R^1, R^2, R^3, and R^4 are H; and

LG^1 and LG^2 are sulfonate esters or ^{18}F, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{76}Br.

[0055] In some embodiments:

a is 0 to 1;

R^1, R^2, R^3, and R^4 are H; and

LG^1 and LG^2 are independently tosylate, mesylate or triflate.

[0056] In some embodiments:

a is 1;

R^1, R^2, R^3, and R^4 are H; and

LG^1 and LG^2 are both tosylate, mesylate or triflate.

[0057] In some embodiments:

a is 0;

R^3, and R^4 are H; and

LG^1 and LG^2 are both tosylate, mesylate or triflate.

[0058] Thus, in various exemplary embodiments, a precursor of an alkylating agent includes but is not limited to disulfonate esters, *e.g.*, ditosylethane, dimesylethane, ditriflylethane, ditosylmethane, dimesylmethane and ditriflylmethane.

[0059] In some embodiments, an alkylating agent can be synthesized by halogenating the precursor thereof. In some embodiments, a halogen may be a radioisotope (*e.g.*, ^{18}F, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{76}Br) and, therefore, also can be a label. In a preferred embodiment, a halogen can be ^{18}F. In some embodiments, ^{18}F can be [^{18}F]fluoride and can be produced by any number of methods as known in the art. In some embodiments, ^{18}F can be produced by irradiating [^{18}O]H_2O with high energy protons in a cyclotron to produce [^{18}F]HF/[^{18}O]H_2O, as known in the art **400**. In some embodiments, [^{18}F]fluoride can be purified by chromatography, including but not limited to, ion-exchange chromatography **410** and other methods, as known in the art.

[0060] Methods of producing other radioisotopes are known in the art (*see, e.g., Handbook of Radiopharmaceuticcals* (Welch and Radvanly, eds., John Wiley & Sons, ©2003) and *Chemists' Views of Imaging Centers* 291-295 (Emran, ed., Penum Press 1995)). For example, methods of producing iodoradionuclides can be found in Finn, *Chemistry Applied to Iodine Radionuclides*, in *Handbook of Radiopharmaceuticals* 423-400 (Welch and Redvanly, eds., John Wiley & Sons, ©2003), and Washburn, *et al., Production and Application of ^{123}I-Labeled M-Iodobenzylguanidine (^{123}I-MIBG)* in *Chemists' Views of Imaging Centers* 291-295 (Emran, ed., Penum Press 1995). Methods of producing bromoradionuclides can be found in Rowland, *et al., Radiobromine for Imaging and Therapy* 441-465 in *Handbook of Radiopharmaceuticcals* 423-400 (Welch and Radvanly, eds., John Wiley & Sons, ©2003).

[0061] In some embodiments, halogenating a precursor can be as described by Block *et al.*, 1987, *J. Label. Compds. Radiopharm.* 24:1029-41. However, in some embodiments, the temperature of the halogenation reaction can be important in determining the yield of the alkylating agent. Therefore, in various exemplary embodiments the temperature of a halogenation reaction can be from about 40°C to less than 80°C, from about 40°C to about 50°C, from about 45°C to about 50°C, or from about 20°C to about 50°C. Generally, without being bound by theory, when halogenating an alkylating agent precursor, the alkylating agent that is synthesized can be more reactive with the halogen than the precursor which results in the synthesis of byproducts, including but not limited to dihaloalkanes. Therefore, lower temperatures (*e.g.*, less than about 50°C) can decrease the synthesis of byproducts. The skilled artisan will appreciate that lower temperatures decrease the rate of the halogenation reaction. Therefore, in some embodiments wherein the halogen is a radioisotope, the temperature and rate of the reaction can be adjusted to minimize the extent of radioisotope decay during the reaction. Determining the range of optimum temperatures and rates of the halogenation reaction is within the abilities of the skilled the artisan. However, in some embodiments reaction temperatures less than about 40°C may be unsuitable when the radioactive isotope has a half-life that is less than about 2 hours. In some embodiments, halogenation can occur in the presence of a catalyst, including but not limited to a Kryptoflix® (*e.g.*, Kryptoflix® 2.2.2) or a basic tetralkylammonium salt (*e.g.*, tetrabutylammonium bicarbonate) **410**. In some embodiments, potassium carbonate can

provide a counter-ion for a halide ion, *e.g.*, [^{18}F]fluoride. However, selecting the source and type of a counter-ion is within the abilities of the skilled artisan and can be influenced by the type of halide ion and/or target compound selected at the discretion of the practitioner. In some embodiments, the halogenation reaction can be agitated by any means, including but not limited to shaking, mixing and bubbling a substantially inert gas into the reaction (*e.g.*, Argon, Nitrogen, Helium and the like) **450**. In some embodiments, the agitation can be maintained as long as possible throughout the reaction.

[0062] In various exemplary embodiments, solid supports or resins can be used to minimize or inhibit the synthesis of dihaloalkyl byproducts. In some embodiments, the solid support or resin binds to a halide ion which can be used to halogenate a precursor. In some embodiments, the alkylating agent precursor can be a disulfonate ester, as described above. In some embodiments, a resin can be a polymer resin, *e.g.*, polystyrene, containing a covalently attached quaternary ammonium salt (*e.g.*, quaternary 4-dialkylaminopyridinium salts) (FIG. 12). In some embodiments a solid support can be QMA.

[0063] In some embodiments, the purified halide ions, *e.g.*, [^{18}F]fluoride, can be dried using methods as known in the art, including but not limited to azeotropic drying under an Argon stream at a temperature from at least about 110°C to at least about 115°C **420** and cooled **430**. In some embodiments, the azeotropic drying temperature may be higher or lower than this range, however, the skilled artisan will appreciate the affect of drying on the suitability of the halide ion for the synthesis of the alkylating agent and downstream uses of the alkylating agent rather than the actual temperature at which drying occurs.

[0064] Once made, an alkylating agent, in alternative embodiments, may be used with or without purification to alkylate or label a target compound **460**. By "purification," "purify," and grammatical equivalents herein are meant to decrease the amount of extraneous matter, *e.g.*, a byproduct, from a substance of interest. Therefore, in some embodiments an alkylating agent, once made, can be used directly to alkylate or label a target compound. In some embodiments, the target compound can be added directly to the vessel used to synthesize the alkylating agent. Therefore, in some embodiments an alkylating agent and an alkylated target compound can be

produced in coupled reaction which in some embodiments may be a "one-pot, two-step" procedure.

[0065] In some embodiments, the amount of the target compound can be liquid at the start of the alkylation reaction and can be from about 0.2 mL to about 0.5 mL. In some embodiments, the target compound can be used neat. In some embodiments, the target compound can be diluted or dissolved in a solvent including but not limited to acetonitrile, dimethylsulfoxide (DMSO), dimethylformamide (DMF) and tetrahydrofuran (THF). Selecting the type of solvent for use with a target compound is within the abilities of the skilled artisan. In various exemplary embodiments, the alkylation of the target compound can occur at about 100°C, about 100°C to about 130°C, or about 100 to about 110°C, or about 100 to about 105°C, for about 10 min. to about 25 min. and can be agitated as described above **460**. Selecting a temperature and reaction period is within the abilities of the skilled artisan.

[0066] In some embodiments, a target compound is alkylated according to Scheme 1:

Scheme 1

$$LG\text{–}(CH_2)_nR^5 \;+\; Q_mZ \;\rightarrow\; Q_mZ(CH_2)_nR^5$$

wherein,

n is an integer from 1 to 3;

R^5 is H or a label (X);

LG is a leaving group;

Q_mZ is an alkylation reactive center of a target compound, wherein, *m* is an integer from 1-4 and each Q is independently H, alkyl, substituted alkyl, cycloalky, aryl and substituted aryl group, and wherein Z is N, O, S, P or C.

[0067] In some embodiments,

n is an integer from 1 to 2;

R^5 is a label (X);

LG is a sulfonate ester;

Q_mZ is an alkylation reactive center of a target compound, wherein, *m* is an integer from 1-4 and each Q is independently H, alkyl, substituted alkyl, cycloalkyl, aryl and substituted aryl, and wherein Z is N, O, S, P or C.

[0068] In some embodiments,

n is an integer from 1 to 2;

X is a radiolabel;

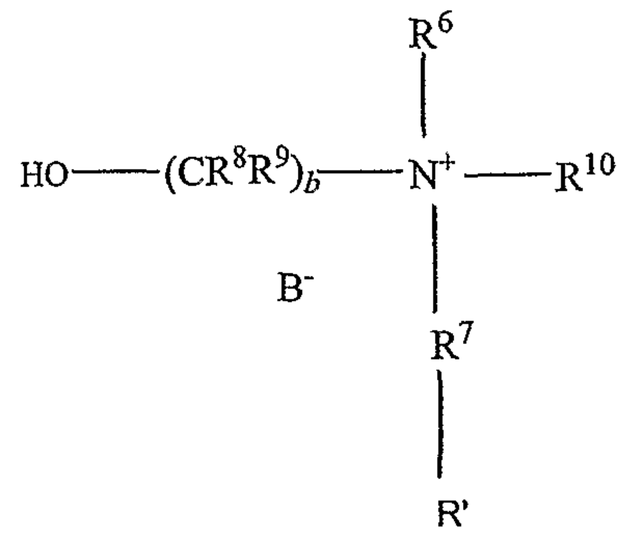
LG is a mesylate, tosylate or triflate;

Q_mZ is an alkylation reactive center of a target compound, wherein, *m* is an integer from 1-3 and each Q_{1-3} is independently methyl and hydroxyethyl, and wherein Z is N.

[0069] Once the target compound is alkylated or labeled, in some embodiments, it can be purified by various means, as known in the art. For example, an alkylated target compound can be purified by electrophoresis, precipitation, extraction, and/or column chromatography **470** (*e.g.*, ion exchange chromatography, molecular exclusion chromatography). Determining any one or more purification steps or methods for purifying an alkylated or labeled target compound is within the abilities of the skilled artisan. Thus, a skilled artisan will appreciate that purification of an alkylated or labeled target compound by for example chromatography can comprise one or more washing steps **480**, **490** prior to elution **500**. In some embodiments, the pH or ionic strength an alkylated or labeled target compound can be subsequently altered **510**, **510**. In some embodiments, an alkylated or labeled target compound can be purified so that it is suitable for use in a living subject **520**.

[0070] By way of exemplification and not limitation, an alkylated target compound can be *N*-fluoromethyl-MQNB (FIG. 4), *N*-fluoromethyl-MPP (FIG. 6), *N*-fluoromethylspiperone (FNMS), [^{18}F]fluoromethyl-neostigmine, [^{18}F]fluoromethyl-tyrosine and 3-(2'-fluoroethyl)spiperone (FESP). In some embodiments, an alkylated target compound also can be labeled. Therefore, the present disclosure, in some embodiments, contemplates the radiolabeled target compounds. Thus, in various exemplary embodiments the fluorine in each of the above can be substitute by ^{18}F, ^{123}I, ^{124}I, ^{125}I, ^{131}I, or ^{76}Br.

[0071] In some embodiments, an alkylated target compound can have a structure of Formula III:



(III)

wherein,

R' is ^{18}F, ^{123}I, ^{124}I, ^{125}I, ^{131}I or ^{76}Br;

B^- is a counterion;

b is an integer from 1 to 3;

R^6, R^7, R^{10} are independently CH_3, C_2H_5, C_3H_7, C_6H_5;

R^8, R^9 are independently H, CH_3, C_2H_5.

