



(51) International Patent Classification:

C07D 271/04 (2006.01) A61K 51/04 (2006.01)

(21) International Application Number:

PCT/US2017/029791

(22) International Filing Date:

27 April 2017 (27.04.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/335,969 13 May 2016 (13.05.2016) US

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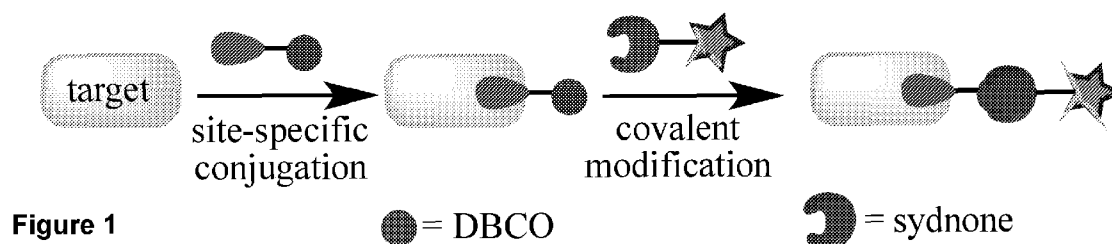
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: NOVEL COMPOSITIONS FOR LABELING BIOMOLECULES AND METHODS USING SAME



(57) Abstract: The present invention includes novel compounds useful for labeling biomolecules. The present invention further includes a novel method of labeling a biomolecule using a compound of the invention. The present invention further includes a novel method of imaging a biomolecule using a compound of the invention.

TITLE OF THE INVENTION

Novel Compositions for Labeling Biomolecules and Methods Using Same

CROSS-REFERENCE TO RELATED APPLICATIONS

5 The present invention claims priority to U.S. Provisional Application No. 62/335,969, filed May 13, 2016, which is hereby incorporated by reference in its entirety herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR
10 DEVELOPMENT

 This invention was made with U.S. government support under Grant No. P50 CA086306 awarded by the National Institutes of Health. The U.S. government has certain rights in the invention.

15 BACKGROUND OF THE INVENTION

 The ability to interrogate the molecular details of a biological process in a living system demands exquisite selectivity and robustness. Chemical reactions to selectively tag a biological target and subsequently image or track that target have been a long-standing goal of organic chemists. Despite the many challenges, careful
20 design and tuning of reaction partners has provided a myriad of bioorthogonal chemical reactions over the past 15 years. Remarkable advancement in the field of bioorthogonal “click” chemistry arose in 2004 when Bertozzi and co-workers introduced a copper-free variant, the strain-promoted azide-alkyne [3+2]
cycloaddition that has enabled cellular component labeling in living systems (Agard
25 et al., 2004, J. Am. Chem. Soc. 126:15046). This reaction exploits the strain activation inherent to cyclooctynes (Gordon et al., 2012, J. Am. Chem. Soc. 134:9199) to avoid the use of a toxic copper(I) catalysts yet retains the kinetics to proceed rapidly in a chemically complex environment, such as in living systems, and has been widely utilized in the chemical biology community (Jewett and Bertozzi,
30 2010, Chem. Soc. Rev. 39:1272; Baskin et al., 2007, Proc. Natl. Acad. Sci. U.S.A. 104:16793; Chang et al., 2010, Proc. Natl. Acad. Sci. U.S.A. 107:1821). Since the initial report of a strain-promoted cycloaddition with a rate of $10^{-3} \text{ M}^{-1} \text{ s}^{-1}$, a great deal of progress has been made towards developing chemoselective reactions for labeling biomolecules with extraordinary fast rate constants (Lang and Chin, 2014, ACS

Chem. Biol. 9:16; Patterson et al., 2014, ACS Chem. Biol. 9:592). In 2008 an alternative bioorthogonal ligation, the inverse-electron-demand Diels-Alder reaction between tetrazines and strained alkenes, was introduced (Devaraj et al., 2008, Bioconjugate Chem. 19:2297; Blackman et al., 2008, J. Am. Chem. Soc. 130:13518) which has reached rate constants up to $10^4 \text{ M}^{-1}\text{s}^{-1}$ (Karver et al., 2011, Bioconjugate Chem. 22:2263; Taylor et al., 2011, J. Am. Chem. Soc. 133:9646; Lang et al., 2012, J. Am. Chem. Soc. 134:10317).

To date, the most common ^{18}F -labeling method for biomolecules utilizes ^{18}F -SFB, a radiolabeled prosthetic group that reacts with the ϵ -amino group of surface-exposed lysine residues (Liu et al., 2011, Mol. Imaging 10:168; Cai et al., 2007, J. Nucl. Med. 48:304; Olafsen et al., 2012, Tumor Biol. 33:669). In addition, site-specific conjugation using 4- ^{18}F -fluorobenzaldehyde (^{18}F -FBA) has also been demonstrated (Cheng et al., 2008, J. Nucl. Med. 49:804). While ^{18}F -SFB has been successfully used to generate ^{18}F -labeled proteins and peptides, labeling with ^{18}F -SFB is far from ideal; in addition to its unselective conjugation, its 3-step synthesis and subsequent protein conjugation results in very poor decay-corrected radiochemical yields of 1.4 – 2.5%. A distinct advantage of ^{18}F -FBA over ^{18}F -SFB is the opportunity for site-specific conjugation; however, its synthesis and protein conjugation is only moderately improved with yields of 13 – 18%.

Bioorthogonal chemical ligations involve two molecular components that selectively react with each other in high yield under physiological conditions without interference from other chemical functionalities and with fast reaction kinetics. The inherent nature of these ligations – extremely rapid, highly-selective, modular, robust – suggest their aptness for the time-sensitive and stringent conditions of ^{18}F -radiolabeling of biomolecules. While over twenty unique bioorthogonal reaction pairs have been established for the selective tagging of biomolecules, few have demonstrated successful applications with regards to ^{18}F -labeling. The most commonly used bioorthogonal ligation for radiolabeling biomolecules applies the azide-alkyne ligation; however, these ^{18}F -labeled compounds are not easily accessible. For example, ^{18}F -fluoroethylazide is highly volatile making synthesis and handling difficult and requiring a specialized reaction set-up. A simplified, one-pot protocol was recently reported for the synthesis of 4- ^{18}F -Fluorobenzyl azide, however, its precursor is generated in a modest 17% yield (Rotstein et al., 2014, Nat. Commun.

5:4365). The synthetic challenges associated with these and other ^{18}F -labeled azides are a considerable limitation to their global use.

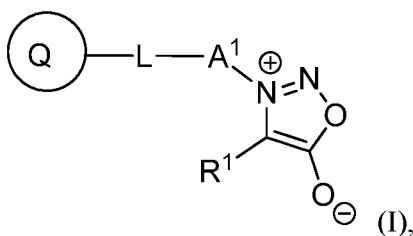
Recently, the bioorthogonal tetrazine-transcyclooctene ligation has been gaining interest for the selective introduction of fluorine-18 into biologically
 5 relevant molecules. However, preparation of an ^{18}F -labeled prosthetic group for this ligation introduces significant synthetic challenges that impede its practicality and global utilization. For example, ^{18}F -transcyclooctene (^{18}F -TCO) is a five-step synthesis, one of which requires a specialized apparatus for the photochemical isomerization of the *cis*-cyclooctyl intermediate to yield the respective *trans*-
 10 cyclooctyl compound in low yield (Royzen et al., 2008, 130:3760; Li et al., 2010, Chem. Commun. 46:8043). In addition, the tetrazine-TCO ligation for ^{18}F labeling requires that the tetrazine be attached to the protein; tetrazines demonstrate poor in vivo stability in most cases, with some disubstituted derivatives showing better stability but slower reaction kinetics (Karver et al., 2011, Bioconjugate Chem.
 15 22:2263).

There is a need in the art for novel compounds and methods for the rapid and site-specific labeling of biomolecules under physiological conditions. The present invention addresses this unmet need.

20

SUMMARY OF THE INVENTION

The present invention relates to a compound of formula (I):



wherein in formula (I):

A¹ is selected from the group consisting of alkyl, heteroalkyl, alkylcycloalkyl, heterocyclyl, alkylheterocyclyl, aryl, alkylaryl, heteroaryl, and alkylheteroaryl,
 25 wherein the alkyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl group is optionally substituted with 0-5 R² groups

R¹ and each occurrence of R² are independently selected from the group consisting of H, -C₁-C₆ alkyl, -C₁-C₆ fluoroalkyl, -C₁-C₆ heteroalkyl, F, ^{18}F , Cl, Br, I,

-CN, -NO₂, -OR³, -SR³, -S(=O)R³, -S(=O)₂R³, -NHS(=O)₂R³, -C(=O)R³, -OC(=O)R³, -CO₂R³, -OCO₂R³, -CH(R³)₂, -N(R³)₂, -C(=O)N(R³)₂, -OC(=O)N(R³)₂, -NHC(=O)NH(R³), -NHC(=O)R³, -NHC(=O)OR³, -C(OH)(R³)₂, and -C(NH₂)(R³)₂;

each occurrence of R³ is independently selected from the group consisting of

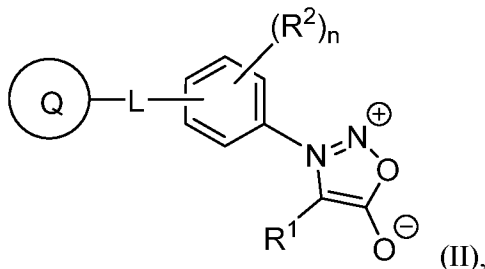
5 H and C₁-C₆ alkyl;

L is a linker; and

Q is an imaging moiety.

a salt thereof, and any combinations thereof.

In one embodiment, A¹ is a phenyl group, wherein the phenyl group is optionally substituted. In another embodiment, L is selected from the group consisting of a bond, an alkyl group, an amide, a poly(alkyl ether), and any combinations thereof. In another embodiment, Q is ¹⁸F. In another embodiment, Q is a fluorescent dye. In another embodiment, the fluorescent dye is BODIPY630. In another embodiment, the compound of formula (I) is a compound of formula (II):



wherein in formula (II):

n is an integer between 0 and 4;

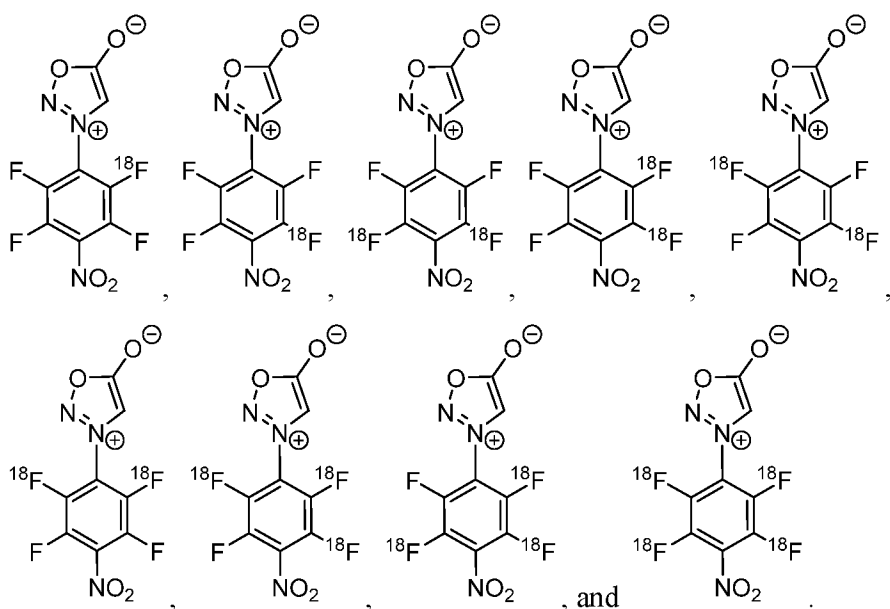
10 R¹ and each occurrence of R² are each independently selected from the group consisting of H, -C₁-C₆ alkyl, -C₁-C₆ fluoroalkyl, -C₁-C₆ heteroalkyl, F, ¹⁸F, Cl, Br, I, -CN, -NO₂, -OR³, -SR³, -S(=O)R³, -S(=O)₂R³, -NHS(=O)₂R³, -C(=O)R³, -OC(=O)R³, -CO₂R³, -OCO₂R³, -CH(R³)₂, -N(R³)₂, -C(=O)N(R³)₂, -OC(=O)N(R³)₂, -NHC(=O)NH(R³), -NHC(=O)R³, -NHC(=O)OR³, -C(OH)(R³)₂, and -C(NH₂)(R³)₂;

15 each occurrence of R³ is independently selected from the group consisting of H and C₁-C₆ alkyl;

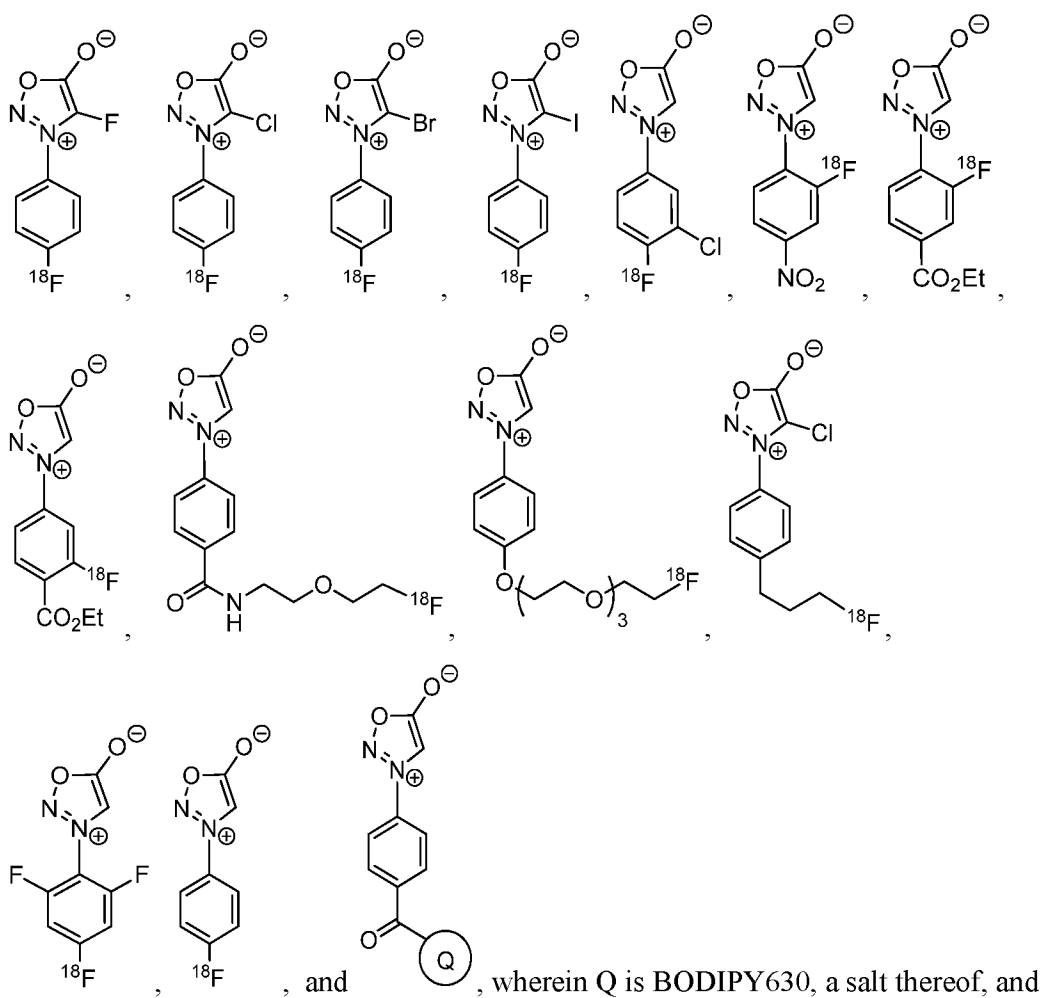
L is a linker; and

Q is an imaging moiety.

