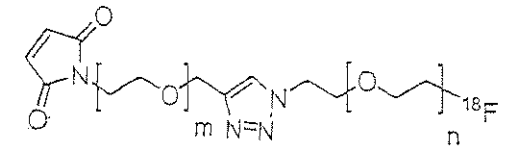
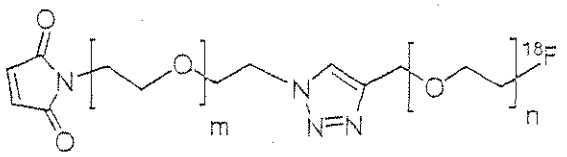
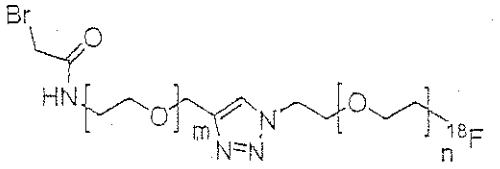
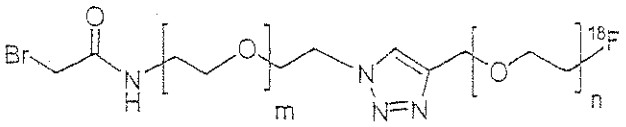
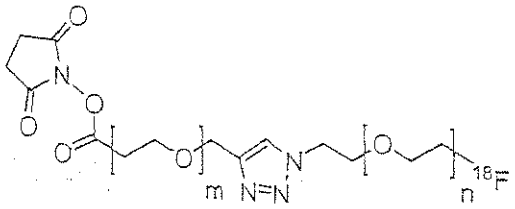
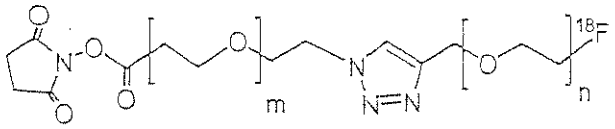
on engineered cysteine residues by prosthetic groups is advantageous as a site-specific method since the presence of cysteine is limited within the proteome, of which the non-reactive disulfide form is dominant (Olafsen et al (2004) Protein Eng Des Sel 17:21-7; Tait et al (2006) J Nucl Med 47:1546-53; Li et al (2008) Bioconjug Chem 19:1684-8). A protein engineering method, PHESELECTOR, employs phage display library to select the optimal amino acid position for cysteine substitution (Junutula et al. (2008) Nat Biotechnol 26:925-32; Junutula et al (2008) J Immunol Methods 332:41-52; US 2007/0092940). Using the PHESELECTOR method, protein stability and binding affinity is retained while the formation of undesired disulfide bonds is minimized providing optimal conjugation efficiencies. This method is used to produce modified Mab containing an available cysteine (ThioMab), from which a Fab fragment (ThioFab) with a reactive thiol group is conveniently generated.

[0010] Prosthetic groups containing the maleimide group, such as [^{18}F]FBEM (Cai et al (2006) J Nucl Med 47:1172-80), [^{18}F]FBAM (Berndt et al (2007) Nucl Med Biol 34:5-15), [^{18}F]FBABM (Li et al (2008) Bioconjug Chem 19:1684-8; Toyokuni et al (2003) Bioconjug Chem 14:1253-9), [^{18}F]FBOM (Wuest et al (2009) Amino Acids 36:283-295), [^{18}F]FDG-MHO (Wuest et al (2008) Bioconjug Chem 19:1202-10), and [^{18}F]FPyMe (de Bruin et al (2005) Bioconjug Chem 16:406-20), have been used to site-specifically introduce ^{18}F into a thiol-bearing protein. These labelling reagents [^{18}F]FBEM, [^{18}F]FBAM, [^{18}F]FBABM, and [^{18}F]FBOM were developed from a common platform where the aromatic precursor, ^{18}F-fluorobenzaldehyde ([^{18}F]FBALD), is coupled with an aminooxy-bearing maleimide precursor. However, the presence of aromatic and (with the exception of [^{18}F]FBOM) aliphatic moieties enhances the lipophilicity of these prosthetic groups, potentially limiting the conjugation efficiency with protein thiol groups present within a hydrophilic environment. Furthermore, these prosthetic groups require long synthesis times and typically provide relatively poor radiochemical yields of ^{18}F-labeled protein.