[0072] Therefore, in various exemplary embodiments an alkylated target compound can be a choline analog (*e.g.*, *N*,*N*-dimethyl-*N*-fluoromethylethanolamine (fluorocholine: FCH), *N*,*N*-dimethyl-*N*-fluoroethylethanolamine (fluoroethylcholine: FECh), [^{18}F]FCH, and [^{18}F]FECh, *N*,*N*-dimethyl-*N*-bromomethylethanolamine (bromocholine: BrCH), *N*,*N*-dimethyl-*N*-bromoethylethanolamine (bromoethylcholine: BrECh), [^{76}Br]BrCH, and [^{76}Br]BrECh, *N*,*N*-dimethyl-*N*-iodomethylethanolamine (iodocholine: ICH), *N*,*N*-dimethyl-*N*-iodoethylethanolamine (iodoethylcholine: IECh), [^{125}I]ICH, and [^{125}I]IECh, and the like.

[0073] In some alternative embodiments, an alkylated target compound can be other than [^{18}F]FCH, [^{18}F]FECh or [^{18}F]FDG (fluorodeoxyglucose), for example, wherein the alkylating agent used to prepare the alkylated compound is purified prior to an alkylation reaction.

[0074] In some embodiments, a process for making an [^{18}F]-labeled target compound (T_c) may comprise [^{18}F]fluoride and X-$(CH_2)_n$-X in a reaction vessel to form [^{18}F]-$(CH_2)_n$-X wherein X is

tosylate, mesylate or triflate and n = 1-4, adding a target compound comprising an alkylation reactive group to the reaction vessel to form [^{18}F]-$(CH_2)_n$-T_c, passing the reaction mixture through a first chromatographic support to bind the [^{18}F]-$(CH_2)_n$-T_c and eluting the [^{18}F]-$(CH_2)_n$-T_c from the support. In some embodiments, the reaction vessel can be in fluid communication with the first chromatographic support. In some embodiments, the first chromatographic support has an outlet which is in fluidic communication with a dispenser for delivering aliquots of eluent to the [^{18}F]-$(CH_2)_n$-T_c to individual vials. In some embodiments, a second chromatographic support can be in fluid communication with and located between the first chromatographic support and the dispenser.

[0075] Once made, the alkylated or labeled target compounds (target compound derivative) may find use *in vivo* or *in vitro* at the discretion of the skilled artisan. In some embodiments, the alkylated or labeled target compound can be used to modify or label a second target compound. As exemplified in FIG. 13, a labeled amino acid can be attached to a peptide. In some embodiments, the method of attachment can employ solid-phase peptide synthesis processes as described for example in Bianco, *et al.*, 2003, *Org. Biomol. Chem.* 1(23):4141-3 (Epub 2003 Oct 22); Deechongkit, *et al.*, 2004, *Org Lett.* 6(4):497-500; Hojo, *et al.*, 2004, *Chem. Pharm. Bull.* (Tokyo) 52(4):422-7; Malkinson, *et a.*, *Org. Lett.* 5(26):5051-4; Merrifield, 1995, *Biopolymers* 37(1):3-4; Merrifield, 1997, *Methods Enzymol.* 289:3-13; Song, *et al.*, 2004, *Bioorg. Med. Chem. Lett.* 14(1):161-5; Stephenson, *et al.*, 2004, *Bioconjug. Chem.* 15(1):128-36; Wang, *et al.*, 1987, *Int. J. Pept. Protein Res.* 30(5):662-667; and U.S. Patent Nos. 4507230, 4816513, 5186898, 5233044, 5258454, 5286846, 5380495, 5444150.

[0076] In some embodiments, a labeled target compound may find use as a therapeutic, imaging agent, radiopharmaceutical, or tracer. Accordingly, in some embodiments, an alkylated or labeled target compound can be use to detect, monitor or analyze a cell or tissue in subject. In some embodiments, the cell or tissue may be benign, malignant, neoplastic and/or cancerous and can be staged by an imaging technique. In some embodiments, the imaging technique can be positron emission tomography (PET). Methods and techniques for performing PET are well-known in the art (*see, e.g.*, U.S. Patent Nos. 4563582, 4642464, 4647779, 4677299, 4733083,

4864138, 4864140, 5027817, 5103098, 5138165, 5210420, 5224037, 5319204, 5378893, 5453623, 5591977, 5602395, 5744802, 5827499, 5998792, 6130430, 6288399, 6434216, 6521893, 6674083, 6718006). Thus, in some embodiments an alkylated or labeled target compound can be formulated according to its intended use at the discretion of the practitioner. For use as an *in vivo* imaging agent, the labeled target compound is formulated to be suitable for intravenous injection (*e.g.*, sterile, non-pyrogenic). In some embodiments, a labeled target compound can be formulated with a pharmaceutically acceptable carrier such as a pharmaceutically acceptable salt and a pharmaceutically effective amount is administered to a subject.

[0077] By "pharmaceutically acceptable salt" herein is meant a salt of a compound of the invention which is made with counterions which is understood in the art to be generally acceptable for pharmaceutical uses and which possesses the desired pharmacological activity of the parent compound. Such salts include: (1) acid addition salts, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or formed with organic acids such as acetic acid, propionic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, 3-(4-hydroxybenzoyl) benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethane-disulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylic acid, glucoheptonic acid, 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, muconic acid and the like; or (2) salts formed when an acidic proton present in the parent compound is replaced by a metal ion, e.g., an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, N-methylglucamine, morpholine, piperidine, dimethylamine, diethylamine and the like. Also included are salts of amino acids such as arginates and the like, and salts of organic acids like glucuronic or galacturonic acids and the like (*see*, *e.g.*, Berge *et al.*, 1977, J. Pharm. Sci. 66:1-19).

[0078] "Pharmaceutically effective amount" or "therapeutically effective amount" refers to an amount sufficient to produce the desired physiological effect or amount capable of achieving the desired result, particularly for diagnosing or treating disorders or disease conditions, including reducing or eliminating one or more symptoms of the disorder or disease or prevention of the disease or condition. Accordingly, in a preferred embodiment, a labeled target compounds can be administered in an mount sufficient to detect, monitor and/or stage a neoplasm in a subject. The amount administered and route of administration is selected at the discretion of the practitioner as known in the art.

[0079] Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can be administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically or intrathecally. Parenteral administration, oral administration, subcutaneous administration and intravenous administration are the preferred methods of administration.

[0080] In various exemplary embodiments, the above methods of synthesizing an alkylating agent, the alkylation or labeling of a target compound, and/or the purification of the alkylated or labeled target compound can be automated. Thus, in some embodiments, the disclosed methods may be implemented on a general purpose or special purpose device, such as, a device having a processor for storing data and/or commands. It will be appreciated that the computing device may be a single computer or a plurality of networked computers and that the several procedures associated with implementing the methods and procedures described herein may be implemented on one or a plurality of computer devices. In some embodiments, the disclosed procedures and methods are implemented on standard server-client network infrastructures with various embodiments of the disclosed features added on top of such infrastructure or compatible herewith. Methods, processes and procedures described herein generally may be implemented in

software, hardware and/or combinations thereof. In some embodiments, as exemplified in FIG. 10, processor **300** can be directed by readable memory **310**. In some embodiments, processor **300** can be programmed to direct the purification of a halogen ion, *e.g.*, [^{18}F]fluoride **100** using QMA **170**. In some embodiments, processor **300** directs elution of [^{18}F]fluoride **100** from QMA **170** using K222/Carbonate **110** and directs the eluate to reaction vessel (RV) **210**. In some embodiments, ditosylmethane **120** can be directed to reaction vessel **210**. To synthesize an alkylating agent, *e.g.*, [^{18}F]fluoromethane tosylate, processor **300** can direct temperature control module **320** to heat reaction vessel **210** to the programmed temperature selected at the discretion of the practitioner and can direct the mixing reactants, for example, by bubbling an inert gas, *e.g.*, Argon **180**. In some embodiments, processor **300** direct the addtion of dimethylamine **130** to RV **210** which can be heated by thermal control module **320** and mixed by Argon **180** to synthesize an alkylated or labeled target compound, *e.g.*, [^{18}F]FCH. EtOH/Water **140** can be directed to reaction vessel **320** by processor **300**, which in some embodiments washes reaction vessel **320** and picks up additional alkylated product. In some embodiments, processor **300** directs the contents of reaction vessel **320** to silica column **200**. Waste **230** does not bind to silica column **200** and, in some embodiments, alkylated product, *e.g.*, [^{18}F]FCH can be eluted by AcOH **150**. AcOH **150** can be removed by directing the eluate over weekly basic ion-exchange resin **240** and alkylated product, *e.g.*, [^{18}F]FCH can be collected into vial **250**. Valve **160** controls flow to and from QMA **170**. Three-way valves **190** and **220** control flow to and/or from silica column **200**, waste **230**, the weekly basic ion-exchange resin **240** and vial **250**.

[0081] In some embodiments, the methods and procedures may be automated by the insertion of 3-way values **190** and **220**, as shown, in a Siemens-CTI Chemical Process Control Unit (CPCU) (CTI, Inc., Knoxville, TN). As known in the art, CPCU has three components: a chemistry process control unit, a control chassis, an operating system and control software designed to be programmable by practitioners to direct a variety of procedures. As known in the art, a CPCU includes an IBM compatible PC system and a standard (STD) bus subsystem. The computer runs on Microsoft® Windows NT® Operating System and the Intellution® Software Package (*see, e.g.*, *[^{18}F]Chemical Process Control Unit*. (CHF.SA 10.0796.0500, CTI, Inc., 1996); and Padgett

et al., 1989, "Computer-controlled radiochemical synthesis: "A chemistry process unit for the automated production of radiopharmaceuticals." *Int. J. Rad. Appl. Instrum* [A]. 40(5):433-45).

[0082] As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural unless the context clearly indicates otherwise. The following examples are offered by way of illustration or exemplification and not by way of limitation. Thus, it is understood that these examples in no way serve to limit the true scope of this disclosure.

7. EXAMPLES

[0083] Reagents and solvents were obtained from Aldrich Chemical Co. and Fisher Scientific and used without further purification unless otherwise specified. Melting points were recorded on an Electrothermal 9100 (Electrothermal Engineering Ltd., Southend-on-Sea, UK) and are uncorrected. NMR spectroscopy was carried out on a Varian 400 MHz instrument (Varian Inc. Palo Alto, CA). Column chromatography was carried out 230 Mesh Silica Gel (Catalog No. 4791010, Whatman Inc., Clifton, NJ). Thin layer chromatography (TLC) was performed on Silica 60 F_{254} analytical plates (E. Merck, Catalog No. 4410222, Whatman Inc., Clifton, NJ), equipped with a Bioscan 200 imaging scanner (Bioscan, Inc., Washington, DC). High pressure liquid chromatography (HPLC) was achieved on a Waters 600 System (Waters Corporation, Milford, MA) with a UV detector (*see, e.g.*, FIGS. 15 and 17) (Catalog No. WAT080690, Waters Corporation, Milford, MA) and radioactivity detector (*see, e.g.*, FIGS. 14 and 16) (Catalog No. FC3200, Bioscan, Inc., Washington, DC) attached in series, using a Partisil SCX column (250 x 4.6 mm, Catalog No. 8173, Alltech Associates, Deerfield, IL) eluted at 1 mL/min with a 20% acetonitrile/water solution containing 0.25 mol/L sodium dihydrogen phosphate. Gas chromatography (GC) was performed on a Hewlett-Packard 6890 GC (Hewlett-Packard Company, Palo Alto, CA) equipped with a CAM column (30 mm x 0.25 μm, Catalog no. 1122132, J&W Scientific, Agilent Technologies, Inc., Palo Alto, CA).