In one embodiment, n is at least 1 and at least one R² is F. In another embodiment, the compound is selected from the group consisting of:

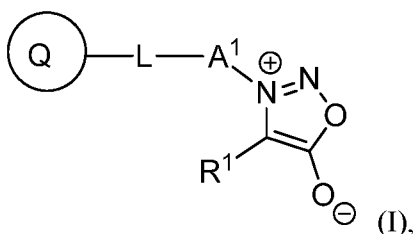


In another embodiment, the compound is selected from the group consisting of:



any combinations thereof.

The present invention also relates to a method for labeling a biomolecule. The method includes the steps of covalently attaching or conjugating a dipolarophile to a biomolecule to form a biomolecule-dipolarophile conjugate, and contacting the biomolecule-dipolarophile conjugate with a compound, wherein the compound undergoes a cycloaddition reaction with the dipolarophile to provide a labeled biomolecule. In one embodiment, the compound is at least one compound of formula (I):



wherein in formula (I):

- A^1 is selected from the group consisting of alkyl, heteroalkyl, alkylcycloalkyl, heterocyclyl, alkylheterocyclyl, aryl, alkylaryl, heteroaryl, and alkylheteroaryl, wherein the alkyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl group is optionally substituted with 0-5 R^2 groups

- R^1 and each occurrence of R^2 are independently selected from the group consisting of H, $-C_1-C_6$ alkyl, $-C_1-C_6$ fluoroalkyl, $-C_1-C_6$ heteroalkyl, F, ^{18}F , Cl, Br, I, $-CN$, $-NO_2$, $-OR^3$, $-SR^3$, $-S(=O)R^3$, $-S(=O)_2R^3$, $-NHS(=O)_2R^3$, $-C(=O)R^3$, $-OC(=O)R^3$, $-CO_2R^3$, $-OCO_2R^3$, $-CH(R^3)_2$, $-N(R^3)_2$, $-C(=O)N(R^3)_2$, $-OC(=O)N(R^3)_2$, $-NHC(=O)NH(R^3)$, $-NHC(=O)R^3$, $-NHC(=O)OR^3$, $-C(OH)(R^3)_2$, and $-C(NH_2)(R^3)_2$;

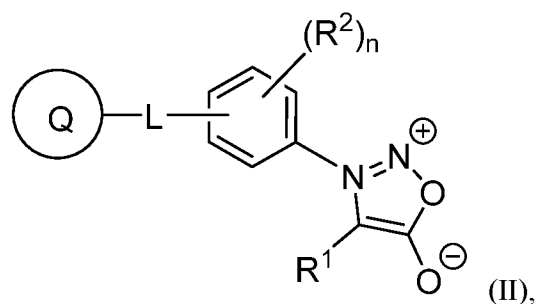
each occurrence of R^3 is independently selected from the group consisting of H and C_1-C_6 alkyl;

- L is a linker; and

Q is an imaging moiety.

a salt thereof, and any combinations thereof.

In one embodiment, A^1 is a phenyl group, wherein the phenyl group is optionally substituted. In another embodiment, L is selected from the group consisting of a bond, an alkyl group, an amide, a poly(alkyl ether) group, and any combinations thereof. In another embodiment, Q is ^{18}F . In another embodiment, Q is a fluorescent dye. In another embodiment, the fluorescent dye is BODIPY630. In another embodiment, the compound of formula (I) is a compound of formula (II):



wherein in formula (II):

n is an integer between 0 and 4;

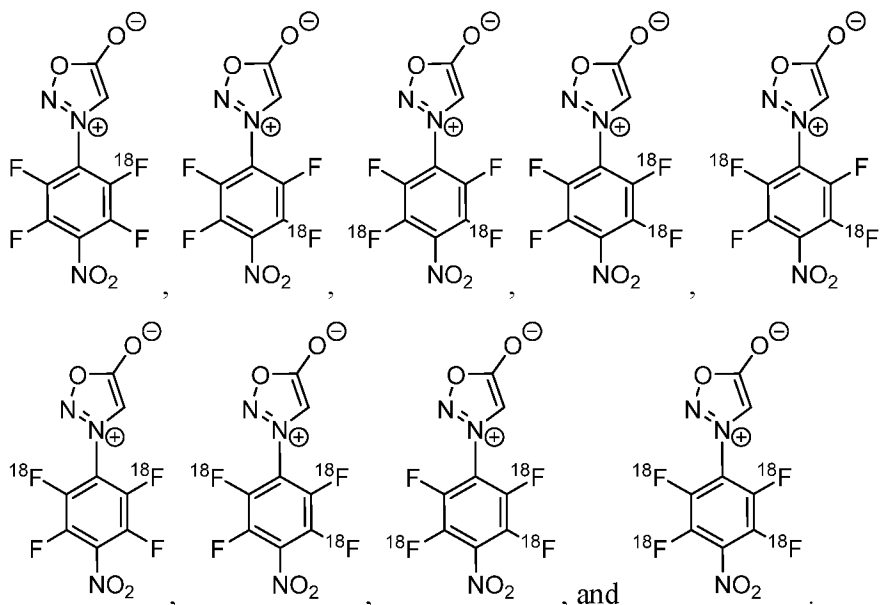
R^1 and each occurrence of R^2 are each independently selected from the group consisting of H, $-C_1-C_6$ alkyl, $-C_1-C_6$ fluoroalkyl, $-C_1-C_6$ heteroalkyl, F, ^{18}F , Cl, Br, I, $-CN$, $-NO_2$, $-OR^3$, $-SR^3$, $-S(=O)R^3$, $-S(=O)_2R^3$, $-NHS(=O)_2R^3$, $-C(=O)R^3$, $-OC(=O)R^3$, $-CO_2R^3$, $-OCO_2R^3$, $-CH(R^3)_2$, $-N(R^3)_2$, $-C(=O)N(R^3)_2$, $-OC(=O)N(R^3)_2$, $-NHC(=O)NH(R^3)$, $-NHC(=O)R^3$, $-NHC(=O)OR^3$, $-C(OH)(R^3)_2$, and $-C(NH_2)(R^3)_2$;

each occurrence of R^3 is independently selected from the group consisting of H and C_1-C_6 alkyl;

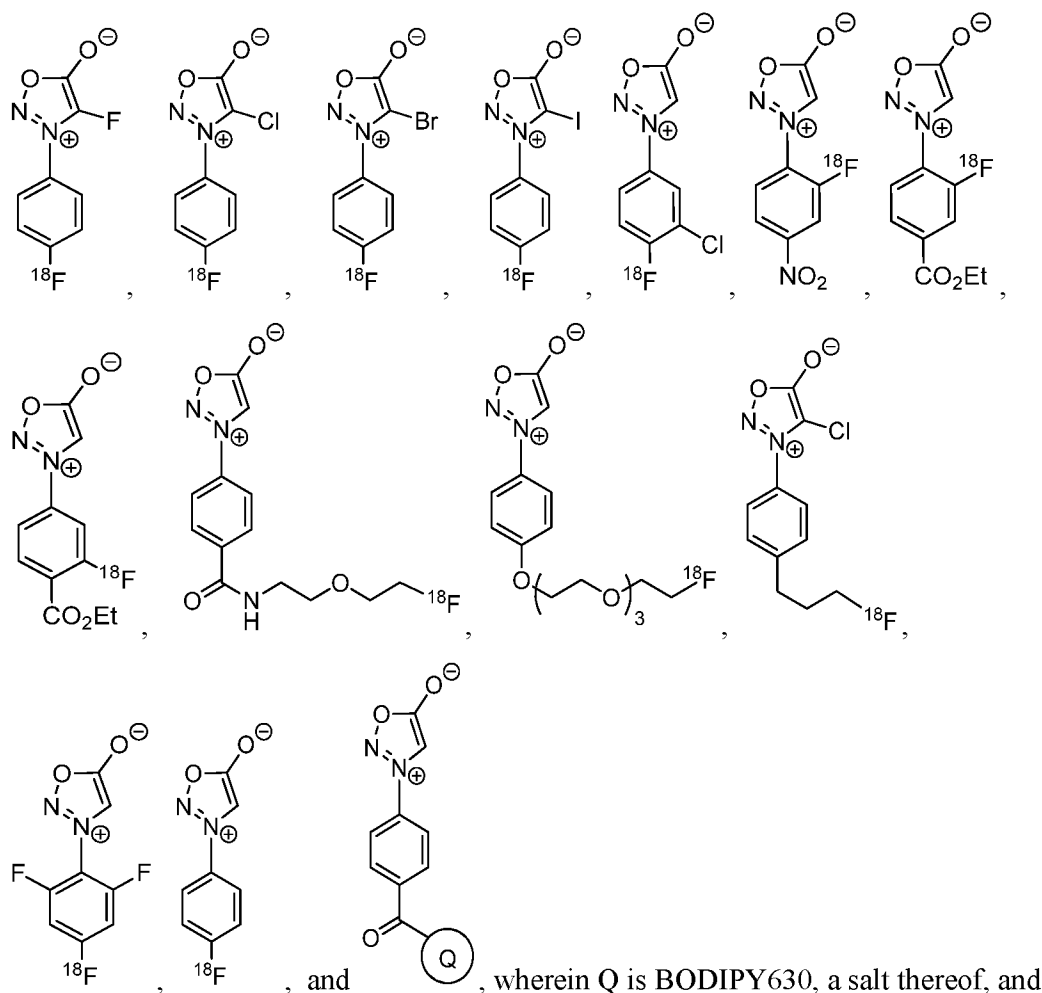
L is a linker; and

Q is an imaging moiety.

In one embodiment, n is at least 1 and at least one R^2 is F. In another embodiment, the compound is selected from the group consisting of:

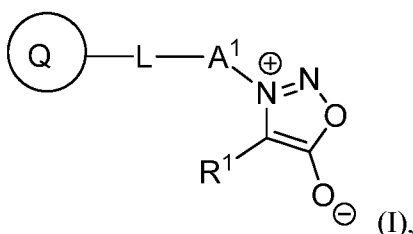


In another embodiment, the compound is selected from the group consisting of:



any combinations thereof. In another embodiment, the dipolarophile is
 5 dibenzocyclooctyne (DBCO). In another embodiment, the dipolarophile is
 biarylazacyclooctynone (BARAC).

The present invention also relates to a method for imaging a biomolecule. The method includes the steps of providing a sample comprising a biomolecule, covalently attaching or conjugating a dipolarophile to a biomolecule to form a biomolecule-dipolarophile conjugate, contacting the biomolecule-dipolarophile conjugate with a compound, wherein the compound undergoes a cycloaddition reaction with the dipolarophile to provide a labeled biomolecule, and detecting the labeled biomolecule, thereby imaging the biomolecule. In one embodiment, the compound is at least one compound of formula (I):



wherein in formula (I):

A¹ is selected from the group consisting of alkyl, heteroalkyl, alkylcycloalkyl, heterocyclyl, alkylheterocyclyl, aryl, alkylaryl, heteroaryl, and alkylheteroaryl,

- 5 wherein the alkyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl group is optionally substituted with 0-5 R² groups

R¹ and each occurrence of R² are independently selected from the group consisting of H, -C₁-C₆ alkyl, -C₁-C₆ fluoroalkyl, -C₁-C₆ heteroalkyl, F, ¹⁸F, Cl, Br, I, -CN, -NO₂, -OR³, -SR³, -S(=O)R³, -S(=O)₂R³, -NHS(=O)₂R³, -C(=O)R³, -OC(=O)R³,
 10 -CO₂R³, -OCO₂R³, -CH(R³)₂, -N(R³)₂, -C(=O)N(R³)₂, -OC(=O)N(R³)₂, -NHC(=O)NH(R³), -NHC(=O)R³, -NHC(=O)OR³, -C(OH)(R³)₂, and -C(NH₂)(R³)₂;

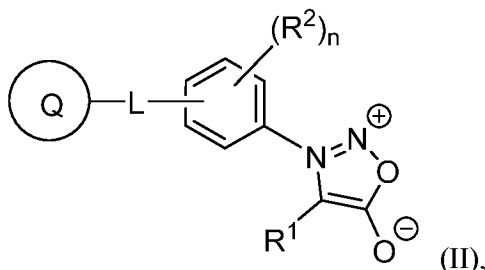
each occurrence of R³ is independently selected from the group consisting of H and C₁-C₆ alkyl;

L is a linker; and

Q is an imaging moiety.

a salt thereof, and any combinations thereof.