[0011] SUMMARY

[0012] A modular platform is provided for rapid preparation of various water-soluble prosthetic groups capable to efficiently introduce ^{18}F into proteins.

[0012a] In a first aspect, there is provided a method of labelling a protein comprising reacting a labelling reagent selected from:

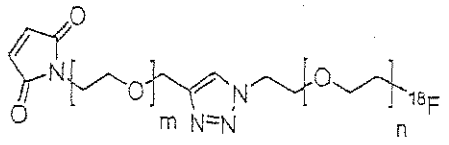
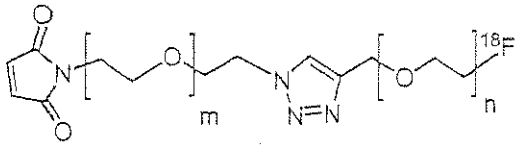
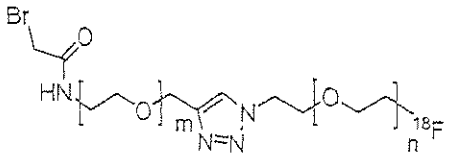
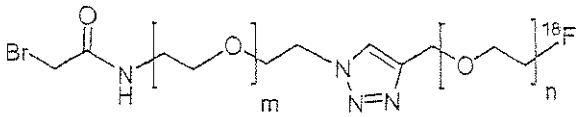
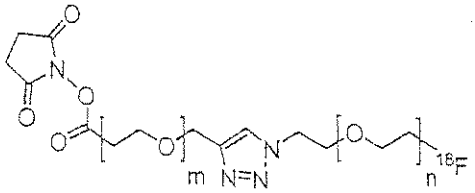
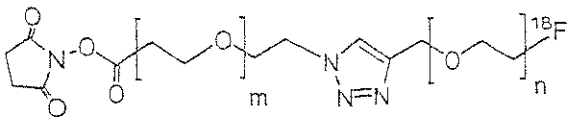







wherein n and m are independently selected from an integer from 2 to 12;

and a protein selected from an antibody, an interferon, a lymphokine, a cytokine, a hormone, or a growth factor,

whereby a labelled protein is formed.

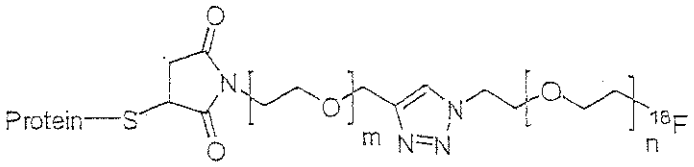
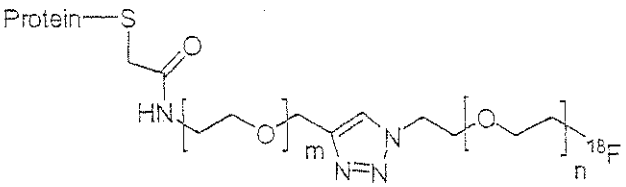
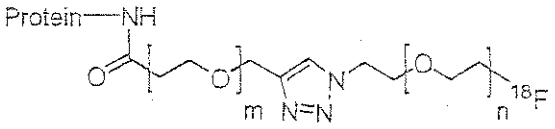
[0013] ~~An aspect of the invention includes~~Disclosed are methods of labelling a protein comprising reacting a labelling reagent and a protein to form a labelled protein. Labelling reagents include:

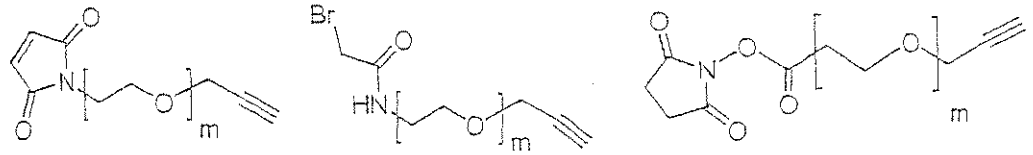
[0014] wherein n and m are independently selected from an integer from 2 to 12.