Example 1: Ditosylmethane:

[0084] Ditosylmethane was prepared by mixing diiodomethane (1.2 g, 4.5 mmol) and two fold of *p*-toluenesulfonate silver salt (2.8 g, 11 mmol) in anhydrous acetonitrile (20 mL). The resulting mixture was refluxed for 16 h. Ditosylmethane was purified using a 230 Mesh Silica Gel Column (Catalog No. 4791010, Whatman, Inc., Clifton, NJ) (30-40% ethylacetate-hexanes) to yield a white crystalline product (1 g, 63%): m.p. 117°C (lit. m.p. 116-117°C); ^{1}H-NMR ($CDCL_3$, 400 MHz) δ 2.45 (s, 6H, CH_3), 5.10 (s, 2H, OCH_2O), 7.25 (d, J = 8 Hz, 2H), 7.60 (d, J = 8 Hz, 2H).

Example 2: *N,N*-Dimethyl-*N* fluoromethylethanolamine (Fluorocholine (FCH)):

[0085] A sealed tube containing 10 mL anhydrous tetrahydrofuran (THF) and 2 mL (20 mmol) *N,N*-dimethylethanolamine was cooled at -78°C. Chlorofluoromethane (5.7 g, 70 mmol, Catalog No. 593-70-4, Synquest Labs, Alachua, FL) was bubbled through the cooled solution for 15 min. The mixture was allowed to slowly warm to room temperature overnight. A white solid formed, which was filtered, washed three times with cold (~5°C) THF and dried under vacuum. FCH was isolated as a hygroscopic white solid (0.5 g, 15%): ^{1}H-NMR (D_2O, 400 MHz) δ 3.24 (s, 3H, CH_3), 3.25 (s, 3H, CH_3), 3.60-3.63 (m, 2H, CH_2), 4.05-4.08 (m, 2H, CH_2OH), 5.43 (bd, J = 45 Hz, 2H, CH_2F).

Example 3: [^{18}F]Fluoromethane tosylate and [^{18}F]fluoroethanetosylate:

[0086] No carrier added [^{18}F]fluorination of 1,1- 1,2- and 1,3-disubstituted alkanes was systematically studied by Block *et. al.* (1987) *J. Label Compds. Radiopharm.* 24:1029-1042. We reproduced the results of Block, *et al.* in the [^{18}F]fluorination of ditosylmethane and 1,2-ditosylethane. Both reactions were heated at 80°C for 5 min. and the products were analyzed by TLC. TLC was performed on Silica 60 F254 analytical plates (Catalog No. 4410222, Whatman Inc., Clifton, NJ) in 30% Ethyl acetate/ hexanes. [^{19}F]fluoride was included in these studies to provide a UV detectable moiety for quality control analysis.

[0087] [^{18}F]Fluorination of 1,2-ditosylethane resulted in an 80% yield of [^{18}F]fluoroethane tosylate (FIG. 1). [^{18}F][^{19}F]difluoroethane was not detected. Therefore, an electrowithdrawing effect was not observed due to the [^{18}F]fluorine at the β position. The 80% yield was achieved

without agitation of the reaction. [^{18}F]fluoroethane tosylate can be produced at lower temperatures but the skilled artisan will appreciate that as the temperature decreases the yield decreases.

[0088] [^{18}F]fluorination of ditosylmethane yielded two labeled products, [^{18}F]difluoromethane and [^{18}F][^{19}F]difluoromethane tosylate, under various conditions. At 80°C, only one product is formed, the byproduct [^{18}F][^{19}F]difluoromethane which is not useful for the synthesis of [18F]fluorocholine. In addition, some free, detectable [^{18}F]fluoride is not uncommon, especially when the reaction is not pushed to completion. As expected by the studies of Block *et al.*, the yield of [^{18}F]fluoromethane tosylate was ~1% (FIG. 2). The low yield of [^{18}F]fluoromethane tosylate may be the result of [^{18}F]fluoromethane tosylate being more reactive than ditosylmethane in SN_2 reactions due to the electrowithdrawing effect of the fluorine at the α position. Due to the very low concentration of [^{18}F]fluoride detected after the reaction, apparently [^{18}F]fluoromethane tosylate may undergo a second fluorination with [^{19}F] carrier present in the reaction vessel to produce [^{18}F][^{19}F]difluoromethane, a very volatile compound (boiling point = -52°C).

[0089] We examined the affects of temperature on the fluorination of ditosylmethane. When the reaction temperature was lowered to about 45-50°C, the production of [^{18}F][^{19}F]difluoromethane decreased and we obtained sufficient amounts of [^{18}F]fluoromethane tosylate to synthesize [^{18}F]FCH (FIG. 2). At temperatures less than about 40°C for up to 20 min., only a small amount of [^{18}F][^{19}F]difluoromethane was detected; however, the reaction rate may be unsuitable for radio-synthesis using some short-lived isotopes. At temperatures greater than about 50°C, the major reaction product was [^{18}F][^{19}F]difluoromethane.

[0090] The fluorination step also was sensitive to agitation. At temperatures ranging from about 40°C to about 50°C, in the absence of agitation, the reaction yielded low amounts of [^{18}F][^{19}F]difluoromethane but also yield low amounts of [^{18}F]fluoromethane tosylate (~10% yield). Performing the [^{18}F]fluorination at temperatures ranging from about 40°C to about 50°C with agitation, produced sufficient quantities of [^{18}F]fluoromethane tosylate (~30% yield) to synthesize [^{18}F]FCH.

Example 4: [^{18}F]*N,N*-Dimethyl-*N*-fluoromethylethanolamine ([^{18}F]FCH):

[0091] [^{18}F]FCH was prepared in a 2-step reaction: fluorination of ditosylmethane with [18F]fluoride followed by an alkylation reaction with [^{18}F]fluoromethane tosylate and dimethylethanolamine using a modified Siemens-CTI Chemical Process Control Unit (CPCU, Catalog No. 3601037, CTI, Knoxville, TN) (FIG. 10). [^{18}F]FCH was purified using a Silica Sep-Pak column (Catalog No. WAT023537, Waters Corporation, Milford, MA). The column was washed with ethanol and water to remove all impurities and [^{18}F]FCH was eluted with 2% acetic acid. The acetic acid was removed using an AG 4-X4 weakly basic ion-exchange resin column (143-3341, Bio-Rad Laboratories, Inc., Hercules, CA) (*see* AG 4 Instruction Manual LIT207 Rev B, Bio-Rad). Two 3-way slider valves, *e.g.*, teflon slider valves, were added to the CPCU to perform the purification (FIG. 10). The software was modified to lower the temperature and to permit agitation for the labeling step.

[0092] To prepare [^{18}F]fluoride, enriched ^{18}O-water was irradiated with an 11 MeV proton (33 μA for 60 min.). After bombardment, the target ^{18}F-HF/^{18}O-water solution was transferred to an anion-exchange QMA cartridge (carbonate form) (Catalog No. WAT054725, Waters Corporation, Milford, MA) using Ar pressure. The no-carrier-added [^{18}F]fluoride was trapped on the QMA cartridge and ^{18}O-water was recovered. The [^{18}F]fluoride was eluted from the cartridge with 1 mL acetonitrile:water solution (19:1) containing 2.2 mM potassium carbonate (K_2CO_3) and 40 mM Kryptofix® 2.2.2 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane, $C_{18}H_{36}N_2O_6$, Catalog No. 291110, Sigma-Aldrich Corp, St. Louis, MO) into the reaction vessel. The mixture was azeotropically dried under an Ar stream at 110°C for 4 min. and allowed to cool for about 60 sec. Ditosylmethane (10 mg, 0.03 mM) in 1 ml anhydrous acetonitrile was added to the dry residue. The mixture was heated at 45-50°C for 5 min. with intermittent Ar gas bubbling (10 sec. for every 20 sec.). Afterwards, 1 mL acetonitrile containing 0.2 mL of *N,N*-dimethylethanolamine was added to the reaction vessel, which was heated at 100°C for 10 min. with intermittent Ar gas bubbling (15 sec. for every 30 sec.) (FIG. 3). The entire mixture was loaded onto a SiO_2 Sep-Pak cartridge (Catalog No. WAT023537, Waters Corporation, Milford, MA) and washed with ethanol (3 x 5 mL) and water (2 x 5 mL). The flow was redirected to the final product vial using two 3-way valves. [^{18}F]FCH was released from the SiO_2 Sep-Pak

cartridge with 5 mL 2% acetic acid followed by 5 ml H_2O. Acetic acid was stripped out of the eluant by an AG 4-X4 weakly basic ion-exchange resin column (Catalog No. 143-3341, Bio-Rad Laboratories, Inc., Hercules, CA) (*see* AG 4 Instruction Manual LIT 207 Rev B, Bio-Rad) and the solution was passed through a 0.2 μm membrane filter (Catalog No. SLGS V255F, Millipore Corp, Billerica, MA) into a sterile vial containing 0.5 ml 23.4% sodium chloride concentrated solution. Alternatively, the SiO_2 Sep-Pak cartridge was replaced by a pre-activated (10 mL 1 N HCl) Accell cartridge (Catalog No. WAT023531, Waters Corp, Millford, MA) and [^{18}F]FCH was eluted with 10 mL saline.

[0093] The results of the alkylation reaction indicated that [^{18}F]fluoromethane tosylate reacted almost quantitatively with *N,N*-dimethylethanolamine in acetonitrile to provide a >90% yield of [^{18}F]FCH. The overall yield of the synthesis of the [18F]fluoromethane tosylate to [^{18}F]FCH was 20%, which may be due to the production of [^{18}F][^{19}F]difluoromethane during the fluorination step. The total synthesis time was less than about 40 min. (*see* FIGS. 16 and 17).

Example 5: *N,N*-Dimethyl-*N*-fluoroethylethanolamine (fluoroethylcholine (FECh)) synthesis:

[0094] 1-Bromo-2-fluoroethane (1 g, 8 mmol) was dissolved in *N,N*-dimethylethanolamine (0.7 g, 8 mmol) and heated at 100°C for 10 min. The resulting mixture was taken up in 10% ethanol/ethylacetate. After removal of the solvent, FECh was obtained as a white solid (1.7 g, 100%). ^{1}H-NMR spectrum was indistinguishable from the spectrum reported by Hara, *et. al.*, 2002, *J. Nucl. Med.* 43(2):187-199.

Example 6: [^{18}F]*N,N*-Dimethyl-*N*-fluoroethylethanolamine ([^{18}F]FECh) synthesis:

[0095] Anhydrous [^{18}F]flouride was obtained as described in Example 3 and reacted with 1,2-bis-ditosylethane (10 mg, 0.03 mmol) in 1 mL anhydrous acetonitrile. The mixture was heated at 80°C for 5 min with intermittent Ar gas bubbling (10 sec for every 20 sec). Afterward, 0.3 mL *N,N*-dimethylethanolamine neat was added to the reaction vessel, which was heated at 100°C for 10 min. with intermittent Ar gas bubbling (15 sec for every 30 sec). [^{18}F]FECh was purified from the reaction mixture as described in Example 3 using a Silica Gel Sep-Pak cartridge.

[0096] Alkylation with [^{18}F]fluoroethane tosylate in *N,N*-dimethylethanolamine neat gave only a

50% yield. The overall yield of [^{18}F]FECh also was 50%, which is higher than the yield of [^{18}F]FCH (20%). This may be a result of very little to no [^{18}F][^{19}F]difluoroethane byproduct being produced during the fluorination step. As described above for [^{18}F]FCH, the total synthesis time was less than about 40 min. (*see* FIGS. 14 and 15).

Example 7: [^{18}F]FCH and [^{18}F]FECh purification:

[0097] [^{18}F]FCH and [^{18}F]FECh are quaternized alkylamines, which can be absorbed strongly onto Silica Gel Sep-Pak cartridges (Mulholland, *et. al.* (1999) *J. Label Componds Radiopharm.* 42:Suppl. 1 s459-s461). [^{18}F]FCH and [^{18}F]FECh tightly held onto the cartridges which allowed most impurities to be quickly removed to waste with an ethanol elution. [^{18}F]FCH and [^{18}F]FECh were eluted with a 2% acetic acid solution and neutralized using a weakly basic ion-exchange resin (AG 4-X4, Catalog No. 140-4341, Bio-Rad Laboraotires, Inc., Hercules, CA).