In one embodiment, A¹ is a phenyl group, wherein the phenyl group is optionally substituted. In another embodiment, L is selected from the group consisting of a bond, an alkyl group, an amide, a poly(alkyl ether) group, and any combinations thereof. In another embodiment, Q is ¹⁸F. In another embodiment, Q is a fluorescent dye. In another embodiment, the fluorescent dye is BODIPY630. In another embodiment, the compound of formula (I) is a compound of formula (II):



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wherein in formula (II):

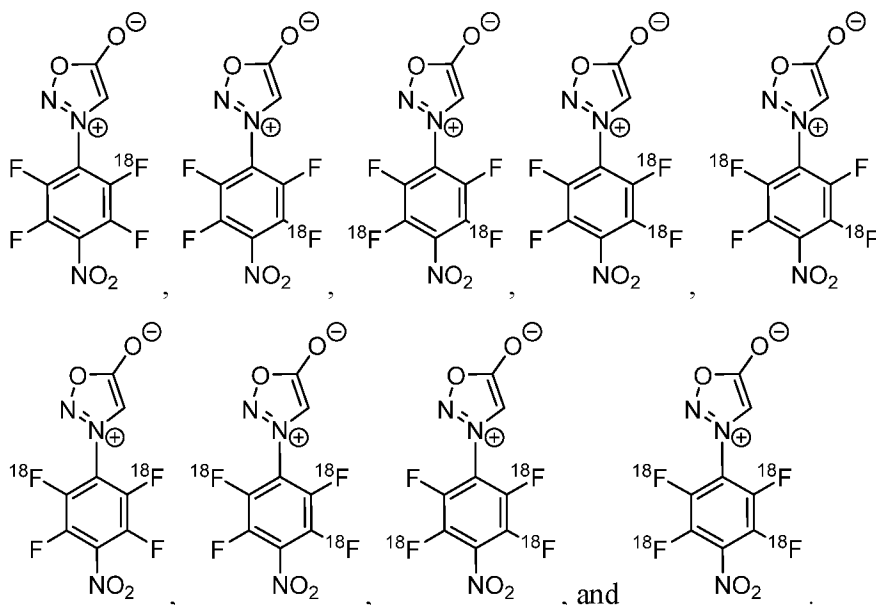
n is an integer between 0 and 4;

- R^1 and each occurrence of R^2 are each independently selected from the group consisting of H, $-C_1-C_6$ alkyl, $-C_1-C_6$ fluoroalkyl, $-C_1-C_6$ heteroalkyl, F, ^{18}F , Cl, Br, I, $-CN$, $-NO_2$, $-OR^6$, $-SR^6$, $-S(=O)R^6$, $-S(=O)_2R^6$, $-NHS(=O)_2R^6$, $-C(=O)R^6$, $-OC(=O)R^6$, $-CO_2R^6$, $-OCO_2R^6$, $-CH(R^6)_2$, $-N(R^6)_2$, $-C(=O)N(R^6)_2$, $-OC(=O)N(R^6)_2$, $-NHC(=O)NH(R^6)$, $-NHC(=O)R^6$, $-NHC(=O)OR^6$, $-C(OH)(R^6)_2$, and $-C(NH_2)(R^6)_2$;
- each occurrence of R^6 is independently selected from the group consisting of H and C_1-C_6 alkyl;

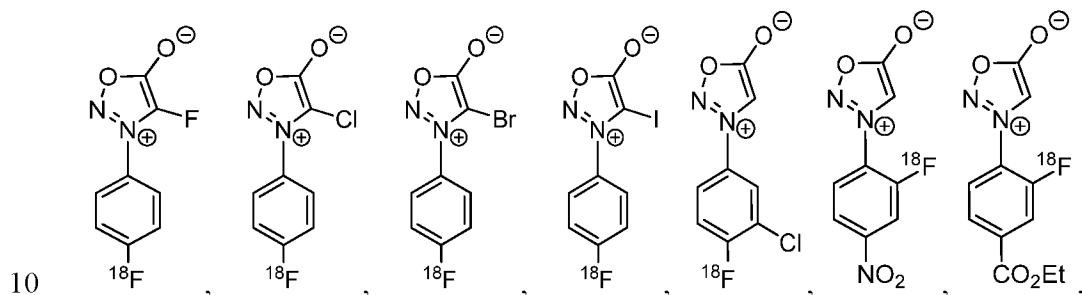
L is a linker; and

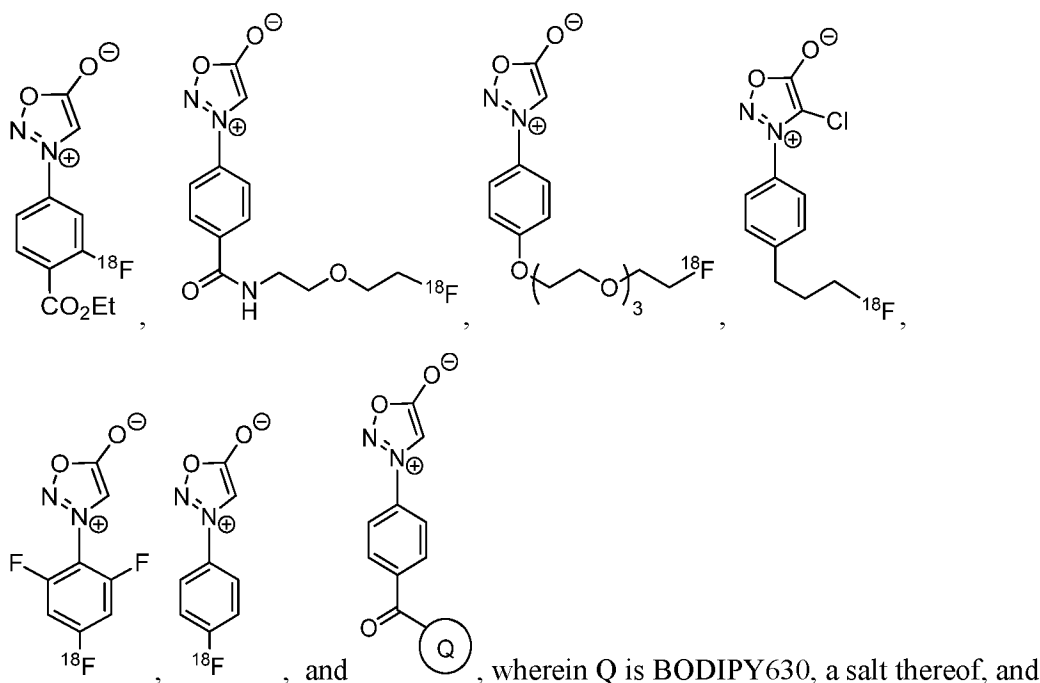
Q is an imaging moiety.

In one embodiment, n is at least 1 and at least one R^2 is F. In another embodiment, the compound is selected from the group consisting of:



In another embodiment, the compound is selected from the group consisting of:





any combinations thereof. In another embodiment, the dipolarophile is dibenzocyclooctyne (DBCO). In another embodiment, the dipolarophile is biarylazacyclooctynone (BARAC). In another embodiment, the biomolecule is imaged using positron emission tomography (PET). In another embodiment, the biomolecule is imaged in vivo.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description of preferred embodiments of the invention will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities of the embodiments shown in the drawings.

Figure 1 is an illustration of an exemplary sydnone-DBCO bioorthogonal chemical reporter strategy.

Figure 2 is a scheme of an exemplary synthesis of a pyrazole employing a [3+2] cycloaddition of phenyl sydnone with strained alkynes.

Figure 3, comprising Figures 3A-3B, illustrates modifications of protein surfaces via bioorthogonal click reactions in tandem. Figure 3A is a scheme illustrating an exemplary synthesis of bioorthogonal click reactions yielding

compounds of the present invention. Dibenzocyclooctyne (DBCO) and 5-norbornene-2-acetic acid (Nor) were appended to bovine serum albumin (BSA) and ovalbumin (OVA) respectively. The labeled proteins BSA-DBCO and OVA-Nor were simultaneously reacted with sydnone-BODIPY (Syd-630) and tetrazine-BODIPY (Tz-504) conjugates. Figure 3B is an image of a gel analysis of DBCO-conjugated BSA and norbornene-conjugated OVA with both Syd-630 and Tz-504 simultaneously for 1-60 min or no reagent (-). The protein loading on the gels was assessed with Coomassie stain.

Figure 4 is a scheme of an exemplary synthesis of a ^{18}F -labeled biomolecule, wherein the ^{18}F -sydnone undergoes 3+2 cycloaddition reaction with DBCO-conjugated biomolecule to afford the ^{18}F -labeled biomolecule.

Figure 5 is a scheme of the reaction rate constants (k) of exemplary bioorthogonal compounds of the present invention for applications in ^{18}F -radiolabeling of biomolecules.

Figure 6, comprising Figures 6A-6C, illustrates the synthesis of exemplary compounds of the invention and experimental data. Figure 6A is a scheme of an exemplary SPAC cycloaddition of various sydnones with bicyclo-[6.1.0]-nonyne (BCN)/biarylazacyclooctynone (BARAC). Figure 6B is a series of graphs of experimental data demonstrating the exponential decay curve of phenylsydnone Abs at 310 nm with BCN and the second order rate constant plot using k_{obs} vs. conc. of excess reagent. Figure 6C is a scheme illustrating the application of exemplary sydnones of the invention as PET probes.

Figure 7 is a scheme of an exemplary synthesis of ^{18}F -labeled sydnones of the present invention.

Figure 8 is a scheme of an exemplary synthesis of a bioorthogonal click reaction yielding a compound of the present invention.

Figure 9 is a scheme of an exemplary mechanism for the [3+2] cycloaddition reaction of a sydnone with an alkyne.

Figure 10 is a scheme of an exemplary synthesis of a bioorthogonal click reaction yielding a compound of the present invention.

Figure 11 is a scheme of an exemplary synthesis of a poly(alkyl ether)-linked fluoro-sydnone compound of the present invention.

Figure 12 is a scheme of an exemplary synthesis of a radiolabeled poly(alkyl ether) sydnone compound of the present invention.

Figure 13 is a scheme of an exemplary synthesis of a compound of the present invention using a bis-sydnone precursor.

Figure 14 is a scheme of an exemplary synthesis of a compound of the present invention using an isotopic exchange reaction and exemplary compounds that
5 can be synthesized using this procedure. If the p-NO₂ reaction is performed at 100 °C for 7 min, 88% of the isotopic exchange product (1) is obtained with 5% of the sydnone exchange product (2).

Figure 15 is a scheme of a method for radiofluorination of a biomolecule (protein, peptide, and the like): conjugation of the biomolecule to a
10 strained alkyne (DIBAC, BCN, and the like) followed by rapid cycloaddition with the purified ¹⁸F-sydnone affords the fluorine-18 labeled biomolecule in complete conversion and >99% (radio)chemical purity.

Figure 16 is an integrated radio-TLC (thin layer chromatography) scan from the synthesis of 4-[¹⁸F]fluorophenyl sydnone, depicted in Figure 13.

Figure 17, comprising Figures 17A and 17B is a radio-HPLC (high
15 performance liquid chromatography) trace of 4-[¹⁸F]fluorophenyl sydnone. Figure 17A is the 254 nm UV trace of the ¹⁹F reference standard. Figure 17B is the radioactivity trace of the purified 4-[¹⁸F]fluorophenyl sydnone. The HPLC conditions were 10% acetonitrile in water to 90% acetonitrile in water over 30 min; both mobile
20 phases contained 0.1% trifluoroacetic acid.

Figure 18 is an HPLC calibration curve for cold (non-radiolabeled) 4-fluorophenyl sydnone.

Figure 19, comprising Figures 19A and 19B, is a radio-HPLC (high
performance liquid chromatography) trace of the reaction mixture from the click
25 reaction between 4-[¹⁸F]fluorophenyl sydnone and DIBAC (depicted in Figure 15). Figure 19A is the 254 nm UV trace of the reaction mixture. Figure 19B is the radioactivity trace of the reaction mixture. The HPLC conditions were 10% acetonitrile in water to 70% acetonitrile in water over 40 min, then to 95% acetonitrile
at 45 min; both mobile phases contained 0.1% trifluoroacetic acid.

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DETAILED DESCRIPTION

The present invention relates to the unexpected discovery of novel sydnones useful for the ¹⁸F-radiolabeling of biomolecules. The compounds of the invention provide a site-selective and rapid method for labeling a biomolecule under

conditions which do not interfere with any biological processes. Thus, the present invention provides compositions and methods useful for labeling a biomolecule. The present invention also provides methods for imaging a biomolecule.

5 Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.

As used herein, each of the following terms has the meaning associated with it in this section.

The articles “a” and “an” are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

“About” as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, more preferably $\pm 5\%$, even more preferably $\pm 1\%$, and still more preferably $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

As used herein, the term “pharmaceutically acceptable” refers to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the compound, and is relatively non-toxic, *i.e.*, the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

As used herein, the language “pharmaceutically acceptable salt” refers to a salt of the administered compounds prepared from pharmaceutically acceptable non-toxic acids, including inorganic acids, organic acids, solvates, hydrates, or clathrates thereof. Examples of such inorganic acids are hydrochloric, hydrobromic, hydroiodic, nitric, sulfuric, phosphoric, acetic, hexafluorophosphoric, citric, gluconic, benzoic, propionic, butyric, sulfosalicylic, maleic, lauric, malic, fumaric, succinic, tartaric, amsonic, pamoic, p-toluenesulfonic, and mesylic. Appropriate organic acids may be selected, for example, from aliphatic, aromatic, carboxylic and sulfonic

classes of organic acids, examples of which are formic, acetic, propionic, succinic, camphorsulfonic, citric, fumaric, gluconic, isethionic, lactic, malic, mucic, tartaric, para-toluenesulfonic, glycolic, glucuronic, maleic, furoic, glutamic, benzoic, anthranilic, salicylic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, pantothenic, benzenesulfonic (besylate), stearic, sulfanilic, alginic, galacturonic, and the like. Furthermore, pharmaceutically acceptable salts include, by way of non-limiting example, alkaline earth metal salts (e.g., calcium or magnesium), alkali metal salts (e.g., sodium-dependent or potassium), and ammonium salts.

As used herein, the terms “imaging agent,” “imaging probe,” or “imaging compound,” means, unless otherwise stated, a molecule which can be detected by its emitted signal, such as positron emission, autofluorescence emission, or optical properties. The method of detection of the compounds may include, but are not necessarily limited to, nuclear scintigraphy, positron emission tomography (PET), single photon emission computed tomography (SPECT), magnetic resonance imaging, magnetic resonance spectroscopy, computed tomography, or a combination thereof depending on the intended use and the imaging methodology available to the medical or research personnel.

As used herein, the term “biomolecule” refers to any molecule produced by a living organism and may be selected from the group consisting of proteins, peptides, polysaccharides, carbohydrates, lipids, as well as analogs and fragments thereof. Preferred examples of biomolecules are proteins and peptides.

As used herein, the terms “bioconjugation” and “conjugation,” unless otherwise stated, refers to the chemical derivatization of a macromolecule with another molecular entity. The molecular entity can be any molecule and can include a small molecule or another macromolecule. Examples of molecular entities include, but are not limited to, compounds of the invention, other macromolecules, polymers or resins, such as polyethylene glycol (PEG) or polystyrene, non-immunogenic high molecular weight compounds, fluorescent, chemiluminescent radioisotope and bioluminescent marker compounds, antibodies, biotin, diagnostic detector molecules, such as a maleimide derivatized fluorescein, coumarin, a metal chelator or any other modifying group. The terms bioconjugation and conjugation are used interchangeably throughout the Specification.