[0015] ~~An aspect of the invention includes~~Disclosed are processes for making a labelling reagent.

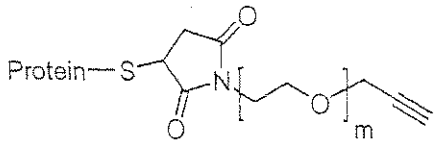
[0015a] In a second aspect, there is provided a method of preparing a labelled protein selected from:

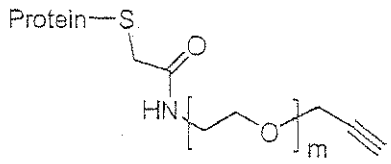




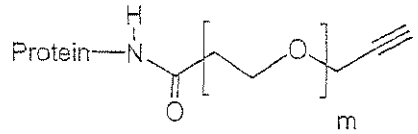
comprising reacting an alkyne reagent selected from:



with a protein selected from an antibody, an interferon, a lymphokine, a cytokine, a hormone, or a growth factor, to form an alkyne-protein selected from:

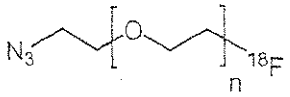






;

and reacting the alkyne-protein with an ^{18}F-PEG-azide reagent having the formula:

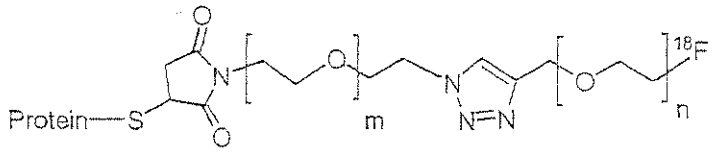


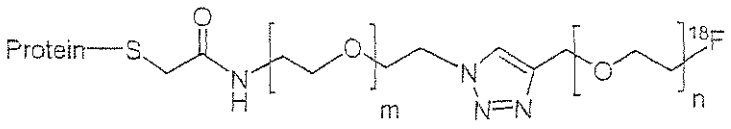
in the presence of a copper catalyst,

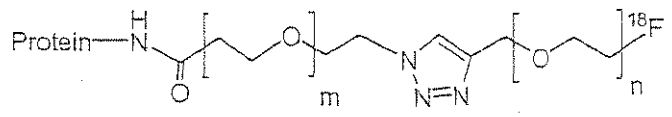
wherein n and m are independently selected from an integer from 2 to 12;

whereby the labelled protein is formed.

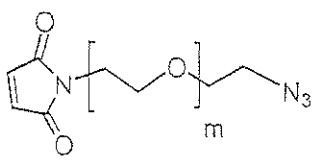
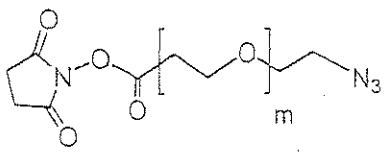
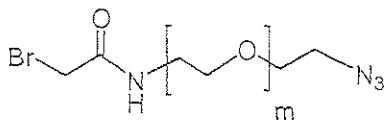
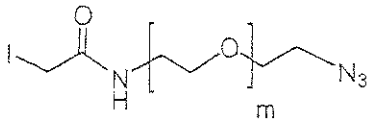
[0015b] In a third aspect, there is provided a method of preparing a labelled protein selected from:



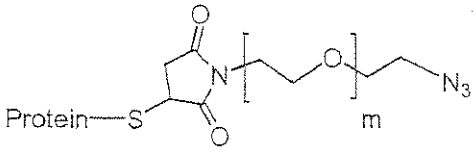
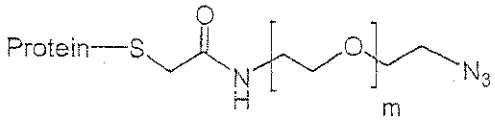
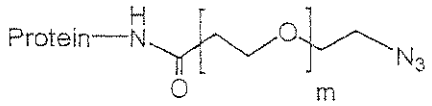




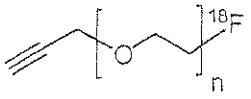
comprising reacting an azide reagent selected from:

with a protein selected from an antibody, an interferon, a lymphokine, a cytokine, a hormone, or a growth factor, to form an azido-protein selected from:

and reacting the azido-protein with an ^{18}F-PEG-alkyne reagent having the formula:

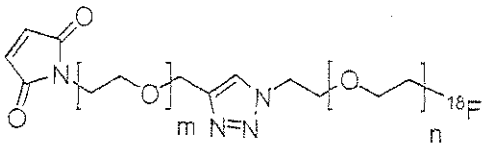


in the presence of a copper catalyst,

wherein n and m are independently selected from an integer from 2 to 12;

whereby the labelled protein is formed.

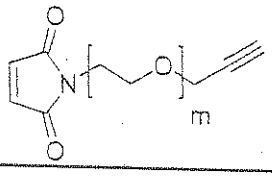
[0015c] In a fourth aspect, there is provided a process for making a labelling reagent having the formula:



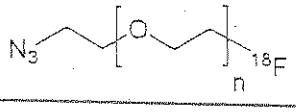
wherein:

n and m are independently selected from an integer from 2 to 12.

comprising reacting a maleimide-PEG-alkyne reagent having the formula:



with an ^{18}F-PEG-azide reagent having the formula:

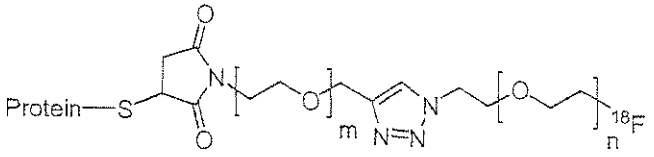


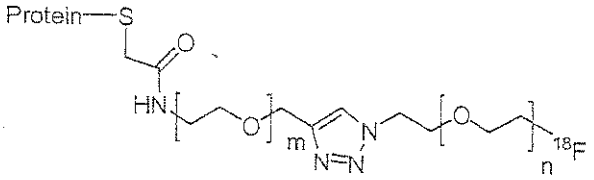
in the presence of a copper catalyst,

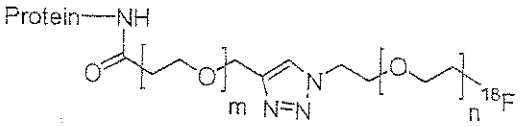
wherein n and m are independently selected from an integer from 2 to 12.

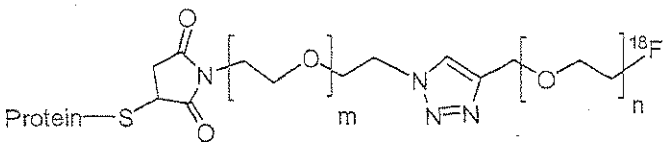
whereby the labelling reagent is formed.