[0098] The radiochemical purity of [^{18}F]FCH and [^{18}F]FECh were determined by HPLC (HPLC 600 System, Waters Corporation, Milford, MA) and TLC (Silica 60 F254 Analytical Plates, Whatman Inc., Clifton, NJ). [^{18}F]FCH and [^{18}F]FECh eluted from the HPLC column at 6.8 and 7.5 min, respectively. HPLC was achieved on a Waters 600 System (Waters Corporation, Milford, MA) with a UV detector (Catalog No. WAT080690, Waters Corporation, Milford, MA) and radioactivity detector (Catalog No. FC3200, Bioscan, Inc., Washington, DC) attached in series, using a Partisil SCX column (250 x 4.6 mm, Catalog No. 8173, Alltech Associates, Deerfield, IL) eluted at 1 mL/min with a 20% acetonitrile/water solution containing 0.25 mol/L sodium dihydrogen phosphate. The residual amounts of *N,N*-dimethylethanolamine in the final solutions were analyzed by GC using a CAM Column designed for amines (Catalog No. 1122132, Agilent Technologies, Inc., Palo Alto, CA). The only chemical contaminants detected by HPLC and GC were *N,N*-dimethylethanolamine (<0.5 mg per batch) and ethanol (<5 mg per batch).

[0099] To lower the *N,N*-dimethylethanolamine contamination, Silica Gel Sep-Pak cartridges were replaced with an Accell cartridge (Hara, *et. al.* (1997) *J. Nucl. Med.* 38(6):842-847). The Accell cartridge was preconditioned with HCl and H_20 and the final products were eluted with saline solution. *N,N*-Dimethylethanolamine was found to be <0.1 mg per batch in the [^{18}F]FCH

preparation. [^{18}F]FECh was not efficiently purified using the Accell cartridge, *i.e.*, about half of the [^{18}F]FECh was found in the waste fraction.

[00100] The final purified solutions in physiologic saline [^{18}F]FCH and [^{18}F]FECh were cultured (1 mL) for microorganisms for growth in enriched thioglycollate at 37°C and trypticase soy broth media at 23°C. No growth was observed for 2 weeks. The final purified solutions were shown to be pyrogen free using the standard methodology for the LAL (Charles River Labs Endosafe, Charleston, SC). A positive control standard is always included in the assay. [^{18}F]FCH is currently in an FDA approved pre-clinical IND safety trial in humans. To date, after about fifty trials, no adverse effects from any [^{18}F]fluorocholine tested has been observed.

[00101] TLC plates were pre-treated in 2% acetic acid and air dried before use. The plates were eluted in 2% acetic acid. The R_f [^{18}F]FCH and [^{18}F]FECh was 0.40, which confirms the HPLC results.

Example 8: Other alkylating agents, alkylation methods and purification methods:

[00102] Alkylating agents:

[00103] Alternatives to the tosylate leaving group for the SN_2 nucleophilic substitution reaction used for preparing [^{18}F]FCH and [^{18}F]FECh include but are not limited to halides and other sulfonate esters, such as, methane sulfonates (*e.g.*, mesylates) and trifluoromethane sulfonates (*e.g.*, triflates). However, as the volatility of an alkylating agent increases maintaining the alkylating agent in solution decreases. Therefore, by increasing the number carbon atoms the volatility can be decreases such that the alkylating agent can be maintained in solution.

[00104] Labeling 1,2-ditritylethane with [^{18}F]fluoride provides >80% yield of [18F]fluoroethane triflate. This is greater than the yield of [^{18}F]fluoroethane tosylate because triflate is the most reactive of the three sulfonate esters (mesylate, tosylate, triflate). Moreover, a higher yield is achieved at the alkylation step with *N,N*-dimethylethanolamine using triflate as the leaving group. Alkylation using 2-[^{18}F]fluoroethane tosylate and *N,N*-dimethylethanolamine provides a 50% yield of [^{18}F]FECh. In contrast, 2-[^{18}F]fluoroethane triflate reacts with *N,N*-

dimethylethanolamine to yield [^{18}F]FECh quantitatively. Thus the overall yield of [^{18}]FECh using 1,2-ditritylethane as the unlabeled precursor is >70%.

[00105] As a result of our studies of the labeling ditosylmethane with [^{18}F]fluoride, the second fluorination is activated by the electrowithdrawing effect of the [^{18}F]fluorine in the α position leading to the synthesis of difluoromethane. Therefore, step(s) to slow or prevent the second activated fluorination are beneficial. Polymer resin supports containing covalently bound quaternary ammonium salts (*see, e.g., J. Labelled. Cpd. Radiopharmceuticals* 26:378-380 (1989), FIG 12) or solid support such as QMA retain no-carrier-added [^{18}F]fluoride from irradiated [^{18}O]H_2O and label ditosylmethane. Polymeric resin processes may not be as efficient as solution reactions but they do slow or prevent the formation of [^{18}F][^{19}F]difluoromethane.

[00106] Alkylation:

[00107] Because sulfonate esters are more reactive than halide esters, [^{18}F]fluoromethane tosylate and [^{18}F]fluoroethane tosylate are suitable for on-column alkylation with *N,N*-dimethylethanolamine. The sulfonate esters are trapped on a Sep-Pak C18 cartridge loaded with the dimethylethanolamine. The [^{18}F]fluoromethylation of *N,N*-dimethylethanolamine thus is streamlined and the alkylation step is faster.

[00108] Purification:

[00109] We have shown that purification of [^{18}F]FCH was performed either using Silica Gel or Accell cartridges. However, [^{18}F]FECh was efficiently purified on Silica Gel but not on an Accell cartridge. This may be due to the alkylation step conditions which require the use of *N,N*-dimethylethanolamine without dilution (neat). With [^{18}F]FCH, alkylation occurs in a solvent, acetonitrile. Therefore, before loading the [^{18}F]FECh reaction mixture onto the Accell cartridge1 mL acetonitrile is added, which permits [^{18}F]FECh to hold on to the Accell cartridge to facilitate purification.

Example 9: Other [^{18}F]-labeled target compounds:

[00110] The disclosed methods can be used to replace target compounds labeled with [^{11}C]alkyl iodides with equivalent compounds labeled with longer-lived halogens, such as, ^{18}F. Therefore,

[^{18}F]fluoroalkane sulfonates can be used for various N, O, S and C alkylations. Examples of quaternary amines that may be [^{18}F]fluoroalkylated by the disclosed methods include MQNB (3-quinuclidinyl benzilate derivatives), a muscarinic ligand (FIG. 5); acetylcholinesterase inhibitor neostigmine (FIG. 6) and its metabolite TMA-phenol; neurotoxin *N*-methyl-4-phenyl-pyridinium are labeled using this methodology. Secondary amines that can be [^{18}F]fluoromethylated according to the disclosed methods include [^{18}F]*N*-methylspiperone ([^{18}F]NMS) and 3-(2'-[^{18}F]fluoroethyl)spiperone ([^{18}F]FESP) (FIG. 7). O-alkylation reactions are used to methylate the amino acid tyrosine in the presence dimethylsulfoxide (DMSO) (FIG. 8). Carboxylic acids are alkylated as shown in FIG. 9). S-alkylation reactions are used to fluoromethylate the target compound of FIG. 9.

CLAIMS:

1. A method of alkylating a target compound comprising:

a) synthesizing an alkylating agent having the formula:

$$X\text{-}(CR^1R^2)_aCR^3R^4 - LG$$

wherein,

a is 0, 1, 2 or 3,

R^1, R^2, R^3 and R^4 are independently H, X or alkyl,

X is a halogen or a label with the proviso that at least one X is ^{18}F,

LG is a leaving group,

wherein said synthesizing comprises a fluoridation reaction; and

b) reacting said alkylating agent with a target compound comprising an alkylation reactive group under conditions suitable for the alkylation of said target compound, wherein said fluoridation reaction occurs at a temperature from about 40°C to about 50°C and wherein said reacting is accomplished without purifying said alkylating agent after said synthesizing.

2. A method of synthesizing an ^{18}F-labeled target compound comprising,

a) synthesizing an alkylating agent having the formula:

$$X\text{-}(CR^1R^2)_aCR^3R^4 - LG$$

wherein,

a is 0, 1, 2 or 3,

R^1, R^2, R^3 and R^4 are independently H, X or alkyl,

X is a halogen or a label with the proviso that at least one X is [^{18}F],

wherein said synthesizing comprises a fluoridation reaction,

LG is a leaving group; and

b) contacting the alkylating agent with a target compound comprising an alkylation reactive group under conditions suitable for the alkylation of said target compound, wherein the target compound is other than [^{18}F]fluorocholine (FCH), [^{18}F]fluoroethylcholine (FECh) or [^{18}F]fluorodeoxyglucose, and wherein said

fluoridation reaction occurs at a temperature from about 40°C to about 50°C and wherein said reacting is accomplished without purifying said alkylating agent after said synthesizing.

3. The method according to claim 1 or 2, wherein LG is tosylate, mesylate or triflate ester.

4. The method according to claim 1, 2 or 3, wherein a precursor of the alkylating agent has the formula LG-$(CR^1R^2)_aCR^3R^4$- LG or LG-$(CH_2)_2$-LG.

5. The method according to claim 1 or 2, wherein said alkylating agent is chosen from the following:

X-CH_2CH_2-LG,

X-CH_2-LG,

CH_3-CH(X)-CH_2-LG,

X-CH_2-CH_2-CH_2-LG

wherein,

X is [^{18}F]; and

LG is a sulfonate ester.

6. The method according to any one of claims 1 to 5, wherein the alkylation reactive group comprises an alkyl, substituted alkyl, alcohol, carboxyl acid, saturated cycloalkyl, unsaturated cycloalkyl, aryl, substituted aryl, saturated heterocycle, unsaturated heterocycle, sulfhydryl, amine, N, O, S or C.

7. The method according to any one of claims 1 to 6, wherein the target compound is morphine, heroin, pethine, tamoxifen, codeine, nicotine, thioproperazine, diazepam, caffeine, flunitrazepam, hexamethonium, methiodide, 3-quinuclidinyl benzilate (MQNB), neostigmine, N-methyl-4-phenyl-pyridinium (MPP), N-methylspiperone (NMS), tyrosine, spiperone, spiroperidol glucose, lactic acid, hexobarbital, thymidine, iodoantipyrine, antipyrine coenzyme Q or dimethylethanolamine.

8. The method according to claim 7, wherein the alkylated target compound is [^{18}F] fluoromethyl-MQNB, [^{18}F]N-fluoromethyl-MPP, [^{18}F]N-fluoromethylspiperone (FNMS), [^{18}F]fluoroethylspiperone, [^{18}F]fluoromethyl-neostigmine, [^{18}F]fluoromethyl-tyrosine, [^{18}F]dimethyl-N-fluoromethylethanolamine or [^{18}F]N,N-dimethyl-N-fluoroethylethanolamine.

9. The method according to claim 1, 2 or 3, wherein when the target compound is dimethylethanolamine, the alkylating agent has the formula:

$$^{18}F\text{-}(CH_2)_a\text{-}LG$$

wherein,

a is 1 or 2, and

LG is the leaving group.

10. The method according to any one of claims 1 to 4, wherein the alkylating agent has the formula:

$$^{18}F\text{-}CH_2\text{-}CH_2\text{-}LG$$

or

$$^{18}F\text{-}CH_2\text{-}LG.$$

11. A computer readable memory storing computer executable instructions to direct a computer to function in a specified manner, comprising

a) executable instructions to direct a computer that controls a synthesis module to synthesize in a reaction vessel an alkylating agent having the formula:

$$X\text{-}(CR^1R^2)_aCR^3R^4 - LG$$

as defined in claim 1, using ^{18}F ions in a fluoridation reaction to make said alkylating agent, wherein said fluoridation reaction occurs at a temperature from about 40° C to about 50° C; and

b) executable instructions directing said computer that controls said synthesis module to directly react in said reaction vessel said alkylating agent with a target compound comprising an alkylation reactive group under conditions suitable to alkylate said target compound with the alklylating agent.