As used herein, the term “alkyl,” by itself or as part of another substituent means, unless otherwise stated, a straight or branched chain hydrocarbon

having the number of carbon atoms designated (*i.e.* C₁₋₆ means one to six carbon atoms) and including straight, branched chain, or cyclic substituent groups. Examples include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, neopentyl, hexyl, and cyclopropylmethyl. Most preferred is (C₁-C₆)alkyl, particularly ethyl, methyl, isopropyl, isobutyl, n-pentyl, n-hexyl and cyclopropylmethyl.

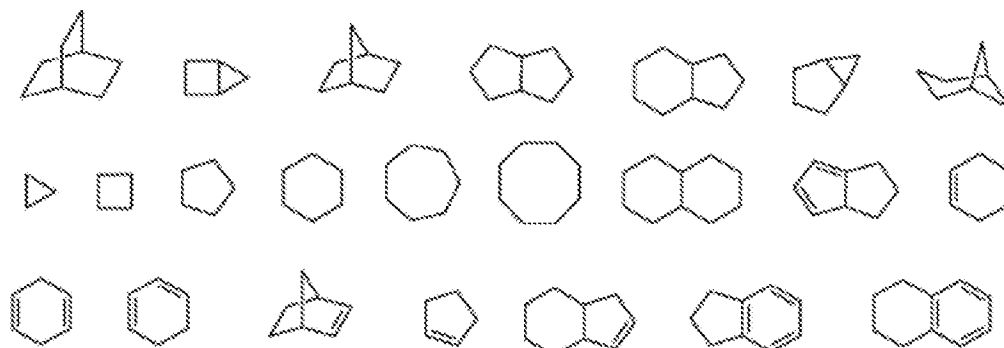
As used herein, the term “substituted alkyl” means alkyl as defined above, substituted by one, two or three substituents selected from the group consisting of halogen, -OH, alkoxy, -NH₂, -N(CH₃)₂, -C(=O)OH, trifluoromethyl, -C≡N, -C(=O)O(C₁-C₄)alkyl, -C(=O)NH₂, -SO₂NH₂, -C(=NH)NH₂, and -NO₂, preferably containing one or two substituents selected from halogen, -OH, alkoxy, -NH₂, trifluoromethyl, -N(CH₃)₂, and -C(=O)OH, more preferably selected from halogen, alkoxy and -OH. Examples of substituted alkyls include, but are not limited to, 2,2-difluoropropyl, 2-carboxycyclopentyl and 3-chloropropyl.

As used herein, the term “heteroalkyl” by itself or in combination with another term means, unless otherwise stated, a stable straight or branched chain alkyl group consisting of the stated number of carbon atoms and one or two heteroatoms selected from the group consisting of O, N, and S, and wherein the nitrogen and sulfur atoms may be optionally oxidized and the nitrogen heteroatom may be optionally quaternized. The heteroatom(s) may be placed at any position of the heteroalkyl group, including between the rest of the heteroalkyl group and the fragment to which it is attached, as well as attached to the most distal carbon atom in the heteroalkyl group. Examples include: -O-CH₂-CH₂-CH₃, -CH₂-CH₂-CH₂-OH, -CH₂-CH₂-NH-CH₃, -CH₂-S-CH₂-CH₃, and -CH₂CH₂-S(=O)-CH₃. Up to two heteroatoms may be consecutive, such as, for example, -CH₂-NH-OCH₃, or -CH₂-CH₂-S-S-CH₃.

As used herein, the term “alkoxy” employed alone or in combination with other terms means, unless otherwise stated, an alkyl group having the designated number of carbon atoms, as defined above, connected to the rest of the molecule via an oxygen atom, such as, for example, methoxy, ethoxy, 1-propoxy, 2-propoxy (isopropoxy) and the higher homologs and isomers. In one embodiment, the alkoxy group is (C₁-C₃) alkoxy, such as ethoxy and methoxy.

As used herein, the term “halo” or “halogen” alone or as part of another substituent means, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom, or isotopes thereof.

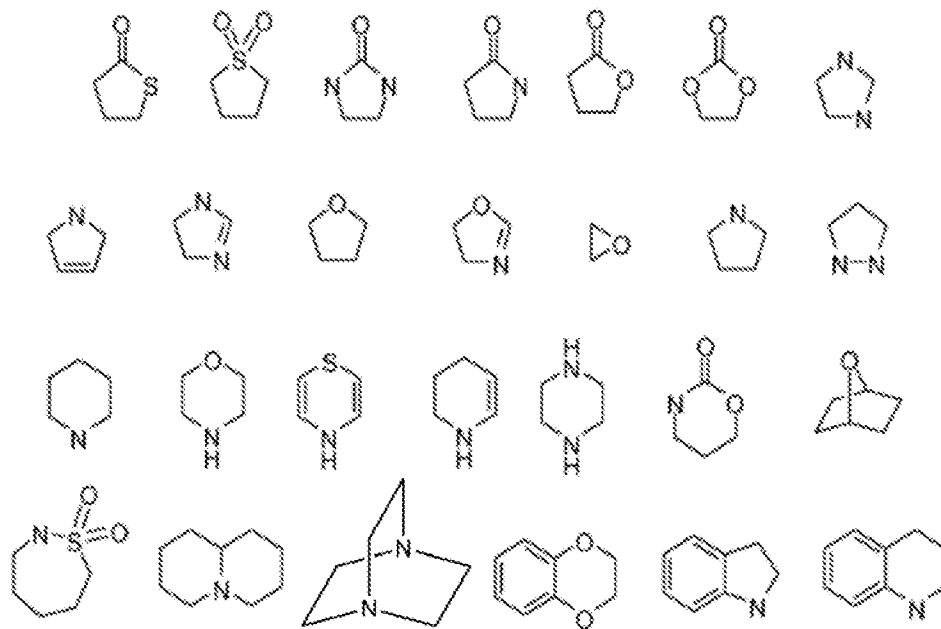
10 As used herein, the term “cycloalkyl” refers to a mono cyclic or polycyclic non-aromatic radical, wherein each of the atoms forming the ring (i.e. skeletal atoms) is a carbon atom. In one embodiment, the cycloalkyl group is saturated or partially unsaturated. In another embodiment, the cycloalkyl group is fused with an aromatic ring. Cycloalkyl groups include groups having from 3 to 10 ring atoms. Illustrative examples of cycloalkyl groups include, but are not limited to, 15 the following moieties:



Monocyclic cycloalkyls include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Dicyclic cycloalkyls 20 include, but are not limited to, tetrahydronaphthyl, indanyl, and tetrahydropentalene. Polycyclic cycloalkyls include adamantane and norbornane. The term cycloalkyl includes “unsaturated nonaromatic carbocyclyl” or “nonaromatic unsaturated carbocyclyl” groups, both of which refer to a nonaromatic carbocycle as defined herein, which contains at least one carbon carbon double bond or one carbon carbon 25 triple bond.

As used herein, the term “heterocycloalkyl” or “heterocyclyl” refers to a heteroalicyclic group containing one to four ring heteroatoms each selected from O, 30 Sand N. In one embodiment, each heterocycloalkyl group has from 4 to 10 atoms in its ring system, with the proviso that the ring of said group does not contain two adjacent O or S atoms. In another embodiment, the heterocycloalkyl group is fused with an aromatic ring. In one embodiment, the nitrogen and sulfur heteroatoms may be optionally oxidized, and the nitrogen atom may be optionally quaternized. The 35 heterocyclic system may be attached, unless otherwise stated, at any heteroatom or carbon atom that affords a stable structure. A heterocycle may be aromatic or non-aromatic in nature. In one embodiment, the heterocycle is a heteroaryl.

An example of a 3-membered heterocycloalkyl group includes, and is not limited to, aziridine. Examples of 4-membered heterocycloalkyl groups include, and are not limited to, azetidine and a beta lactam. Examples of 5-membered heterocycloalkyl groups include, and are not limited to, pyrrolidine, oxazolidine and thiazolidinedione. Examples of 6-membered heterocycloalkyl groups include, and are not limited to, piperidine, morpholine and piperazine. Other non-limiting examples of heterocycloalkyl groups are:



Examples of non-aromatic heterocycles include monocyclic groups such as aziridine, oxirane, thiirane, azetidine, oxetane, thietane, pyrrolidine, pyrroline, pyrazolidine, imidazoline, dioxolane, sulfolane, 2,3-dihydrofuran, 2,5-dihydrofuran, tetrahydrofuran, thiophane, piperidine, 1,2,3,6-tetrahydropyridine, 1,4-dihydropyridine, piperazine, morpholine, thiomorpholine, pyran, 2,3-dihydropyran, tetrahydropyran, 1,4-dioxane, 1,3-dioxane, homopiperazine, homopiperidine, 1,3-dioxepane, 4,7-dihydro-1,3-dioxepin, and hexamethyleneoxide.

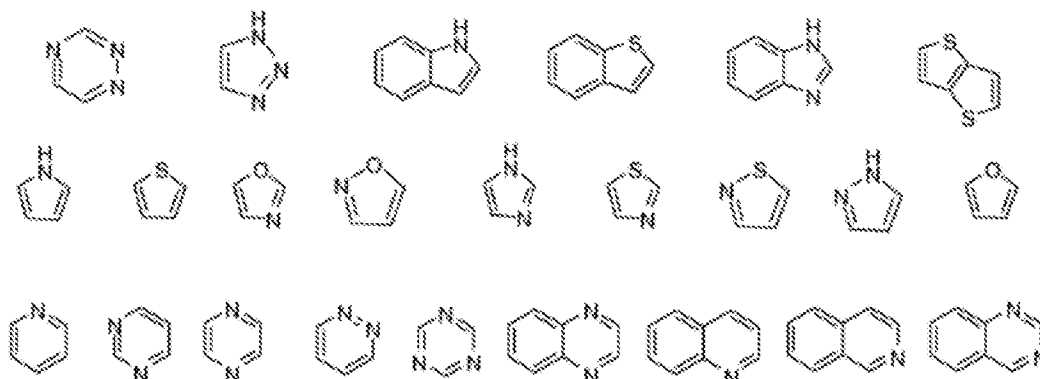
As used herein, the term “aromatic” refers to a carbocycle or heterocycle with one or more polyunsaturated rings and having aromatic character, i.e. having $(4n + 2)$ delocalized π (pi) electrons, where n is an integer.

As used herein, the term “aryl,” employed alone or in combination with other terms, means, unless otherwise stated, a carbocyclic aromatic system containing one or more rings (typically one, two or three rings), wherein such rings may be attached together in a pendent manner, such as a biphenyl, or may be fused,

such as naphthalene. Examples of aryl groups include phenyl, anthracyl, and naphthyl.

As used herein, the term “aryl-(C₁-C₃)alkyl” means a functional group wherein a one- to three-carbon alkylene chain is attached to an aryl group, *e.g.*, -CH₂CH₂-phenyl. Preferred is aryl-CH₂- and aryl-CH(CH₃)-. The term “substituted aryl-(C₁-C₃)alkyl” means an aryl-(C₁-C₃)alkyl functional group in which the aryl group is substituted. Preferred is substituted aryl(CH₂)-. Similarly, the term “heteroaryl-(C₁-C₃)alkyl” means a functional group wherein a one to three carbon alkylene chain is attached to a heteroaryl group, *e.g.*, -CH₂CH₂-pyridyl. In one embodiment, the heteroaryl-(C₁-C₃)alkyl is heteroaryl-(CH₂)-. The term “substituted heteroaryl-(C₁-C₃)alkyl” means a heteroaryl-(C₁-C₃)alkyl functional group in which the heteroaryl group is substituted. In one embodiment, the substituted heteroaryl-(C₁-C₃)alkyl is substituted heteroaryl-(CH₂)-.

As used herein, the term “heteroaryl” or “heteroaromatic” refers to a heterocycle having aromatic character. A polycyclic heteroaryl may include one or more rings that are partially saturated. Examples include the following moieties:



Examples of heteroaryl groups also include pyridyl, pyrazinyl, pyrimidinyl (particularly 2- and 4-pyrimidinyl), pyridazinyl, thienyl, furyl, pyrrolyl (particularly 2-pyrrolyl), imidazolyl, thiazolyl, oxazolyl, pyrazolyl (particularly 3- and 5-pyrazolyl), isothiazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,3,4-triazolyl, tetrazolyl, 1,2,3-thiadiazolyl, 1,2,3-oxadiazolyl, 1,3,4-thiadiazolyl and 1,3,4-oxadiazolyl. Examples of polycyclic heterocycles and heteroaryls include indolyl (particularly 3-, 4-, 5-, 6- and 7-indolyl), indolinyl, quinolyl, tetrahydroquinolyl, isoquinolyl (particularly 1- and 5-isoquinolyl), 1,2,3,4-tetrahydroisoquinolyl, cinnolinyl, quinoxalinyl (particularly 2- and 5-quinoxalinyl), quinazolinyl, phthalazinyl,

1,8-naphthyridinyl, 1,4-benzodioxanyl, coumarin, dihydrocoumarin,
1,5-naphthyridinyl, benzofuryl (particularly 3-, 4-, 5-, 6- and 7-benzofuryl),
2,3-dihydrobenzofuryl, 1,2-benzisoxazolyl, benzothienyl (particularly 3-, 4-, 5-, 6-,
and 7-benzothienyl), benzoxazolyl, benzothiazolyl (particularly 2-benzothiazolyl and
5 5-benzothiazolyl), purinyl, benzimidazolyl (particularly 2-benzimidazolyl),
benzotriazolyl, thioxanthinyl, carbazolyl, carbolinyl, acridinyl, pyrrolizidinyl, and
quinolizidinyl.

As used herein, the term “substituted” means that an atom or group of
atoms has replaced hydrogen as the substituent attached to another group. The term
10 “substituted” further refers to any level of substitution, namely mono-, di-, tri-, tetra-,
or penta-substitution, where such substitution is permitted. The substituents are
independently selected, and substitution may be at any chemically accessible position.
In one embodiment, the substituents vary in number between one and four. In another
embodiment, the substituents vary in number between one and three. In yet another
15 embodiment, the substituents vary in number between one and two.

As used herein, the term “optionally substituted” means that the
referenced group may be substituted or unsubstituted. In one embodiment, the
referenced group is optionally substituted with zero substituents, i.e., the referenced
group is unsubstituted. In another embodiment, the referenced group is optionally
20 substituted with one or more additional group(s) individually and independently
selected from groups described herein.