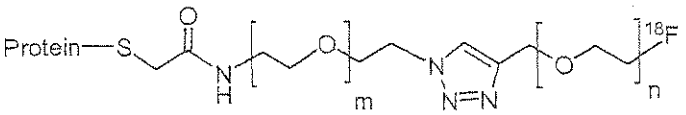
[0016] ~~An aspect of the invention includes~~Disclosed are labelled proteins selected from:

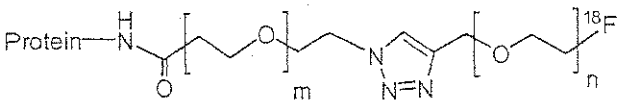




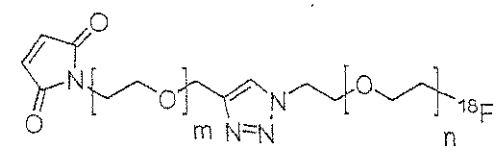


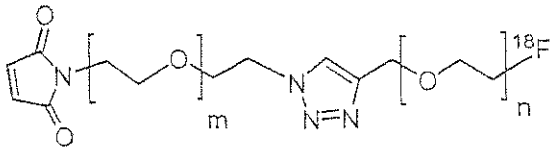


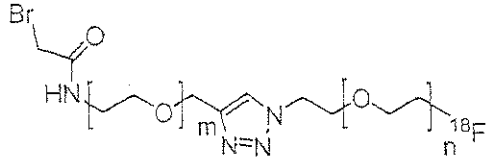


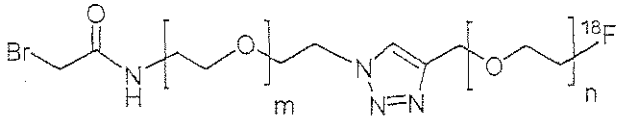


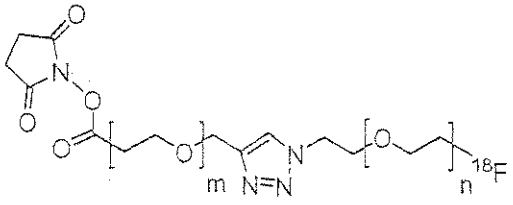
[0016a] In a fifth aspect, there is provided a labelling reagent selected from:

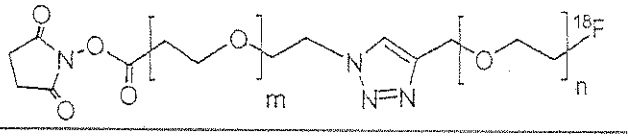






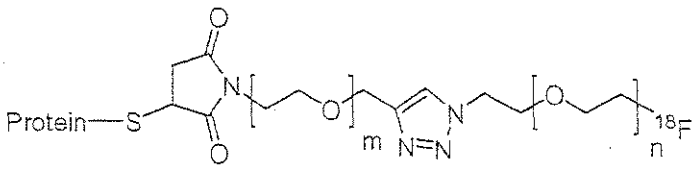


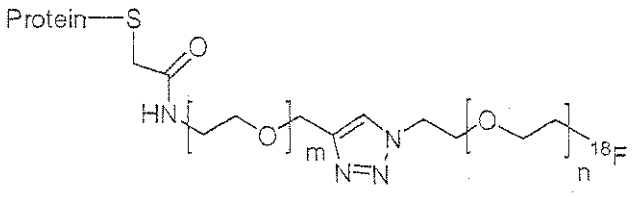


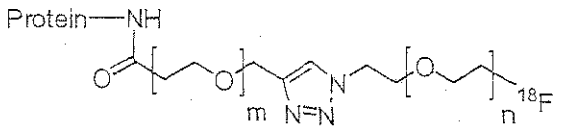


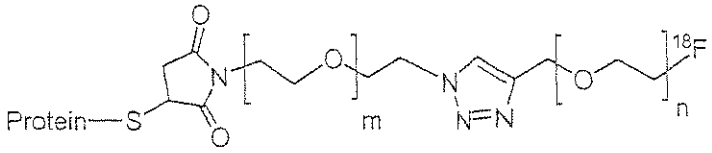
wherein n and m are independently selected from an integer from 2 to 12.