12. The computer readable memory according to claim 11, further comprising:

c) executable instructions directing said computer that controls said synthesis module to bind the alkylated target compound to a first solid support, wherein said reaction vessel and said first solid support are in fluidic communication.

13. The computer readable memory according to claim 12, further comprising:

d) executable instructions directing said computer that controls said synthesis module to elute said alkylated target compound from said first solid support.

14. The computer readable memory according to claim 13, further comprising:

e) executable instructions directing said computer that controls said synthesis module to bind the eluted alkylated target compound to a second solid support, wherein said first and second solid supports are in fluidic communication, and to elute said alkylated target compound from said second solid support.

15. A system comprising a synthesis module and a computer comprising (i) at least one processor, (ii) a memory and (iii) at least one program, wherein the at least one program is stored in the memory and executable by the at least one processor, the at least one program comprising instructions to control said synthesis module, wherein:

a. said instructions to direct said computer to control said synthesis module, wherein said synthesis module comprises a reaction vessel, and said control of said synthesis module comprises executing the synthesis of an alkylating agent, wherein said alkylating agent has the formula:

$$X\text{-}(CR^1R^2)_aCR^3R^4\text{-}LG$$

wherein,

a is an integer from 0 to 3,

R^1, R^2, R^3, and R^4 are independently H or X,

X is a halogen or a label with the proviso that at least one X is ^{18}F, wherein a fluoridation reaction is used to make said alkylating agent, wherein said fluoridation reaction occurs at a temperature from about 40^{o} C to about 50^{o} C and

LG is a leaving group; and

b. said instructions direct said computer to control said synthesis module to react in said reaction vessel said alkylating agent with a target compound under conditions suitable for alkylation of said target compound, wherein said alkylation occurs without first isolating said alkylating agent and wherein synthesis of said alkylating agent occurs at a temperature from about 40° C to about 50° C.

16. The system according to claim 15, wherein said reaction vessel is associated with a thermal control module.

17. The system according to claim 15 or 16, wherein said computer readable memory further comprises executable instructions to direct mixing of said alkylating agent with said target compound.

18. The system according to claim 17, wherein said mixing is accomplished by application of an inert gas to said reaction vessel.

19. The system according to claim 18, wherein said inert gas is argon.

20. The system according to any one of claims 15 to 19, wherein said computer readable memory further comprises executable instructions to direct said processor to direct purification of a halogen ion, wherein said halogen ion is used in said synthesis of said alkylating agent.

21. The system according to claim 20, wherein subsequent to said purification, said processor further directs elution of the purified halogen ion to said reaction vessel.

22. The system according to any one of claims 15 to 21, wherein synthesis of said alkylating agent and alkylation of said target compound occur in the same reaction vessel.

23. The system according to claim 22, wherein said alkylating agent is not purified before reacting said alkylating agent with said target compound.

24. The system according to any one of claims 15 to 23, wherein said alkylating agent has the formula $^{18}F - CH2 - LG$.

25. The system according to any one of claims 15 to 24, wherein LG is a sulfonate ester.

26. The system according to any one of claims 15 to 24, wherein LG is selected from the group consisting of tosylate, mesylate and triflate ester.

FIGURE 1

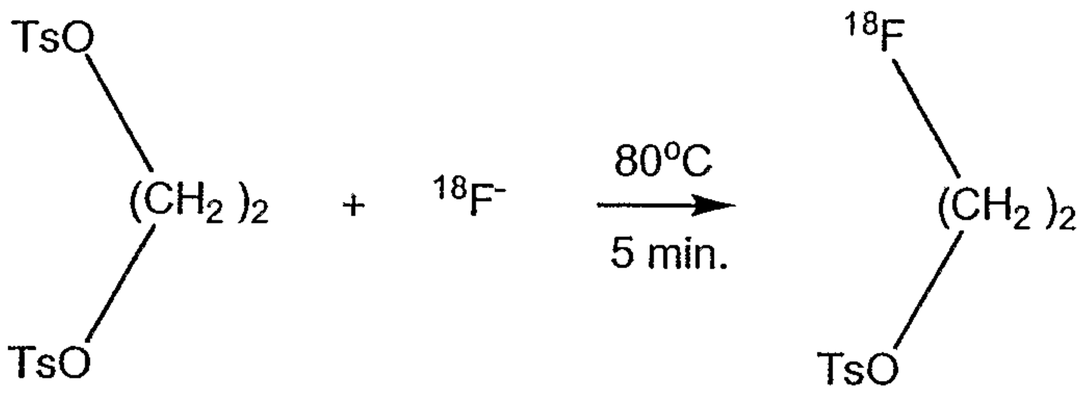


FIGURE 2

FIGURE 3

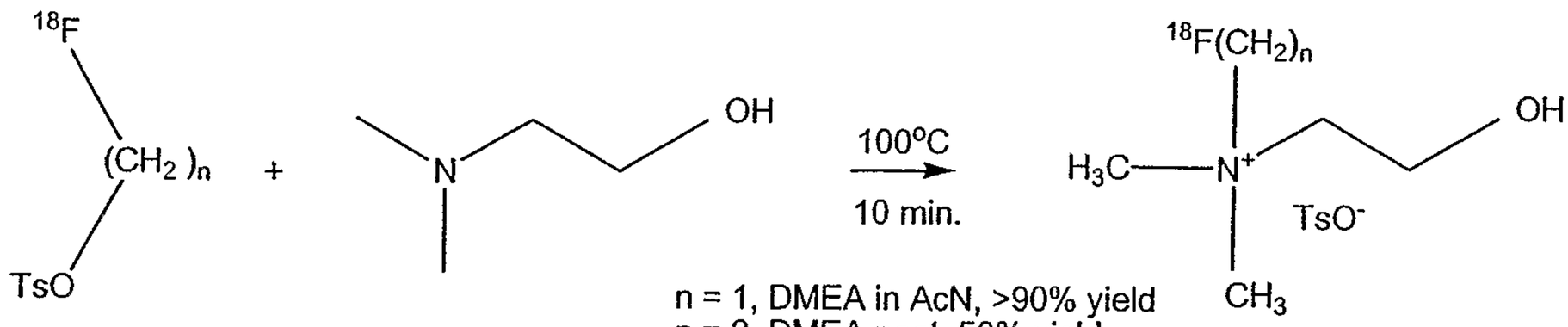
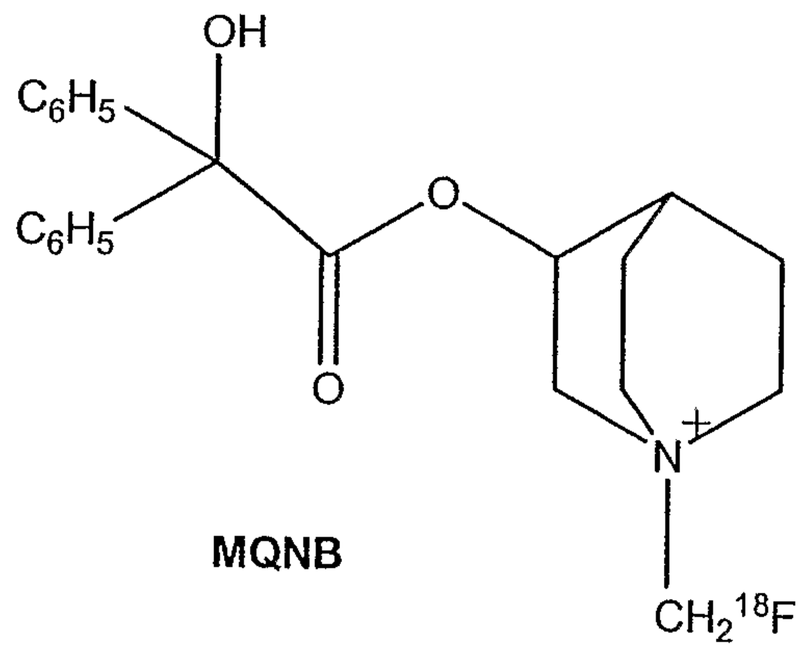


FIGURE 4



MQNB

FIGURE 5

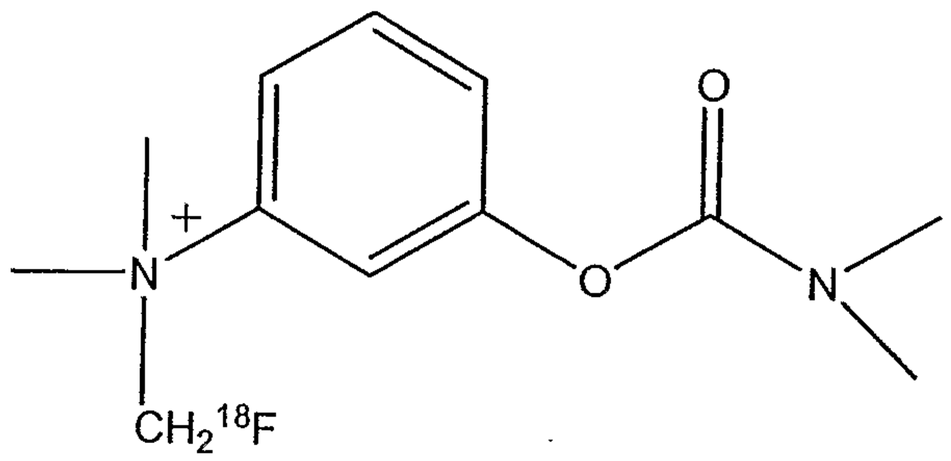
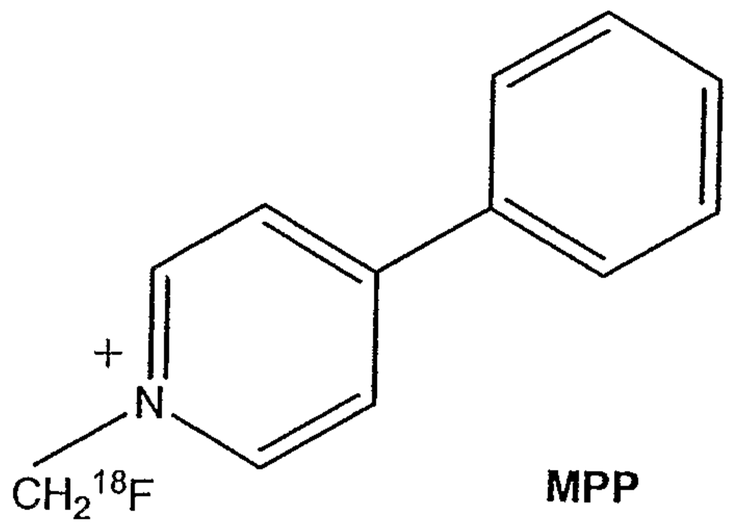