In one embodiment, the substituents are independently selected from
the group consisting of oxo, halogen, -CN, -NH₂, -OH, -NH(CH₃), -N(CH₃)₂, alkyl
(including straight chain, branched and/or unsaturated alkyl), substituted or
25 unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, fluoro alkyl,
substituted or unsubstituted heteroalkyl, substituted or unsubstituted alkoxy,
fluoroalkoxy, -S-alkyl, S(=O)₂alkyl, -C(=O)NH[substituted or unsubstituted alkyl], or
substituted or unsubstituted phenyl], -C(=O)N[H or alkyl]₂, -OC(=O)N[substituted or
unsubstituted alkyl]₂, -NHC(=O)NH[substituted or unsubstituted alkyl], or substituted
30 or unsubstituted phenyl], -NHC(=O)alkyl, -N[substituted or unsubstituted
alkyl]C(=O)[substituted or unsubstituted alkyl], -NHC(=O)[substituted or
unsubstituted alkyl], -C(OH)[substituted or unsubstituted alkyl]₂, and -
C(NH₂)[substituted or unsubstituted alkyl]₂. In another embodiment, by way of
example, an optional substituent is selected from oxo, fluorine, chlorine, bromine,

iodine, -CN, -NH₂, -OH, -NH(CH₃), -N(CH₃)₂, -CH₃, -CH₂CH₃, -CH(CH₃)₂, -CF₃, -CH₂CF₃, -OCH₃, -OCH₂CH₃, -OCH(CH₃)₂, -OCF₃, -OCH₂CF₃, -S(=O)₂-CH₃, -C(=O)NH₂, -C(=O)-NHCH₃, -NHC(=O)NHCH₃, -C(=O)CH₃, and -C(=O)OH. In yet

5 one embodiment, the substituents are independently selected from the group consisting of C₁₋₆ alkyl, -OH, C₁₋₆ alkoxy, halo, amino, acetamido, oxo and nitro. In yet another embodiment, the substituents are independently selected from the group consisting of C₁₋₆ alkyl, C₁₋₆ alkoxy, halo, acetamido, and nitro. As used herein, where a substituent is an alkyl or alkoxy group, the carbon chain may be branched, straight or cyclic, with straight being preferred.

10 Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges
15 as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

20

Description

The present invention relates to the unexpected discovery of novel sydnones useful for the ¹⁸F-radiolabeling of biomolecules. The compounds of the invention provide a site-selective and rapid method for labeling a biomolecule under
25 conditions which do not interfere with any biological processes. Thus, the present invention provides compositions and methods useful for labeling a biomolecule. The present invention also provides methods for imaging a biomolecule.

Until recently, the potential role of sydnones as a bioorthogonal reaction partner for labeling biomolecules via a [3+2] cycloaddition was relatively
30 overlooked. First discovered in 1935, sydnones are remarkably stable mesoionic heterocycles that contain a 1,5-dipolar azomethinimine moiety (Earl and Mackney, 1935, J. Chem. Soc. 899). Huisgen reported, upon treatment with alkynes at 170 °C, sydnones undergo a [3+2] cycloaddition to form pyrazoles, via a bicyclic intermediate which eliminates carbon dioxide (Huisgen et al., 1962, Angev. Chem. Int. Ed. 1:48).

Using a high-throughput immunoassay technique, Taran and co-workers found that sydnone undergo 1,3-dipolar cycloaddition with alkynes at 50 °C in the presence of copper (Kolodych et al., 2013, *Angew. Chem. Int. Ed.* 52:12056). Upon further optimization, they identified a copper phenanthroline complex that catalyzes the
5 cycloaddition to produce 1,4-pyrazoles at room temperature in high yield. Building on precedent of the azide-alkyne cycloaddition, Chin and co-workers recently described the strain-promoted [3+2] cycloaddition reaction between phenyl sydnone and exo-((1R,8S)-bicyclo[6.1.0]non-4-yn-9-yl)methanol (BCN) at ambient temperature to afford the corresponding pyrazole in 30 minutes, with an isolated yield of 99%
10 (Wallace and Chin, 2014, *Chem. Sci.* 5:1742). The pseudo-first order rate constant in 55:45 MeOH-H₂O was determined to be 0.054 M⁻¹s⁻¹.

Following this report, Taran et al. introduced a series of sydnone bearing substituents at the 3 and 4 positions of the mesoionic sydnone ring (Plougastel et al., 2014, *Chem. Commun.* 50:9376). Their results indicated a significant effect on
15 the rate constant of the cycloaddition reaction, with the 4-chlorinated sydnone undergoing cycloaddition with BCN 30-fold faster than the non-chlorinated analogue, yielding a rate constant of 1.593 M⁻¹s⁻¹. Rapid reactions with high intrinsic second-order rate constants, such as the sydnone-DBCO reaction, are beneficial for labeling on a short time-scale or for labeling material in low abundance. Thus, the present
20 invention is based in part on the reactivity of sydnone and tuning their kinetics to be suitable for ¹⁸F-labeling of biomolecules (Figure 1).

Current technology used for the ¹⁸F-labeling of biomolecules includes inverse-electron-demand Diels-Alder reactions between tetrazine and strained alkenes, in addition to “click” chemistry between azides and alkynes. A key
25 advantage of sydnone over a tetrazine ligation lies in the combination of its exceptionally easy preparation (no specialized apparatus needed) with high second-order rate constant, comparable to the fastest bioorthogonal reactions reported to date. The stability and scope to tune the reaction rates by varying the substitutions on the sydnone ring over the existing alkyl/aryl azides demonstrates that sydnone may be
30 useful as new bioorthogonal probes for imaging, such as for ¹⁸F-PET imaging.

Reliable and site-specific conjugation chemistries for coupling chemical reporters to biomolecules are critical not only for the field of molecular imaging, but also for the advancement of molecular and cellular biology. The availability of a simple, robust ¹⁸F-chemistry platform for site-selective biomolecule

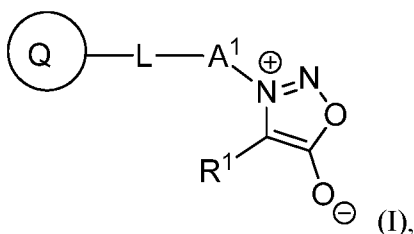
labeling will impact molecular imaging and, therefore, clinical practice reduced to standard-of-care by advances in chemical methods. Currently, simple and efficient chemical methods for the ^{18}F -labeling of biomolecules are severely limited. The compositions and methods described herein provide a highly selective, efficient, and rapid approach to labeling biomolecules with a chemical reporter, such as a radionuclide or fluorescent dye.

The methods of the invention include the use of biorthogonal chemistry to achieve bioconjugation using the [3+2] cycloaddition reaction between a radiolabeled compound of the present invention and a suitable dipolarophile to deliver the radiolabeled compound to the biomolecule. The compounds of the invention can be attached to the biomolecule predictably and with specificity, and are otherwise inert in biological systems.

Compounds

The compounds of the present invention may be synthesized using techniques well-known in the art of organic synthesis. The starting materials and intermediates required for the synthesis may be obtained from commercial sources or synthesized according to methods known to those skilled in the art.

In one aspect, the compound of the invention is a compound of formula (I), or a salt, solvate, or *N*-oxide thereof:



wherein in formula (I):

A^1 is selected from the group consisting of alkyl, heteroalkyl, alkylcycloalkyl, heterocyclyl, alkylheterocyclyl, aryl, alkylaryl, heteroaryl, and alkylheteroaryl, wherein the alkyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl group is optionally substituted with 0-5 R^2 groups

R^1 and each occurrence of R^2 are independently selected from the group consisting of H, $-\text{C}_1\text{-C}_6$ alkyl, $-\text{C}_1\text{-C}_6$ fluoroalkyl, $-\text{C}_1\text{-C}_6$ heteroalkyl, F, ^{18}F , Cl, Br, I, $-\text{CN}$, $-\text{NO}_2$, $-\text{OR}^3$, $-\text{SR}^3$, $-\text{S}(=\text{O})\text{R}^3$, $-\text{S}(=\text{O})_2\text{R}^3$, $-\text{NHS}(=\text{O})_2\text{R}^3$, $-\text{C}(=\text{O})\text{R}^3$, $-\text{OC}(=\text{O})\text{R}^3$,

-CO₂R³, -OCO₂R³, -CH(R³)₂, -N(R³)₂, -C(=O)N(R³)₂, -OC(=O)N(R³)₂, -NHC(=O)NH(R³), -NHC(=O)R³, -NHC(=O)OR³, -C(OH)(R³)₂, and -C(NH₂)(R³)₂;

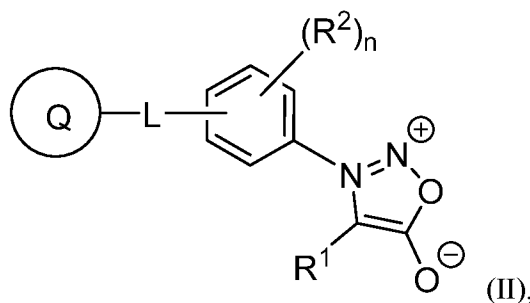
each occurrence of R³ is independently selected from the group consisting of H and C₁-C₆ alkyl;

5 L is a linker; and

Q is an imaging moiety.

In one embodiment, A¹ is aryl. In one embodiment, the A¹ is a phenyl group, wherein the phenyl group is optionally substituted.

In another aspect, the compound of the invention is a compound of
10 formula (II), or a salt, solvate, or *N*-oxide thereof:



wherein in formula (II):

n is an integer between 0 and 4;

R¹ and each occurrence of R² are each independently selected from the group
15 consisting of H, -C₁-C₆ alkyl, -C₁-C₆ fluoroalkyl, -C₁-C₆ heteroalkyl, F, ¹⁸F, Cl, Br, I, -CN, -NO₂, -OR³, -SR³, -S(=O)R³, -S(=O)₂R³, -NHS(=O)₂R³, -C(=O)R³, -OC(=O)R³, -CO₂R³, -OCO₂R³, -CH(R³)₂, -N(R³)₂, -C(=O)N(R³)₂, -OC(=O)N(R³)₂, -NHC(=O)NH(R³), -NHC(=O)R³, -NHC(=O)OR³, -C(OH)(R³)₂, and -C(NH₂)(R³)₂;

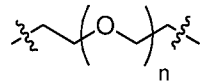
each occurrence of R³ is independently selected from the group consisting of
20 H and C₁-C₆ alkyl;

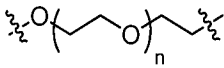
L is a linker; and

Q is an imaging moiety.

In one embodiment, n is at least 1 and at least one R² is F. In another embodiment, n is at least two and at least two R² are F. In some embodiments, the aryl
25 ring is substituted with one or more R². In one embodiment, R² is a nitro group. In another embodiment, R² is an alkyl ester. In one embodiment, n is four and all occurrences of R² are F. In one embodiment, n is four, and one or more occurrences of R² are ¹⁸F.

The linker L may be any suitable linker, as would be understood by one of ordinary skill in the art. Examples of linkers include, but are not limited to, an alkyl group, a benzyl group, an aryl group, a heteroaryl group, a cycloalkyl group, an amide group, an ester, a sulfonamide, a carbamate, a carbonate, a sulfone, an ether, an oxime, a hydrazine, a urea, a thiourea, a phosphate, a poly(alkyl ether), a heteroatom, or combinations thereof, wherein the alkyl, benzyl, aryl, heteroaryl, cycloalkyl, amide, ester, sulfonamide, carbamate, carbonate, sulfone, ether, oxime, hydrazine, urea, thiourea, phosphate, and poly(alkyl ether) may be optionally substituted. In one embodiment, L is a bond. For example, when the L is a bond, A¹ is bonded directly to the ¹⁸F atom. In one embodiment, L is alkyl. In another embodiment, L is selected from the group consisting of a bond, an alkyl group, an amide, a poly(alkyl ether), and any combinations thereof. In another embodiment, L is comprised of an alkyl group

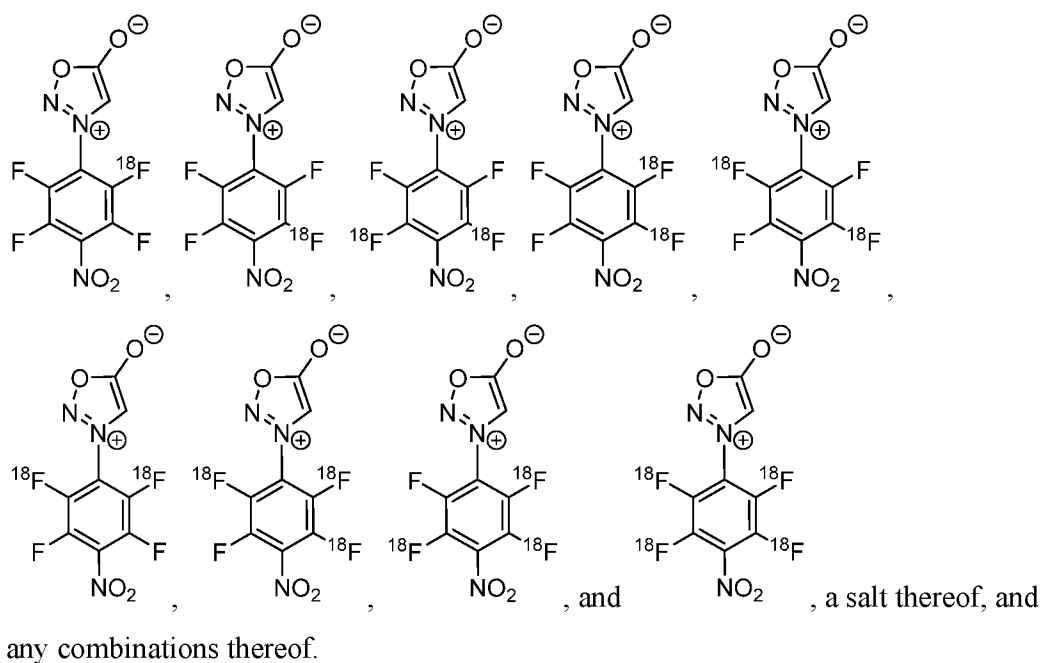
and a poly(alkyl ether) group. In one embodiment, L is , wherein n is

an integer from 1 to 20. In another embodiment, L is , wherein n is an integer from 1 to 20. In some embodiments of the invention, L is bound to the aryl ring at a position *para* to the sydnone. In other embodiments, L is bound to the aryl ring at a position *meta* or *ortho* to the sydnone.