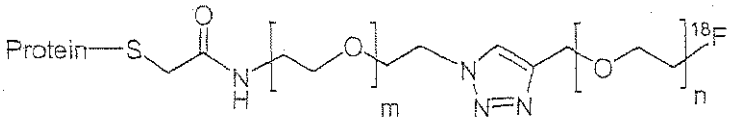
[0016b] In a sixth aspect, there is provided a labelled protein selected from:

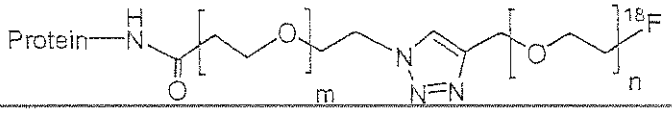








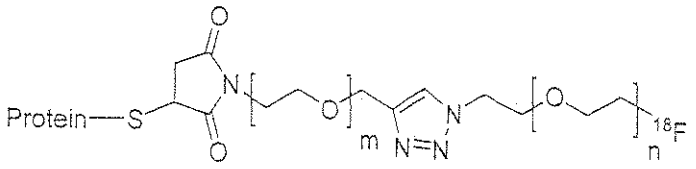


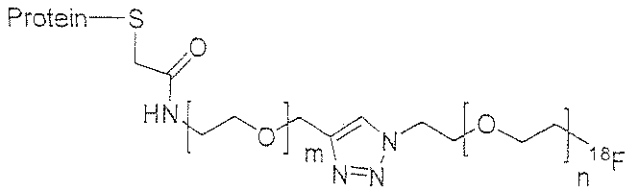


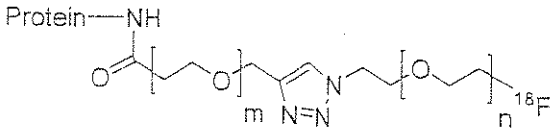
wherein n and m are independently selected from an integer from 2 to 12; and the Protein is selected from an antibody, an interferon, a lymphokine, a cytokine, a hormone, and a growth factor.

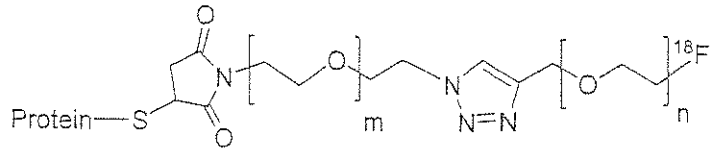
[0017] ~~An aspect of the invention includes~~Disclosed are pharmaceutical compositions comprising a labelled protein and one or more pharmaceutically acceptable carrier, glidant, diluent, or excipient.

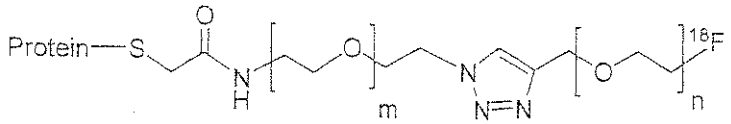
[0017a] In a seventh aspect, there is provided a pharmaceutical composition comprising a labelled protein and one or more pharmaceutically acceptable carrier, glidant, diluent, or excipient, wherein the labelled protein is selected from