FIGURE 6



MPP

FIGURE 7

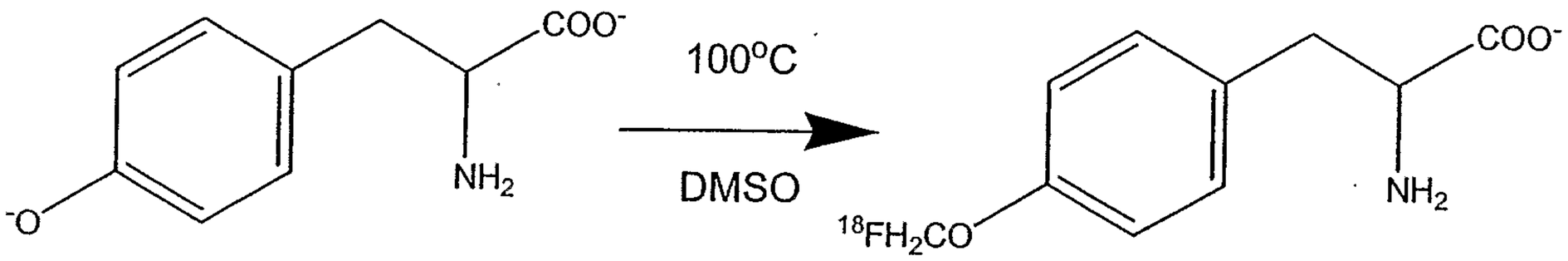


FIGURE 8

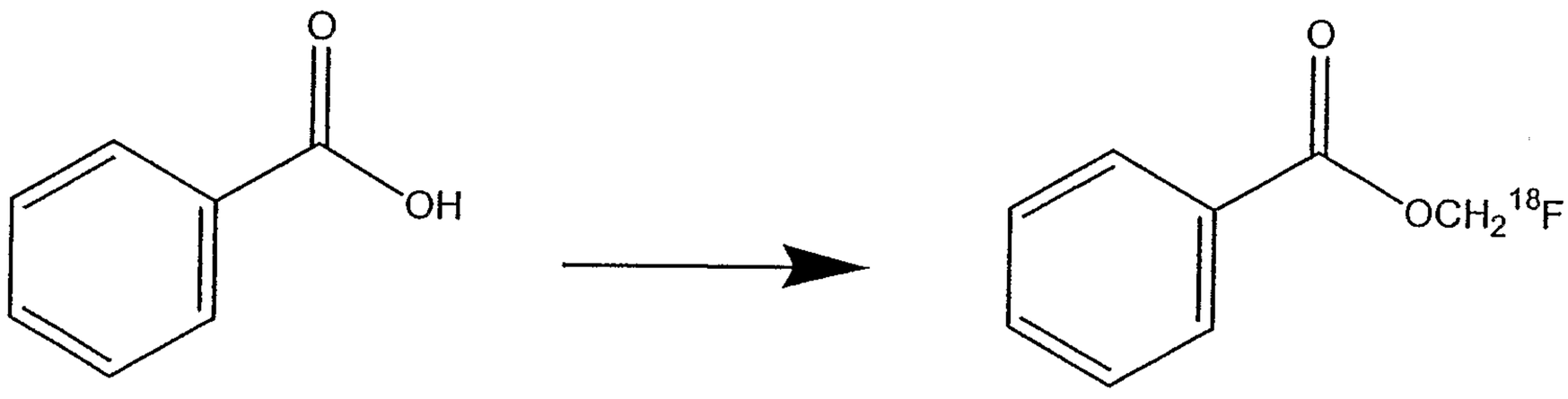


FIGURE 9

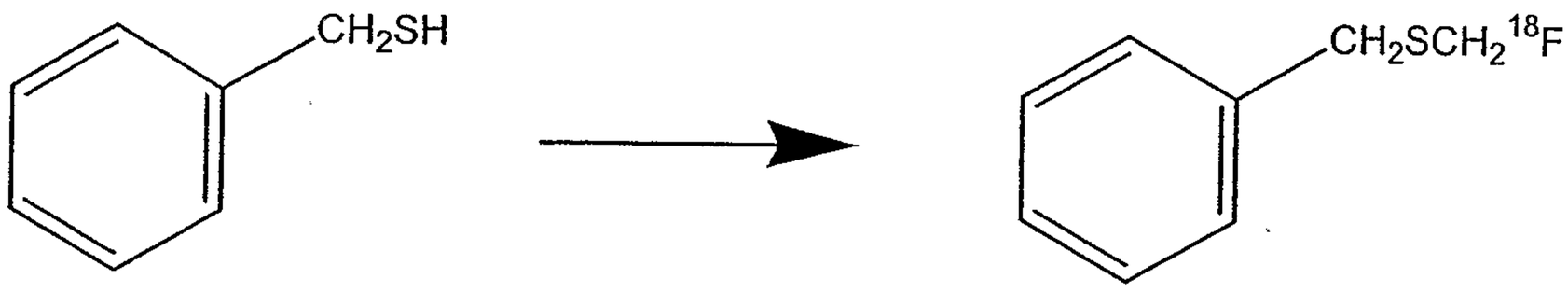


FIGURE 12

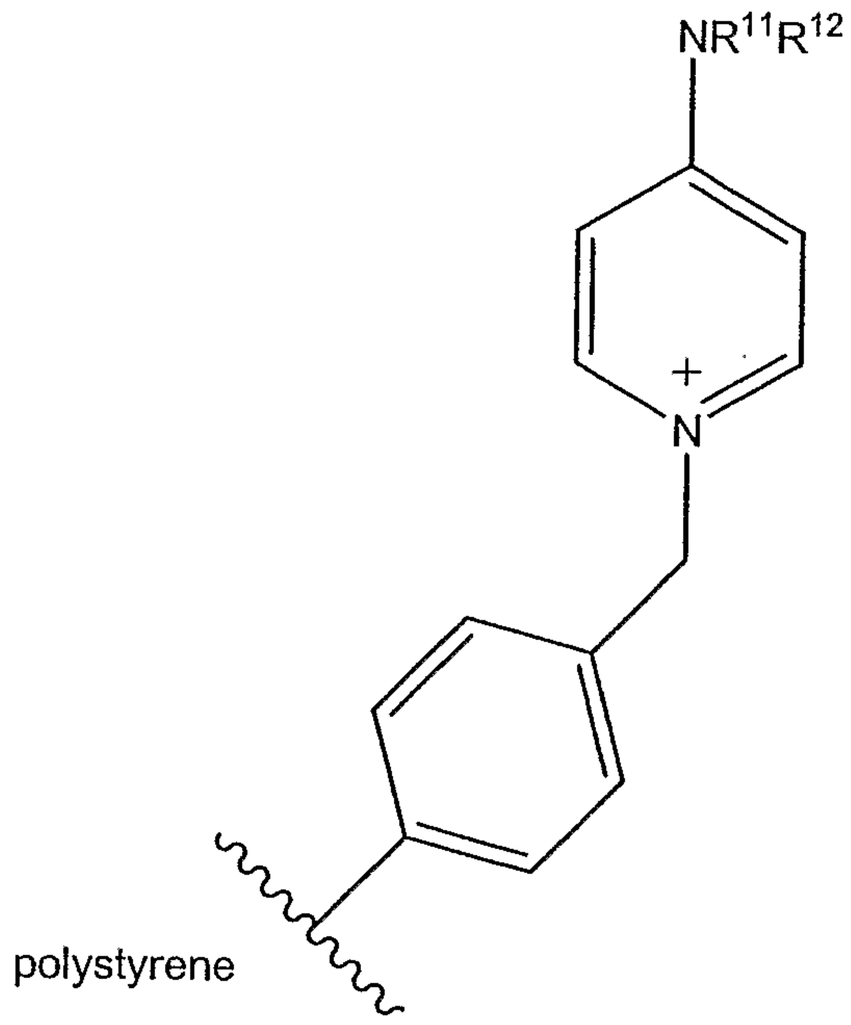


FIGURE 10

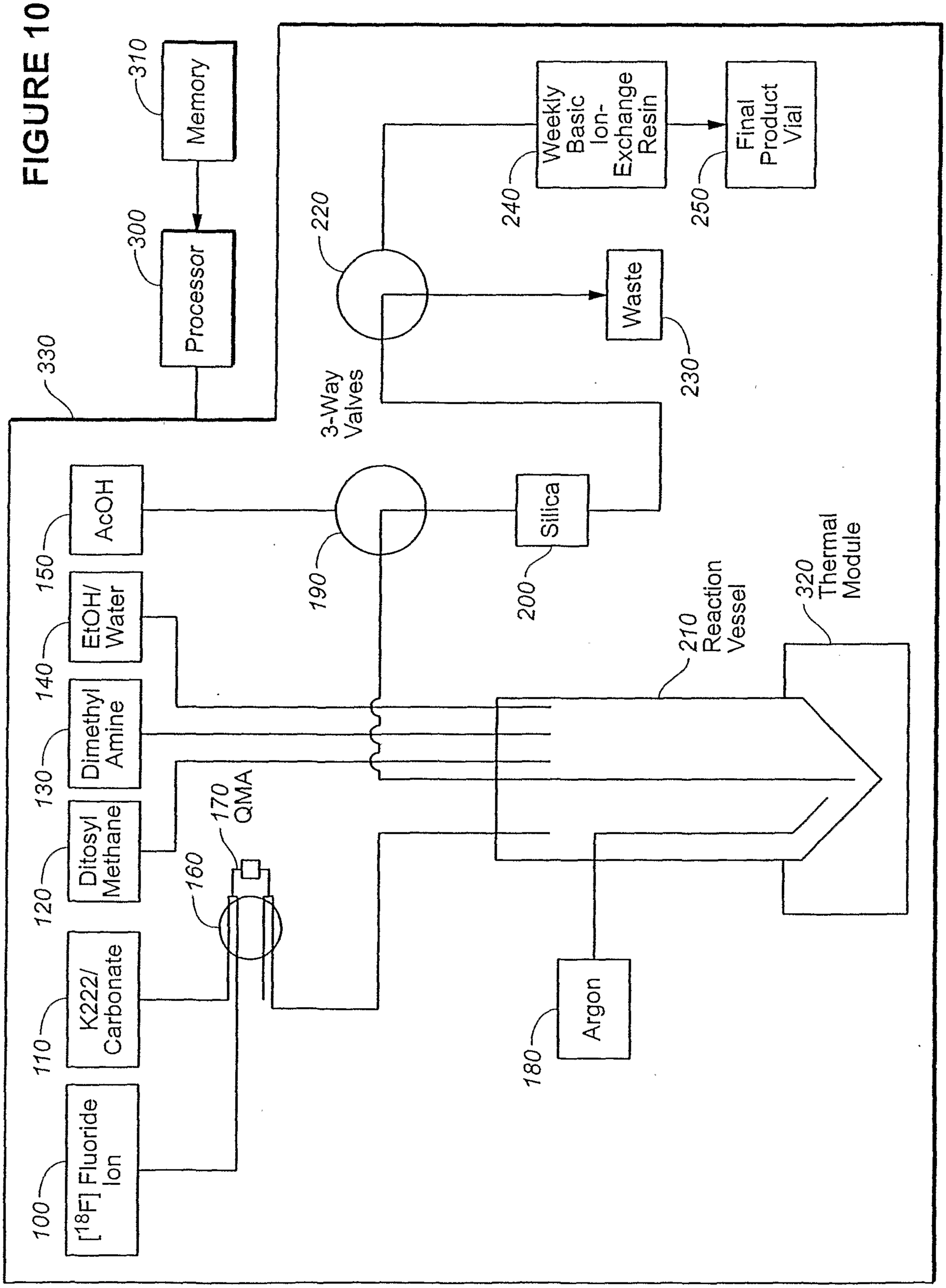

Memory
310
Processor
300
330
3-Way Valves
220
Weekly Basic Ion-Exchange Resin
240
Final Product Vial
250
Waste
230
AcOH
150
EtOH/ Water
140
Dimethyl Amine
130
Ditosyl Methane
120
K222/ Carbonate
110
[18F] Fluoride Ion
100
190
Silica
200
210 Reaction Vessel
320 Thermal Module
170 QMA
160
Argon
180