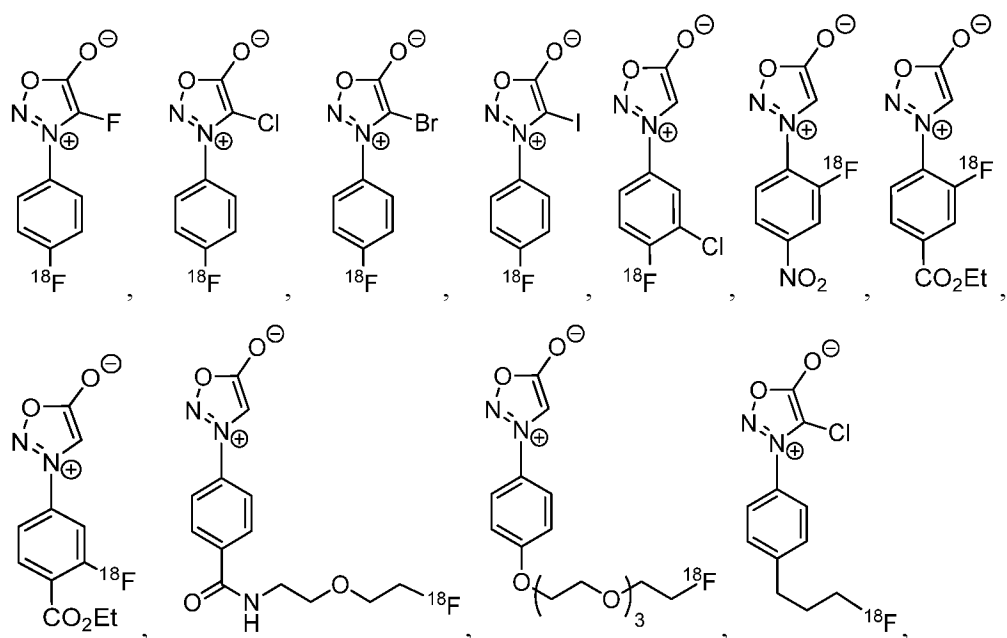
Q may be any suitable imaging moiety. Imaging moieties include those that are well known to those skilled in the art, and include those moieties that may be useful in the generation of diagnostic images by diagnostic techniques well known to the ordinarily skilled artisan. Non-limiting examples of imaging moieties include radioisotopes, paramagnetic species, echogenic entities, and fluorescent moiety, such as a fluorescent dye. In one embodiment, Q is a radioisotope. Examples of radioisotopes include and are not limited to ²H, ³H, ¹¹C, ¹³C, ¹⁴C, ³⁶Cl, ¹⁸F, ¹²³I, ¹²⁵I, ¹³N, ¹⁵N, ¹⁵O, ¹⁷O, ¹⁸O, ³²P, and ³⁵S. In one embodiment, Q is ¹⁸F. In another embodiment, Q is a fluorescent dye. Examples of fluorescent dyes include, but are not limited to, BODIPY dyes such as BODIPY630 and BODIPY504, POPO-1, TOTO-3, TAMRA, BOXT0, BEBO, SYBR DX, SYTOX dyes, SYTO dyes, Alexa dyes, fluorescein, rhodamine, propidium iodide, Hoechst dyes, tetramethylrhodamine, R-phycoerythrin, Cy-3, Cy-5, Cy-7, Texas Red, Phar-Red, allophycocyanin (APC), fluorescein amine, eosin, dansyl, umbelliferone, 5-carboxyfluorescein (FAM), 2'7'-

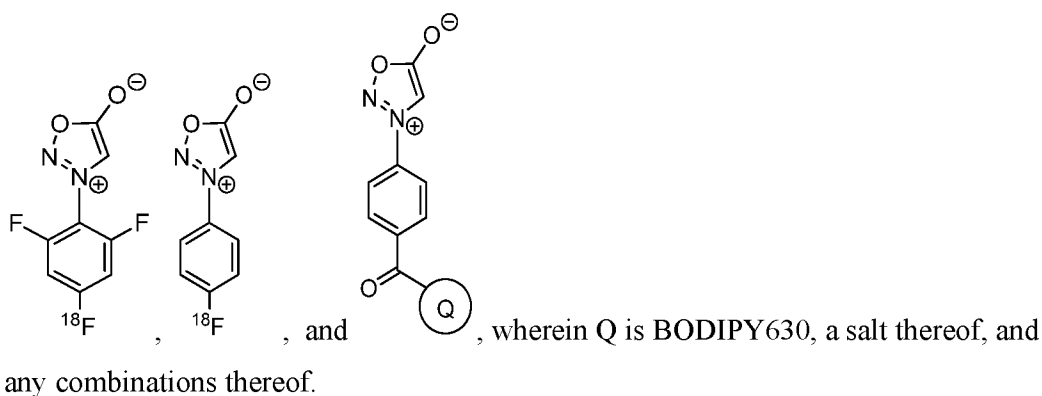
dimethoxy-4'5'-dichloro-6-carboxyfluorescein (JOE), 6 carboxyrhodamine (R6G),
 N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), 6-carboxy-X-rhodamine
 (ROX), 4-(4'-dimethylaminophenylazo) benzoic acid (DABCYL), 5-(2'-aminoethyl)
 aminonaphthalene-1-sulfonic acid (EDANS), 8-Anilino-1-naphthalenesulfonic acid
 5 ammonium salt (ANS), 4-acetamido-4'-isothiocyanatostilbene-2,2' disulfonic acid,
 acridine, acridine isothiocyanate, acridine orange (*N,N,N',N'*-tetramethylacridine-3,6-
 diamine), *R*-amino-N-(3-vinylsulfonyl)phenylnaphthalimide-3,5, disulfonate (Lucifer
 Yellow VS), N-(4-anilino-1-naphthyl)maleimide, anthranilamide, Brilliant Yellow,
 coumarin, 7-amino-4-methylcoumarin, 7-amino-4-trifluoromethylcoumarin
 10 (Coumarin 151), cyanosine, 2-(4-amidinophenyl)-1H-indole-6-carboxamide
 (DAPI), 5',5''-dibromopyrogallol-sulfonephthalein (Bromopyrogallol Red), 7-
 diethylamino-3-(4'-isothiocyanatophenyl)-4-methylcoumarin diethylenetriamine
 pentaacetate, 4,4'-diisothiocyanatodihydro-stilbene-2,2'-disulfonic acid, 4,4'-
 diisothiocyanatostilbene-2,2'-disulfonic acid, 4-dimethylaminophenylazophenyl-4'-
 15 isothiocyanate (DABITC), eosin isothiocyanate, erythrosin B, erythrosin
 isothiocyanate, ethidium, 5-(4,6-dichlorotriazin-2-yl) aminofluorescein (DTAF),
 QFITC (XRITC), fluorescamine, IR144, IR1446, Malachite Green isothiocyanate, 4-
 methylumbelliferone, ortho cresolphthalein, nitrotyrosine, pararosaniline, Phenol Red,
 B-phycoerythrin, o-phthalaldehyde, pyrene, pyrene butyrate, succinimidyl 1-pyrene
 20 butyrate, Reactive Red 4 (Cibacron.RTM. Brilliant Red 3B-A), lissamine rhodamine
 B sulfonyl chloride, rhodamine B, rhodamine 123, rhodamine X, sulforhodamine B,
 sulforhodamine 101, sulfonyl chloride derivative of sulforhodamine 101, tetramethyl
 rhodamine, thiazole orange, riboflavin, rosolic acid, and terbium chelate derivatives.
 In one embodiment, the fluorescent dye is BODIPY630. In another embodiment, the
 25 fluorescent dye is BODIPY540.

In one embodiment, the compound is selected from the group
 consisting of:



In another embodiment, the compound is selected from the group consisting of:

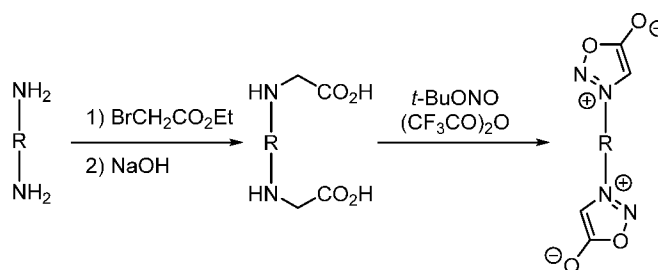




Preparation of the Compounds of the Invention

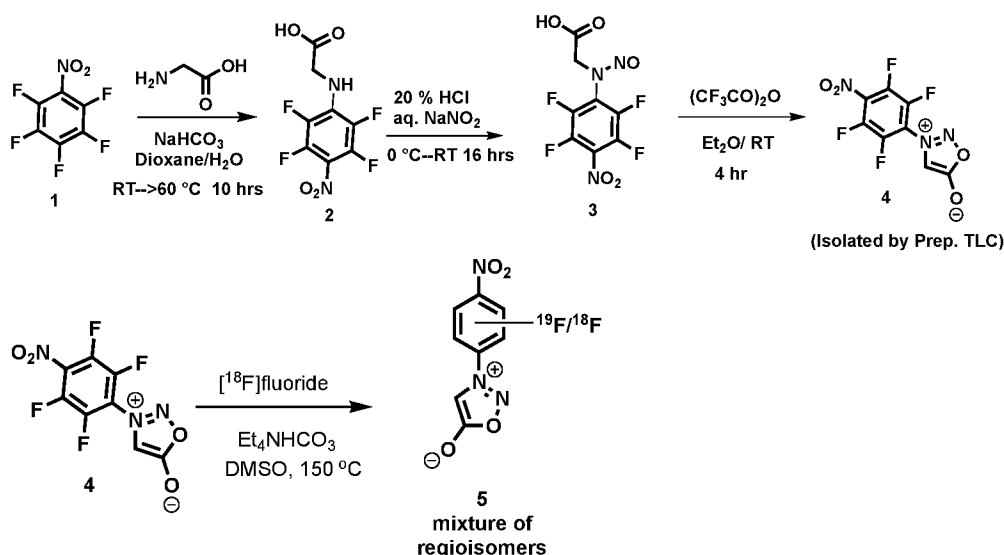
5 Compounds of Formula (I)-(III) may be prepared by the general schemes described herein, using the synthetic method known by those skilled in the art. The following examples illustrate non-limiting embodiments of the invention.

In a non-limiting embodiment, the synthesis of a compound of formula (I) may be accomplished by bis-alkylating a bis-amine with a bromoacetate and then saponifying the resulting bis-ester. Alternatively, the bis-acid can be obtained in one
 10 step from $\text{ClCH}_2\text{CO}_2\text{Na}$. The bis-acid can then be treated with *t*-BuONO and a dehydrating agent such as $(\text{CF}_3\text{CO})_2\text{O}$ or carbonyldiimidazole (CDI) to afford a bisynnone (Figure 13).



15

In one embodiment, the synthesis of a compound of formula (I) may be accomplished by the following synthetic scheme:

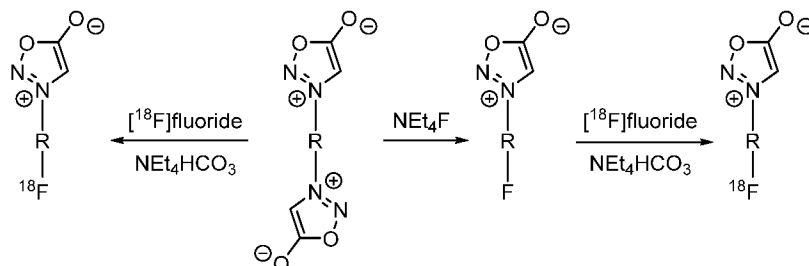


Compounds described herein also include isotopically-labeled

- 5 compounds wherein one or more atoms is replaced by an atom having the same atomic number, but an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes suitable for inclusion in the compounds described herein include and are not limited to ^2H , ^3H , ^{11}C , ^{13}C , ^{14}C , ^{36}Cl , ^{18}F , ^{123}I , ^{125}I , ^{13}N , ^{15}N , ^{15}O , ^{17}O , ^{18}O , ^{32}P , and ^{35}S . In one embodiment, the
- 10 compounds include ^{18}F . In one embodiment, isotopically-labeled compounds are useful in drug and/or substrate tissue distribution studies. In another embodiment, substitution with heavier isotopes such as deuterium affords greater metabolic stability (for example, increased in vivo half-life or reduced dosage requirements). In yet another embodiment, substitution with positron emitting isotopes, such as ^{11}C , ^{18}F ,
- 15 ^{15}O and ^{13}N , is useful in Positron Emission Topography (PET) studies for examining substrate receptor occupancy. Isotopically-labeled compounds are prepared by any suitable method or by processes using an appropriate isotopically-labeled reagent in place of the non-labeled reagent otherwise employed.

- Incorporation of ^{18}F into a compound of the invention can be
- 20 performed using any method known in the art, as would be understood by one of ordinary skill in the art. In one non-limiting embodiment, one sydnone moiety of the bissydnone can be displaced with radiolabeled ^{18}F in the presence of an alkyl ammonium base to generate a radiolabeled sydnone of the present invention (Figure 13). In another embodiment, the bissydnone is treated with a non-radiolabeled
- 25 fluoride to generate a stable intermediate which can be stored and transported if

necessary. In one embodiment, the non-labeled compound can be subjected to isotopic exchange with ^{18}F to generate the radiolabeled compound (Figure 14).



The compounds of the invention may possess one or more

- 5 stereocenters, and each stereocenter may exist independently in either the R or S configuration. In one embodiment, compounds described herein are present in optically active or racemic forms. It is to be understood that the compounds described herein encompass racemic, optically-active, regioisomeric and stereoisomeric forms, or combinations thereof that possess the therapeutically useful properties described
- 10 herein. Preparation of optically active forms is achieved in any suitable manner, including by way of non-limiting example, by resolution of the racemic form with recrystallization techniques, synthesis from optically-active starting materials, chiral synthesis, or chromatographic separation using a chiral stationary phase. In one embodiment, a mixture of one or more isomer is utilized as the therapeutic compound
- 15 described herein. In another embodiment, compounds described herein contain one or more chiral centers. These compounds are prepared by any means, including stereoselective synthesis, enantioselective synthesis and/or separation of a mixture of enantiomers and/ or diastereomers. Resolution of compounds and isomers thereof is achieved by any means including, by way of non-limiting example, chemical
- 20 processes, enzymatic processes, fractional crystallization, distillation, and chromatography.

The methods and formulations described herein include the use of N-oxides (if appropriate), crystalline forms (also known as polymorphs), solvates, amorphous phases, and/or pharmaceutically acceptable salts of compounds having the

25 structure of any compound of the invention, as well as metabolites and active metabolites of these compounds having the same type of activity. Solvates include water, ether (e.g., tetrahydrofuran, methyl tert-butyl ether) or alcohol (e.g., ethanol) solvates, acetates and the like. In one embodiment, the compounds described herein exist in solvated forms with pharmaceutically acceptable solvents such as water, and

ethanol. In another embodiment, the compounds described herein exist in unsolvated form.

In one embodiment, the compounds of the invention may exist as tautomers. All tautomers are included within the scope of the compounds presented
5 herein.