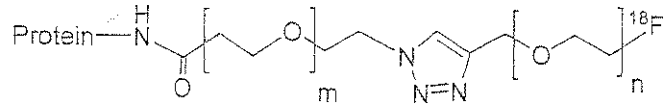








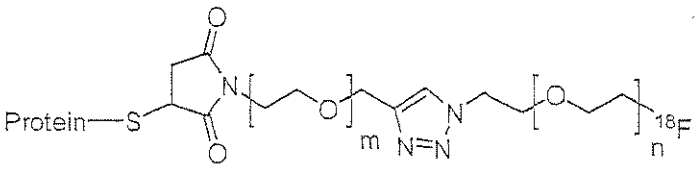


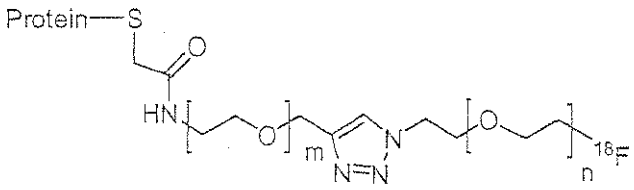


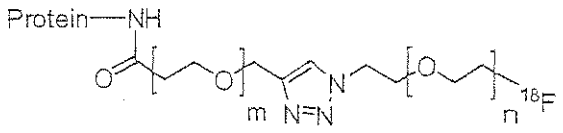
wherein n and m are independently selected from an integer from 2 to 12; and the protein is selected from an antibody, an interferon, a lymphokine, a cytokine, a hormone, and a growth factor.

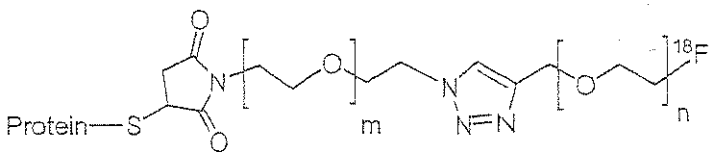
[0018] ~~An aspect of the invention includes~~Disclosed is a method of imaging comprising administering a labelled protein to an animal; and detecting in vivo the presence of the labelled protein by imaging.

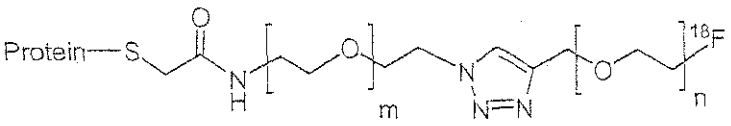
[0018a] In an eighth aspect, there is provided a method of imaging comprising: detecting the presence of a labelled protein by imaging an animal that has been administered the labelled protein, wherein the labelled protein is selected from:

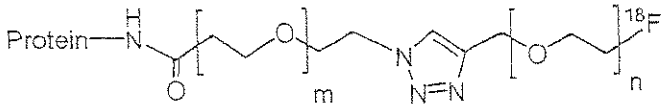












wherein n and m are independently selected from an integer from 2 to 12; and the protein is selected from an antibody, an interferon, a lymphokine, a cytokine, a hormone, and a growth factor.

[0019] BRIEF DESCRIPTION OF THE DRAWINGS

[0020] Figure 1 shows an exemplary synthetic route for radiolabeling Fab fragments using [^{18}F]FPEGMA. PEG = 2-12 ethyloxy units

[0021] Figure 2 shows an exemplary synthetic route for radiolabeling Fab fragments using [^{18}F]FPEGMA.

[0022] Figure 3 shows synthesis of intermediates **2** and **4**. Reagents: i. NaH, propargyl bromide; ii. maleimide, PPh_3, DIAD; iii. TsCl, pyridine; iv. $TBAHCO_3$, [^{18}F]fluoride

[0023] Figure 4 shows synthesis of [^{18}F]FPEGMA **5** and radiolabeling of a ThioFab to give [^{18}F]FPEGMA-Thio4D5Fab **6**. Reagents: v. $CuSO_4.H_2O$, BPDS, sodium ascorbate; vi. phosphate buffer pH 8

[0024] Figure 5 shows (a) Representative microPET images (coronal slices through tumor) of animal before (day -1) and after (day 1) 17-AAG therapy (top) compared to control animal (bottom). (b) Uptake of the ^{18}F-4D5ThioFab in the tumor tissue before and after the therapy.

[0025] DETAILED DESCRIPTION OF THE EXEMPLARY EMBODIMENTS

[0026] Reference will now be made in detail to certain embodiments of the invention, examples of which are illustrated in the accompanying structures and formulas. While the