FIGURE 11

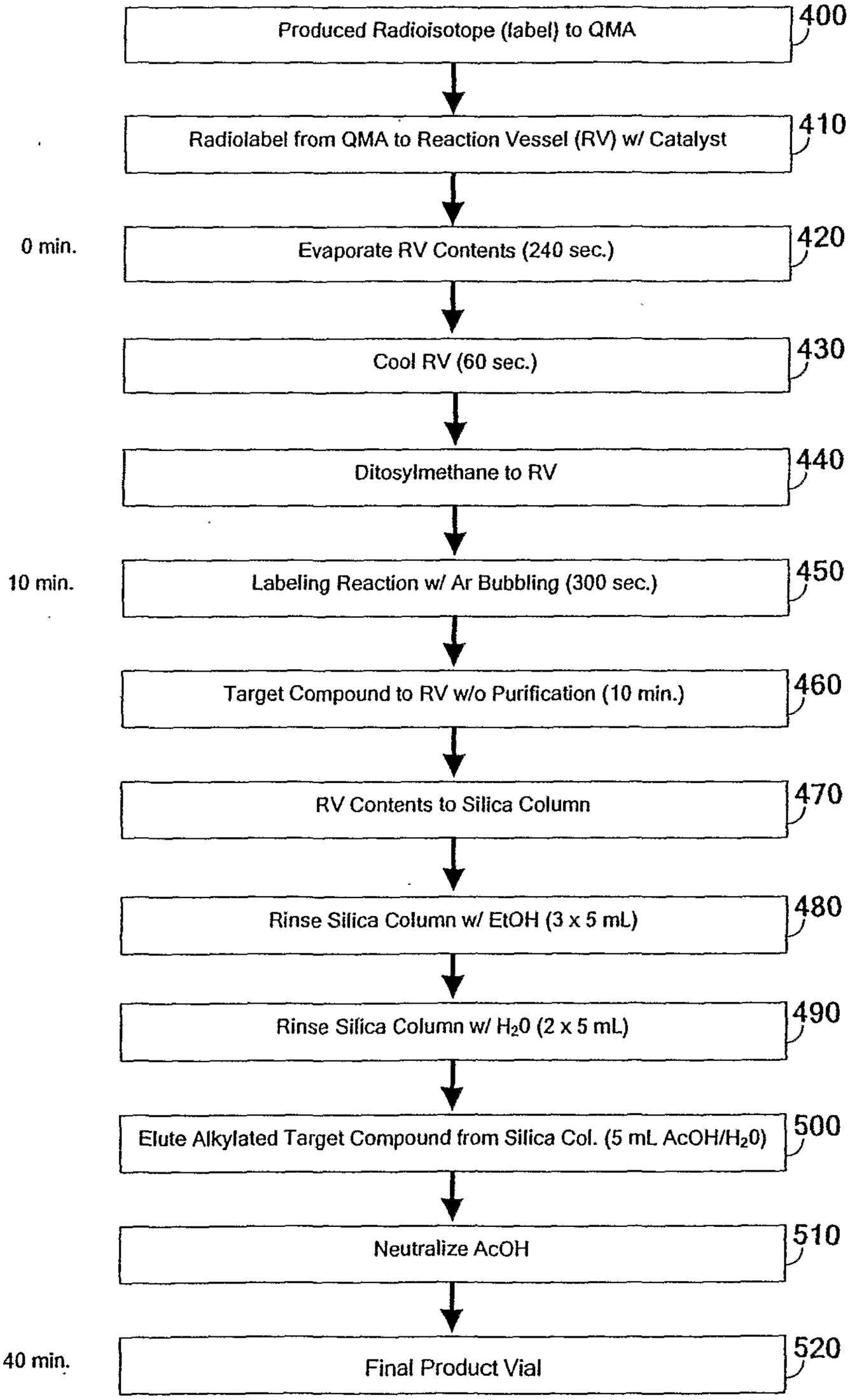

Produced Radioisotope (label) to QMA
400
Radiolabel from QMA to Reaction Vessel (RV) w/ Catalyst
410
0 min.
Evaporate RV Contents (240 sec.)
420
Cool RV (60 sec.)
430
Ditosylmethane to RV
440
10 min.
Labeling Reaction w/ Ar Bubbling (300 sec.)
450
Target Compound to RV w/o Purification (10 min.)
460
RV Contents to Silica Column
470
Rinse Silica Column w/ EtOH (3 x 5 mL)
480
Rinse Silica Column w/ H20 (2 x 5 mL)
490
Elute Alkylated Target Compound from Silica Col. (5 mL AcOH/H20)
500
Neutralize AcOH
510
40 min.
Final Product Vial
520