In one embodiment, sites on, for example, the aromatic ring portion of compounds of the invention are susceptible to various metabolic reactions. Incorporation of appropriate substituents on the aromatic ring structures may reduce, minimize or eliminate this metabolic pathway. In one embodiment, the appropriate
10 substituent to decrease or eliminate the susceptibility of the aromatic ring to metabolic reactions is, by way of example only, a deuterium, a halogen, or an alkyl group.

In one embodiment, the compounds described herein are labeled by other means, including, but not limited to, the use of chromophores or fluorescent moieties, bioluminescent labels, or chemiluminescent labels.

15 The compounds described herein, and other related compounds having different substituents are synthesized using techniques and materials described herein and as described, for example, in Fieser & Fieser's Reagents for Organic Synthesis, Volumes 1-17 (John Wiley and Sons, 1991); Rodd's Chemistry of Carbon Compounds, Volumes 1-5 and Supplementals (Elsevier Science Publishers, 1989);
20 Organic Reactions, Volumes 1-40 (John Wiley and Sons, 1991), Larock's Comprehensive Organic Transformations (VCH Publishers Inc., 1989), March, Advanced Organic Chemistry 4th Ed., (Wiley 1992); Carey & Sundberg, Advanced Organic Chemistry 4th Ed., Vols. A and B (Plenum 2000,2001), and Green & Wuts, Protective Groups in Organic Synthesis 3rd Ed., (Wiley 1999) (all of which are
25 incorporated by reference for such disclosure). General methods for the preparation of compound as described herein are modified by the use of appropriate reagents and conditions, for the introduction of the various moieties found in the formula as provided herein.

Compounds described herein are synthesized using any suitable
30 procedures starting from compounds that are available from commercial sources, or are prepared using procedures described herein.

In one embodiment, reactive functional groups, such as hydroxyl, amino, imino, thio or carboxy groups, are protected in order to avoid their unwanted participation in reactions. Protecting groups are used to block some or all of the

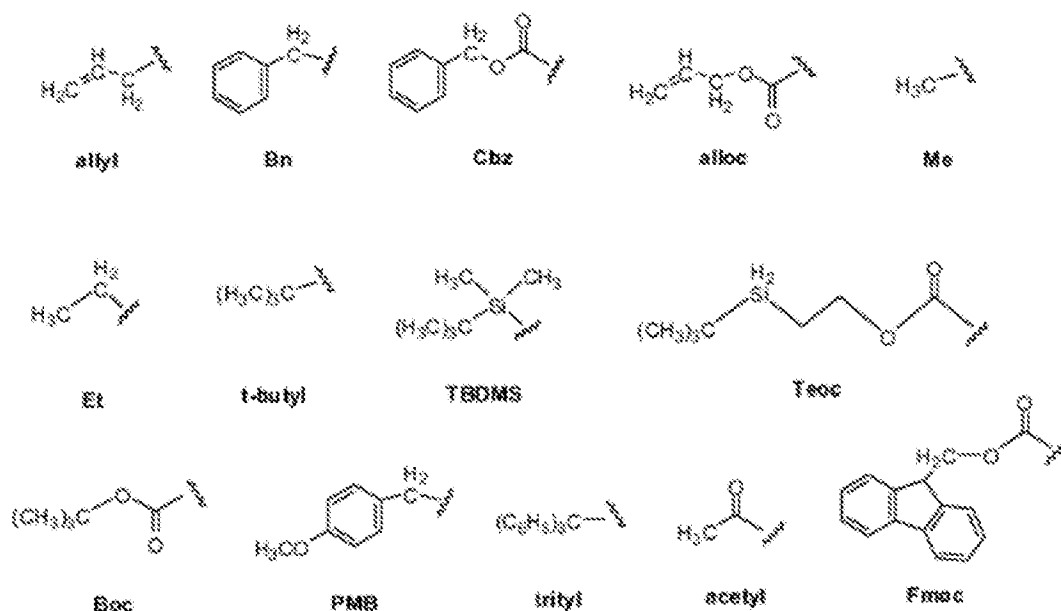
reactive moieties and prevent such groups from participating in chemical reactions until the protective group is removed. In another embodiment, each protective group is removable by a different means. Protective groups that are cleaved under totally disparate reaction conditions fulfill the requirement of differential removal.

5 In one embodiment, protective groups are removed by acid, base, reducing conditions (such as, for example, hydrogenolysis), and/or oxidative conditions. Groups such as trityl, dimethoxytrityl, acetal and t-butyldimethylsilyl are acid labile and are used to protect carboxy and hydroxy reactive moieties in the presence of amino groups protected with Cbz groups, which are removable by
10 hydrogenolysis, and Fmoc groups, which are base labile. Carboxylic acid and hydroxy reactive moieties are blocked with base labile groups such as, but not limited to, methyl, ethyl, and acetyl, in the presence of amines that are blocked with acid labile groups, such as t-butyl carbamate, or with carbamates that are both acid and base stable but hydrolytically removable.

15 In one embodiment, carboxylic acid and hydroxy reactive moieties are blocked with hydrolytically removable protective groups such as the benzyl group, while amine groups capable of hydrogen bonding with acids are blocked with base labile groups such as Fmoc. Carboxylic acid reactive moieties are protected by conversion to simple ester compounds as exemplified herein, which include
20 conversion to alkyl esters, or are blocked with oxidatively-removable protective groups such as 2,4-dimethoxybenzyl, while co-existing amino groups are blocked with fluoride labile silyl carbamates.

 Allyl blocking groups are useful in the presence of acid- and base-protecting groups since the former are stable and are subsequently removed by metal
25 or pi-acid catalysts. For example, an allyl-blocked carboxylic acid is deprotected with a palladium-catalyzed reaction in the presence of acid labile t-butyl carbamate or base-labile acetate amine protecting groups. Yet another form of protecting group is a resin to which a compound or intermediate is attached. As long as the residue is attached to the resin, that functional group is blocked and does not react. Once
30 released from the resin, the functional group is available to react.

 Typically blocking/protecting groups may be selected from:



Other protecting groups, plus a detailed description of techniques applicable to the creation of protecting groups and their removal are described in

- 10 Greene & Wuts, *Protective Groups in Organic Synthesis*, 3rd Ed., John Wiley & Sons, New York, NY, 1999, and Kocienski, *Protective Groups*, Thieme Verlag, New York, NY, 1994, which are incorporated herein by reference for such disclosure.

10 Labeling and Imaging of Biomolecules

In one aspect, the present invention provides a method for labeling a biomolecule. In one embodiment, the method for labeling a biomolecule comprises the steps of covalently attaching or conjugating a dipolarophile to a biomolecule to

20 form a biomolecule-dipolarophile conjugate, contacting the biomolecule-dipolarophile conjugate with a compound of the invention, wherein the compound undergoes a cycloaddition reaction with the dipolarophile to provide a labeled biomolecule.

In one embodiment, the method comprises the step of covalently attaching or conjugating a dipolarophile to a biomolecule to form a biomolecule-

25 dipolarophile conjugate. Preferred examples of biomolecules are proteins and peptides. In one embodiment, the biomolecule is a protein. In another embodiment, the biomolecule is bovine serum albumin (BSA). In another embodiment, the biomolecule is ovalbumin (OVA). In another embodiment, the biomolecule is an

engineered antibody fragment. A non-limiting example of an engineered antibody fragment is anti-PSCA A2 cys-diabody.

The dipolarophile can be any dipolarophile that is capable of undergoing a [3+2] cycloaddition reaction with a sydnone, as would be understood by one of ordinary skill in the art. Non-limiting examples of dipolarophiles include alkynes, such as ethynyl and propynyl. In one embodiment, the dipolarophile is an alkyne. Particularly advantageous dipolarophiles are those that are highly stable in aqueous environments, such as dibenzocyclooctyne (DBCO), biarylazacyclooctynone (BARAC), and derivatives thereof. In one embodiment, the dipolarophile is selected from the group consisting of dibenzocyclooctyne (DBCO) and a derivative thereof. In another embodiment, the dipolarophile is dibenzocyclooctyne (DBCO). In another embodiment, the dipolarophile is selected from the group consisting of biarylazacyclooctynone (BARAC) and a derivative thereof. In another embodiment, the dipolarophile is biarylazacyclooctynone (BARAC).

The biomolecule may be attached to the dipolarophile using any method known in the art. For example, the dipolarophile may comprise a moiety capable of being conjugated to the biomolecule. Examples of such moieties include an N-hydroxysuccinimide (NHS) ester and maleimide.

In one embodiment, the method further comprises the step of contacting the biomolecule-dipolarophile conjugate with a compound of the invention, wherein the compound undergoes a cycloaddition reaction with the dipolarophile to provide a labeled biomolecule. The cycloaddition reaction that occurs between the dipolarophile and the compound of the invention results in the formation of a pyrazole moiety at the dipolarophile site (Figure 4). The pyrazole moiety is comprised of an imaging moiety, which functions as a label for imaging, thereby incorporating a label into the biomolecule. For example, when the imaging moiety is ^{18}F , the biomolecule is labeled with ^{18}F and can subsequently be imaged.

In another aspect, the present invention provides a method for labeling a biomolecule. The method comprises the step of contacting a dipolarophile with a compound of the invention, wherein the compound undergoes a cycloaddition reaction with the dipolarophile to provide a pyrazole moiety. The method further comprises the step of covalently attaching or conjugating the pyrazole moiety to a biomolecule to provide a labeled biomolecule. The pyrazole moiety may be attached to the biomolecule using any method known in the art. For example, the pyrazole

moiety may comprise a moiety capable of being conjugated to the biomolecule. Examples of such moieties include an N-hydroxysuccinimide (NHS) ester and maleimide.

In another aspect, the present method provides a method for imaging a biomolecule. In one embodiment, the method comprises the steps of providing a sample comprising a biomolecule, covalently attaching or conjugating a dipolarophile to a biomolecule to form a biomolecule-dipolarophile conjugate, contacting the biomolecule-dipolarophile conjugate with a compound, wherein the compound undergoes a cycloaddition reaction with the dipolarophile to provide a labeled biomolecule, and detecting the labeled biomolecule, thereby imaging the biomolecule.

The biomolecule may be imaged using any method of detection known in the art. If the biomolecule is labeled with ^{18}F , the biomolecule can be detected by its emitted signal, such as autofluorescence emission or optical properties of the labeled biomolecule. The methods of detection for ^{18}F -labeled biomolecules may include, but are not necessarily limited to, nuclear scintigraphy, positron emission tomography (PET), single photon emission computed tomography (SPECT), magnetic resonance imaging, magnetic resonance spectroscopy, computed tomography, or a combination thereof depending on the intended use and the imaging methodology available to the medical or research personnel. In one embodiment, the labeled biomolecule is imaged using positron emission tomography (PET). The methods of detection for biomolecules labeled with fluorescent dyes includes, but not limited to, CCD cameras, video cameras, photographic film, laser scanning devices, fluorometers, photodiodes, quantum counters, epifluorescence microscopes, scanning microscopes, flow cytometers, fluorescence microplate readers, or by amplification devices such as photomultiplier tubes. In some embodiments, the biomolecule is imaged in vitro. In other embodiments, the biomolecule is imaged in vivo.

The sample can generally be any type of sample. For example, the sample can be a cell or group of cells, an organism, cell lysates, a cell culture medium, a bioreactor sample, and so on. In one embodiment, the sample is a cell. In one embodiment, the cell is a live cell. Alternatively, the sample can be a non-biological sample. The cells can be any type of cell, such as bacterial cells, fungal cells, insect cells, and mammalian cells. The sample can be a solid, a liquid, or a suspension. The sample can be a biological fluid such as blood, plasma, or urine. The sample can be a material immobilized in a gel, on a membrane, bound to a bead or

beads, arranged in an array, and so on. The sample can be a partially or fully purified preparation in a buffer or in water.

Those skilled in the art recognizes, or is able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures, embodiments, claims, and examples described herein. Such equivalents were considered to be within the scope of this invention and covered by the claims appended hereto. For example, it should be understood, that modifications in reaction conditions, including but not limited to reaction times, reaction size/volume, and experimental reagents, such as solvents, catalysts, pressures, atmospheric conditions, e.g., nitrogen atmosphere, and reducing/oxidizing agents, with art-recognized alternatives and using no more than routine experimentation, are within the scope of the present application.

It is to be understood that wherever values and ranges are provided herein, all values and ranges encompassed by these values and ranges, are meant to be encompassed within the scope of the present invention. Moreover, all values that fall within these ranges, as well as the upper or lower limits of a range of values, are also contemplated by the present application.

The following examples further illustrate aspects of the present invention. However, they are in no way a limitation of the teachings or disclosure of the present invention as set forth herein.

EXPERIMENTAL EXAMPLES

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

30

Example 1: Rapid and Selective Cycloaddition Reaction for Applications in Molecular Imaging

The results described herein demonstrate the reactivity of sydnone and explore the scope of this reaction and tuning its kinetics to be suitable for ^{18}F -

labeling of proteins (Figure 1). The electronics of various sydnone derivatives were studied with the goal of increasing their reaction kinetics to obtain a rapid reaction that could be utilized for ^{18}F -chemistry. The rate constant reported for the sydnone-exo-((1R,8S)-bicyclo[6.1.0]non-4-yn-9-yl)methanol (BCN) reaction is $0.054 \text{ M}^{-1}\text{s}^{-1}$, comparable to the well-known azide-alkyne ligation. Nearly twenty sydnone derivatives were synthesized, their electronics altered, and the second-order rate constants were studied for the reactions between these derivatives with BCN. Depending on the substrate, rate constants from $0.003 - 23.0 \text{ M}^{-1}\text{s}^{-1}$ were observed. Electron-poor derivatives were found to give faster rates.

During this investigation, two dipolarphiles were identified [the strained alkynes dibenzocyclooctyne (DBCO) (**6**) and biarylazacyclooctynone (BARAC) (**8**)] that undergo rapid [3+2] cycloaddition with sydnones (Figure 2). These bioorthogonal reactions possess second-order rate constants greater than $10^2 \text{ M}^{-1}\text{s}^{-1}$ orders of magnitude faster than the previously reported sydnone-BCN ligation, and are completed in less than five seconds. Rapid reactions with high intrinsic second-order rate constants, such as the sydnone-DBCO reaction, are beneficial for labeling on a short time-scale or for labeling material in low abundance, such is the case when working with fluorine-18.⁶ Fluorine-18 has a half-life of 109 minutes and is accessible only in picomolar concentrations for labeling reactions. As a result of its extraordinary reaction kinetics and selectivity, the sydnone-DBCO ligation may be useful for applications in ^{18}F -labeling of biomolecules.