FIGURE 13

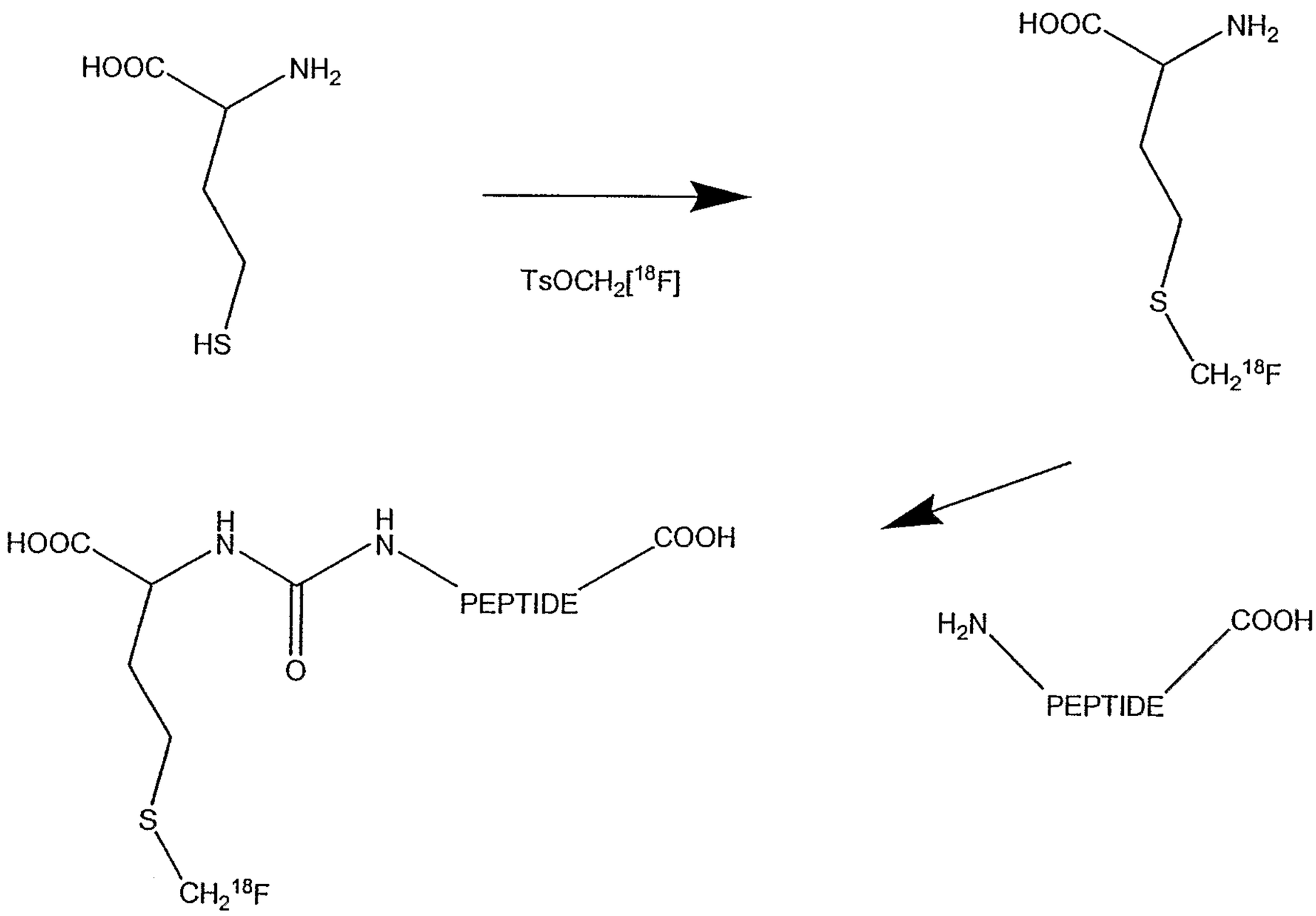

HOOC
NH2
HS
TsOCH2[18F]
HOOC
NH2
S
CH2 18F
HOOC
H
N
H
N
COOH
PEPTIDE
O
S
CH2 18F
H2N
COOH
PEPTIDE

FIGURE 14

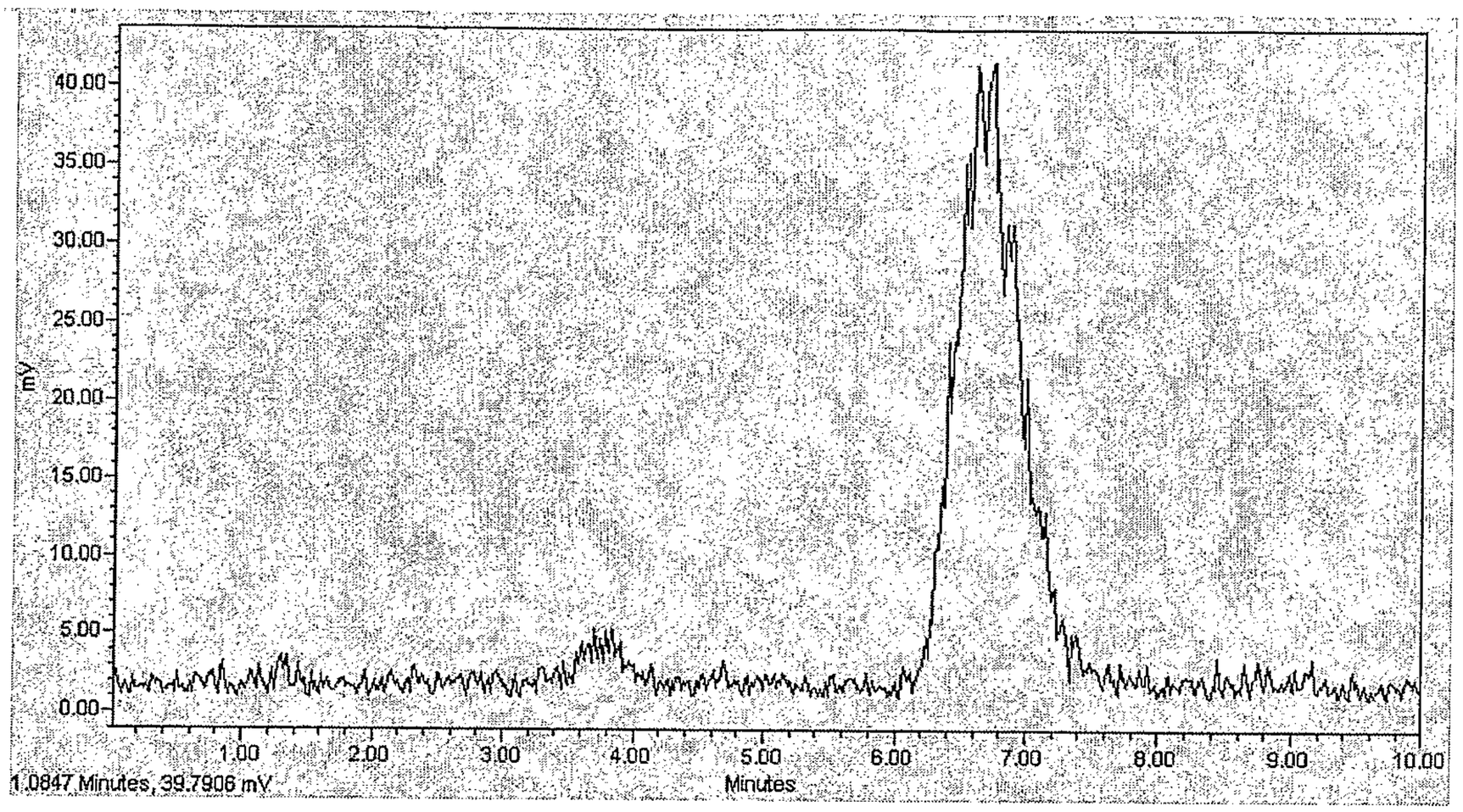


FIGURE 15

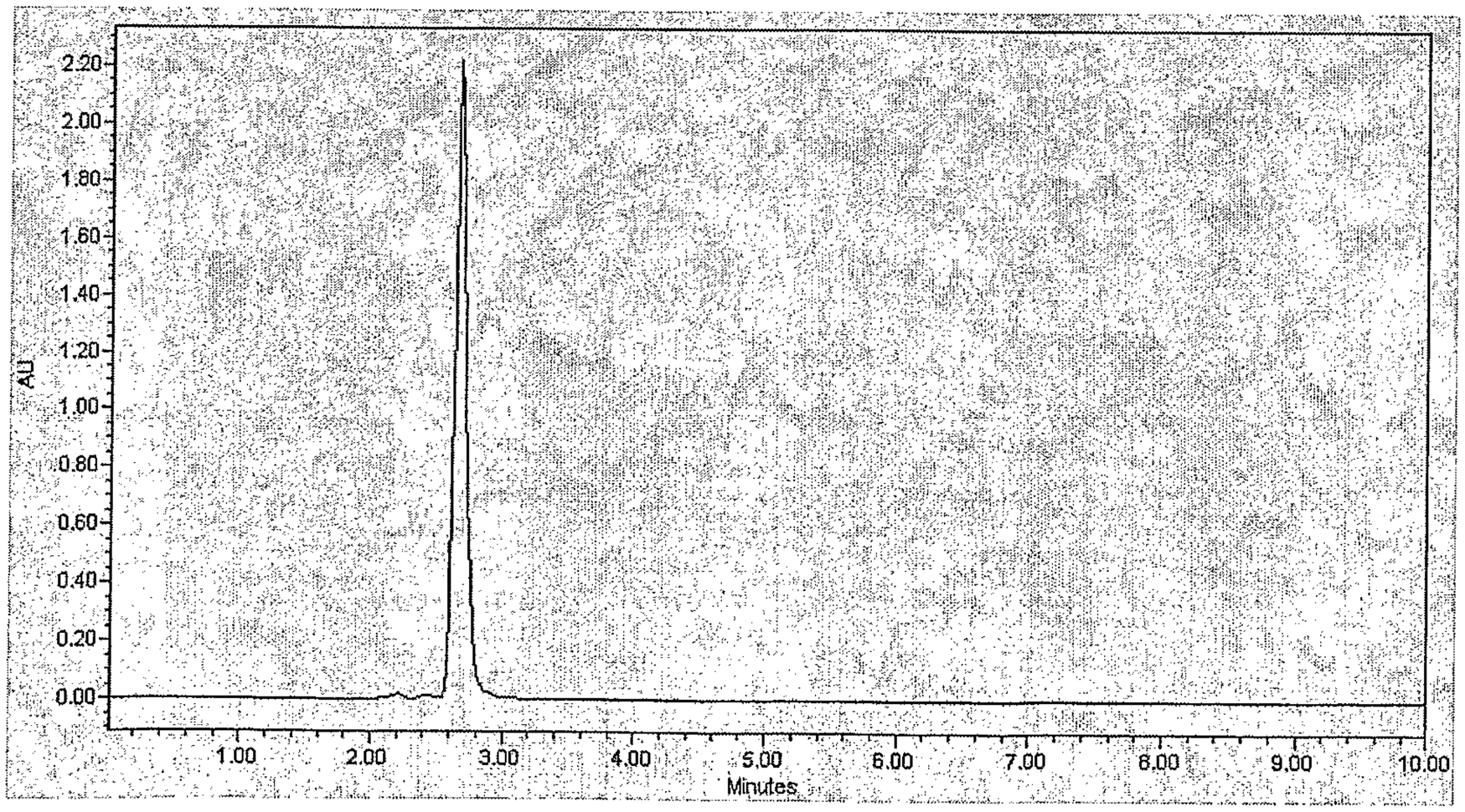


FIGURE 16

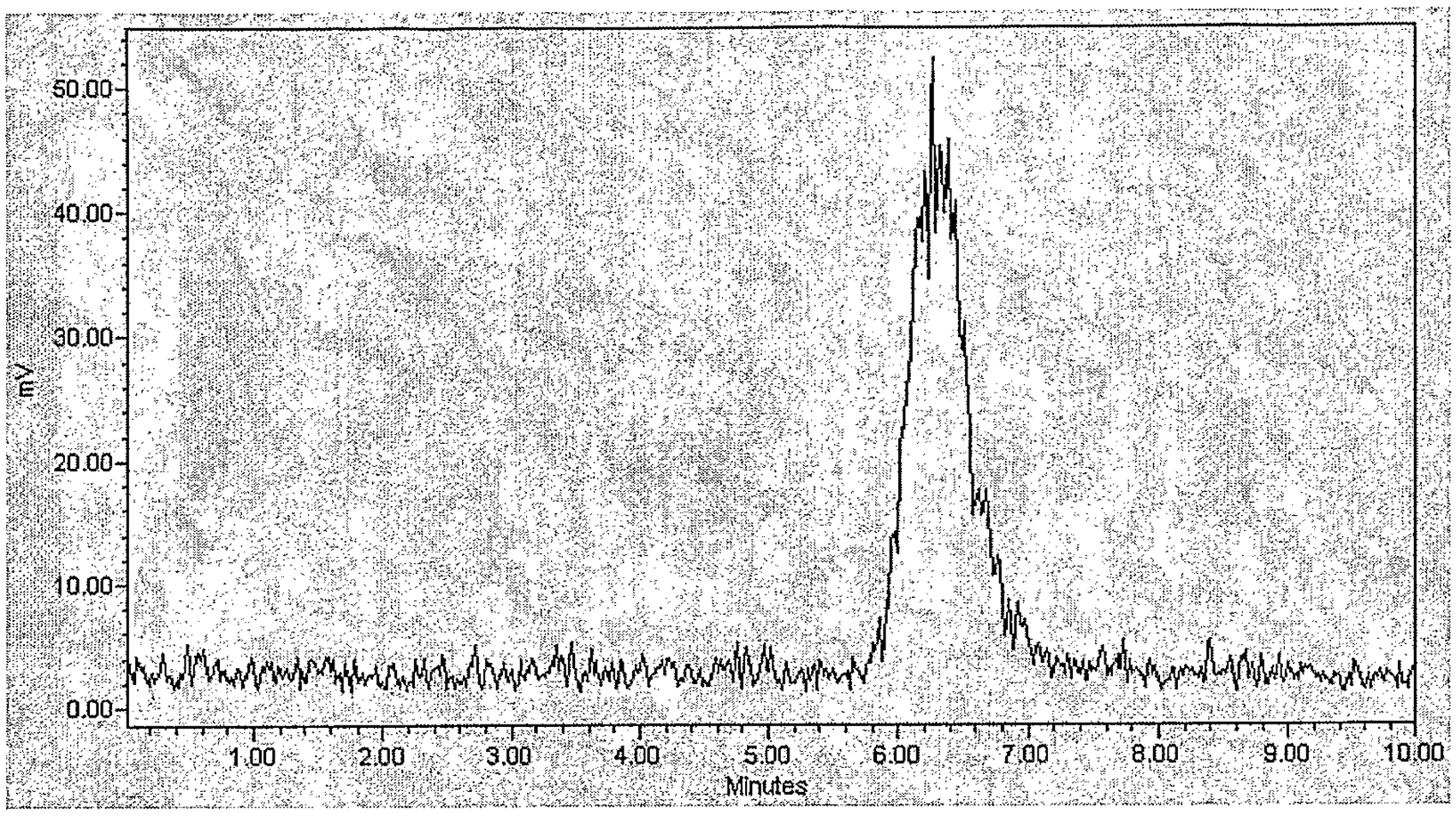
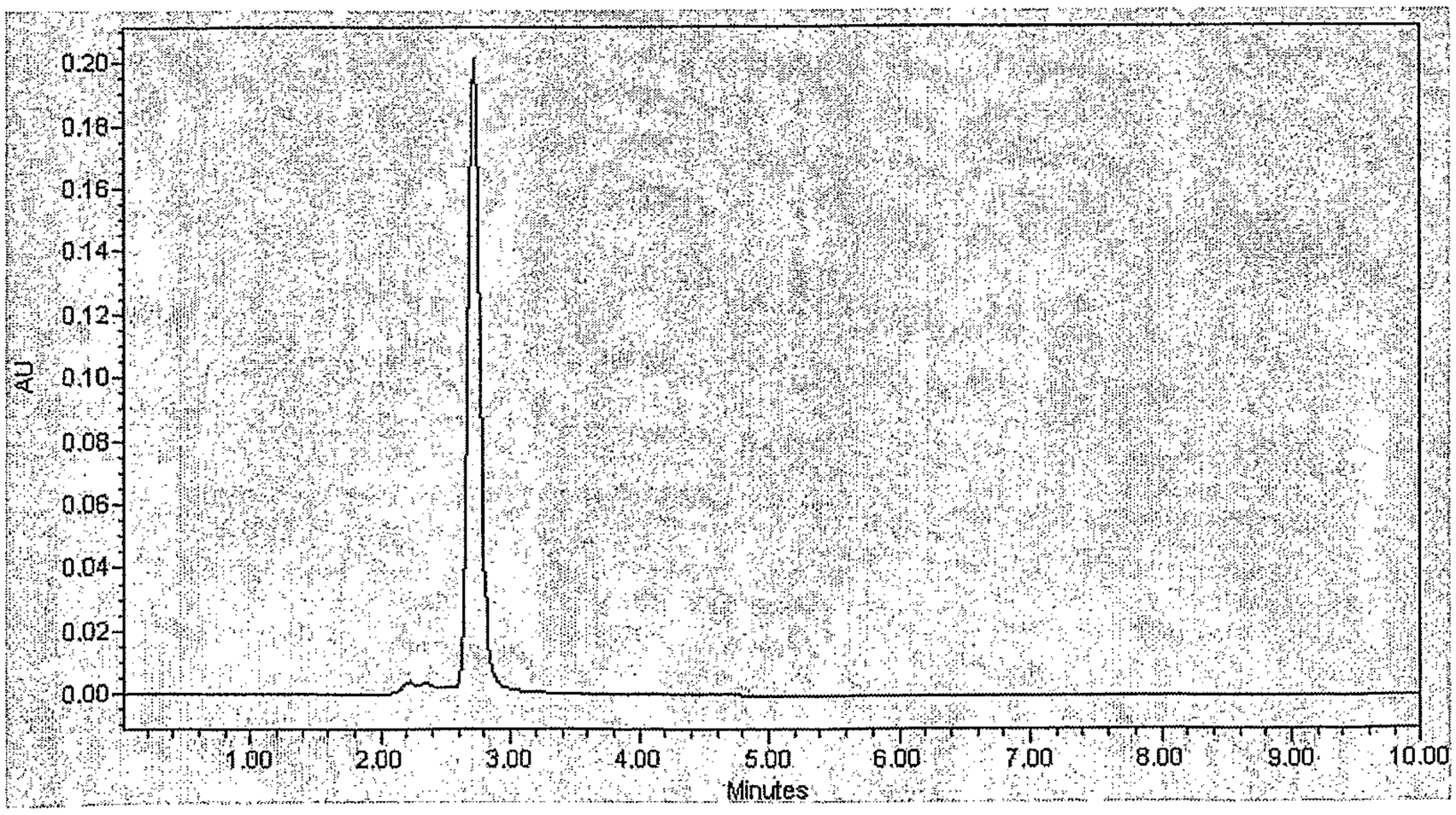



FIGURE 17