To demonstrate the utility of this approach for protein labeling, a protein conjugation experiment was performed using bovine serum albumin (BSA) and ovalbumin (OVA) as model proteins (Figure 3). A mutually orthogonal reaction pair, the tetrazine-norbornene ligation, was identified and utilized to demonstrate dual fluorescence protein labeling in tandem. The DBCO and norbornene moieties were appended to the surface of BSA and OVA respectively, using standard coupling conditions (Figure 3). The modified proteins (BSA-DBCO, OVA-Nor) were treated with either a sydnone-BODIPY630 conjugate (Syd-630) or a tetrazine-BODIPY504 conjugate (Tz-504) or both and analyzed via in gel fluorescence imaging. When the modified proteins were jointly treated with Syd-630, only BSA-DBCO was fluorescently labeled, in agreement with observed kinetic data (lane 1, Figure 3). Likewise, in the presence of Tz-504, OVA-Nor showed selective labeling without any detection of BSA-DBCO fluorescence labeling (lane 2, Figure 3). Co-administration

of both fluorophore conjugates in “one pot” resulted in the concurrent labeling of both proteins (lane 6, Figure 3). In agreement with observed kinetic experiments, the protein labeling ligations demonstrate time-dependence with the BSA-DBCO labeling occurring very rapidly, exhibiting fluorescence in less than 1 min (lane 3, Figure 3).

- 5 The slower tetrazine-norbornene ligation started to show protein labeling after 15 min (lane 4, Figure 3). Unmodified proteins BSA and OVA, which do not contain the dipolarphiles DBCO and norbornene, showed no fluorescence labeling after exposure to Syd-630 and Tz-504, indicating the absence of nonspecific reactivity. These experiments demonstrate the exquisite specificity of the synone and tetrazine imaging
10 agents for DBCO- and norbornene-modified proteins respectively as well as their mutual compatibility within a single environment.

Example 2: Rapid strain induced syndnone-alkyne cycloaddition reactions for
bioorthogonal PET imaging

- 15 Described herein is a novel copper-free cycloaddition reaction between syndnone derivatives and the biarylazacyclooctynone (BARAC). These functionalized syndnones are used as 1,3-dipoles for metal-free cycloaddition partners with ring-strained alkynes. The highly stable syndnones display excellent reaction kinetics with BARAC. Extremely selective and high yielding bioorthogonal click chemistry
20 reactions have been widely studied and used as tools for radiolabelling biomolecules and small molecules for positron emission tomography (PET) (Figure 6C) (Thirumurugan et al., 2013, Chem. Rev. 113:4905-4979). The novel copper-free cycloaddition reaction between syndnone derivatives and the biarylazacyclooctynone (BARAC) described herein is the fastest reported cycloaddition reaction with BARAC
25 to date, with rates of 10^1 to 10^2 M⁻¹s⁻¹.

- 3-Arylsyndnone derivatives **16** were synthesized via intramolecular cyclization of *N*-nitroso-*N*-aryl amino acids with trifluoroacetic anhydride. The rate constant for the cycloaddition between syndnone and BARAC or BCN was determined under *pseudo*-first order conditions and followed *via* the exponential decay in UV
30 absorbance over time (Figure 6B).

The reactions of syndnones with BCN or BARAC proceed at room temperature, in aqueous media and without a transition-metal catalyst (Figure 6A). The *N*-3-phenyl ring and C-4 substitutions on the syndnone significantly impacted the reaction rate constants. Improved reaction rates were observed with electron deficient

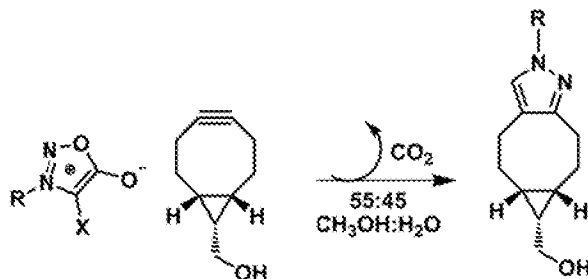
sydnones and 4-chlorosydnones; the cycloaddition reaction of phenyl sydnone with BARAC is nearly 10^4 -fold faster than with BCN.

5 Sydnones **21** were synthesized in two-steps from N-arylglycines **19** (Figure 7). ^{18}F -labeled sydnones **24** are synthesized from precursor **23**.

10 Second order reaction kinetics of various sydnones and strained alkynes were measured on a UV-Visible spectrophotometer (Figure 6B). The decay in absorbance of sydnone was monitored over time by reacting sydnones with excess of strained alkynes. Figure 6B illustrates the calculation of rate constant of the cycloaddition between phenylsydnone and BCN ($k_2 = 0.019 \text{ M}^{-1}\text{s}^{-1}$).

15 Diverse variety of arylsydnones were synthesized and their reaction kinetics were studied with BCN. The cycloaddition reactions were conducted in methanol: water (55:45) at room temperature (23 °C). Table 1 lists the calculated second order rate kinetics associated with the cycloaddition reaction between BCN and various sydnone derivatives.

Table 1: Second order rate kinetics of the cycloaddition reaction between BCN and various sydnone derivatives



Entry	R	X	Second order rate $k(M^{-1}s^{-1})$
1	C_6H_5	H	0.0194
2	4- NO_2 - C_6H_4	H	0.1543
3	4- NH_2 - C_6H_4	H	0.0027
4	4- NO_2 -2- FC_6H_3	H	0.1494
5	4- NH_2 -2- FC_6H_3	H	0.0029
6	4- NO_2 -2- CF_3 - C_6H_3	H	0.0137
7	4-COOH- C_6H_4	H	0.0103
8	4-COOH-3- FC_6H_3	H	0.0106
9	4-COOC ₂ H ₅ - C_6H_4	H	0.0045
10	4-COOC ₂ H ₅ -3- FC_6H_3	H	0.0832
11	3-COOC ₂ H ₅ -2- FC_6H_3	H	0.0772
12	4- NO_2 - C_6F_4	H	23.133
13	4- NH_2 - C_6F_4	H	0.031
14	C_6H_5	Br	0.4049

These results demonstrate that the second order rate constant of sydnones were improved by introduction of fluorine on the phenyl ring at the C-3 position when compared to their parent compounds. Furthermore, the introduction of electron donating substituents results in an observed decrease in reaction rates, while 4-nitro-2,3,5,6-tetrafluorophenyl at C-3 was found to dramatically increase the rate of cycloaddition. A proposed mechanism for the cycloaddition reaction can be seen in Figure 9. These results demonstrate the synthesis and kinetic studies of diverse variety of arylsydnones with BCN, and reveal the effect of fluorophenyl substitutions on cycloaddition reaction rates. Other examples of click reactions between sydnones and alkynes can be found in Figures 8 and 10.

Example 3: Synthesis of radiolabeled sydnones from bis-syndnone

Materials and Methods

No-carrier-added [^{18}F]fluoride was produced by the $^{18}O(p,n)^{18}F$ nuclear reaction in a Siemens RDS-112 cyclotron at 11 MeV using a 1 mL tantalum target with havar foil. Unless otherwise stated, reagents and solvents were commercially available and used without further purification. HPLC grade acetonitrile and trifluoroacetic acid were purchased from Fisher Scientific. Anhydrous

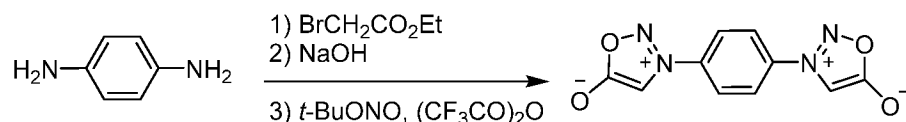
acetonitrile, dimethyl sulfoxide and tetraethylammonium bicarbonate were purchased from Sigma-Aldrich. Sterile product vials were purchased from Hollister-Stier. QMA-light Sep-Paks and tC18 light cartridges were purchased from Waters Corporation. HPLC purifications were performed on a Knauer Smartline HPLC system with inline
5 Knauer UV (254 nm) detector and gamma-radiation coincidence detector and counter (Bioscan Inc.). Semi-preparative HPLC was performed using Phenomenex reverse-phase Luna column (10 × 250 mm, 5 µm) with a flow rate of 4 mL/min. Final purity and identity of compounds were determined by analytical HPLC analysis performed with a Phenomenex reverse-phase Luna column (4.6 × 250 mm, 5 µm) with a flow
10 rate of 1 mL/min. All chromatograms were collected by a GinaStar (Raytest USA, Inc.) analog to digital converter and GinaStar software.

Dry [¹⁸F]TEAF was prepared using an ELIXYS automated radiosynthesizer (Sofie Biosciences). [¹⁸F]Fluoride was delivered to the ELIXYS in [¹⁸O]H₂O (1 mL) via nitrogen gas push and trapped on a QMA cartridge to remove the [¹⁸O]H₂O.
15 Trapped [¹⁸F]fluoride was subsequently eluted into the reaction vial using a solution containing Et₄NHCO₃ (9-10 mg) in acetonitrile and water (1 mL, 8:2). Contents in the reaction vial were subjected to a nitrogen stream and evaporated by heating the vial to 110 °C while applying a vacuum for 3.5 min, with stirring. Acetonitrile (1.3 mL) was passed through the QMA cartridge to wash remaining activity into the reaction vial.
20 The combined contents in the reaction vial were dried by azeotropic distillation (heating to 110 °C under vacuum) for 2 min. Anhydrous acetonitrile (1.3 mL) was directly added to the reaction vial and azeotropic distillation was repeated once more until dryness, approximately 3-4 min. The reaction vial was cooled to 30 °C under nitrogen pressure and DMSO (1 mL) was added to provide anhydrous [¹⁸F]TEAF
25 which was used for subsequent reactions.

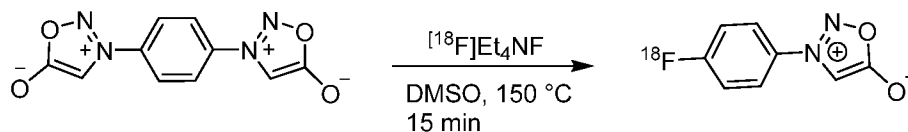
General experimental procedure: Radiochemistry experiments were conducted in a 4 mL glass vial containing *N*-arylsydnone (4-5 mg) with 250-350 µCi of [¹⁸F]TEAF in 150-200 µL of DMSO. The contents were heated to 150 °C for 5-20 min and progress of the reaction was observed by radio-TLC. An aliquot of the crude
30 reaction mixture was spotted on a silica gel coated TLC plate, developed in a glass chamber with acetonitrile:water (95:5) as the eluent. The radiochemical conversion (RCC) was calculated by dividing the integrated area of the ¹⁸F-fluorinated product peak by the total integrated area of all peaks on the TLC and multiplying by 100 to

convert to percentage units. Analytical HPLC was used to confirm product identity and purity via UV absorbance at 254 nm, by coinjection with the ^{19}F -reference standard. An aliquot of the crude reaction mixture (10 μL) was added to the ^{19}F -reference standard (1 mg/mL) in acetonitrile (10 μL) and the sample was injected into the analytical HPLC.

Preparation of radiolabeled sydnones



To a suspension of aryl amine (10 mmol) and sodium acetate (12 mmol) in ethanol (20 mL) was added ethyl bromoacetate (12 mmol). The mixture was refluxed for 7 h, left overnight at room temperature, and poured into crushed ice. The precipitate formed was filtrated and dried. To the intermediate ester in water/tetrahydrofuran (1:1) (25 mL) was added sodium hydroxide (1.1 mmol), and the mixture was stirred at 100 °C for 30 min then to room temperature for 4 hrs. The reaction mixture was acidified to pH 2 by dropwise addition of a 2 M solution of HCl. The resulting precipitate was filtered, washed with water, and purified by recrystallization from a mixture ethanol/water to afford N-arylglycine. To a mixture of aryl glycine (1 mmol) in anhydrous THF (10 mL) was added *t*-Butyl nitrite (1.1). The mixture was stirred at room temperature for 30 min and trifluoroacetic anhydride (1.15 mmol) was added. After 1 h stirring at room temperature, ethyl acetate was added and the reaction was quenched with a saturated solution of sodium bicarbonate. The aqueous layer was extracted with ethyl acetate and the organic layers were combined, dried over MgSO_4 and evaporated to obtain the bis-sydnones (Figure 13).



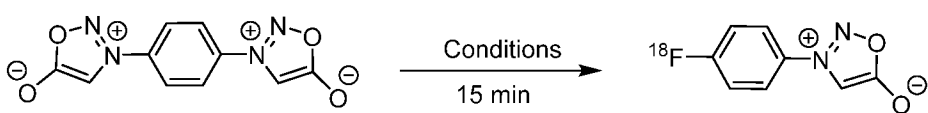
To anhydrous $[^{18}\text{F}]\text{Et}_4\text{NF}$ (15-45 mCi), prepared using an ELIXYS synthesis module, was added phenyl bis-sydnones (4-5 mg) in 0.5 mL of DMSO. The contents were stirred at 150 °C for 15 min. The reaction mixture was cooled to room temperature and diluted with 4 mL water before purification via semi-preparative HPLC (5% Acetonitrile in water (both 0.1% TFA) 0-5 min then to 50% acetonitrile in

water over 40 min). The HPLC fraction was collected (retention time, 21 min) in 30 mL of water and passed through tC18 Sep-Pak, which was preactivated by sequential washing of ethanol (5 mL) and water (10 mL). The product was eluted with diethyl ether (2 mL) or acetonitrile (1.5 mL). The product identity and purity were

5 determined by radio-HPLC and radio-TLC. Non-decay corrected radiochemical yields were calculated from the amount of activity trapped on QMA cartridge to product isolated after HPLC purification.

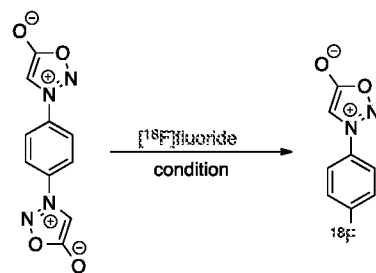
Table 2: Base optimization in the synthesis of radiolabeled sydnones

10



Entry	Qty. Bissyd/ DMSO (mL)	Base (Qty) (solvent system)	Temp(°C)/ Time(min)	n.d.c. RCY [%]
1.	7.2 mg/0.8mL	NEt ₄ HCO ₃ (10 mg) (9:1 ACN:H ₂ O)	150°C/15	17
2.	7.2 mg/0.8 mL	NEt ₄ HCO ₃ (11 mg) (9:1 ACN:H ₂ O)	130°C/15	9
3.	6.5 mg/0.8 mL	K ₂ CO ₃ /K222 (1.2 mg/11 mg) (7:3 ACN: H ₂ O)	150°C/10	15
4.	4 mg/ 0.8 mL	K ₂ CO ₃ /K222 (1.8 mg/12 mg) (7:3 ACN: H ₂ O)	150 °C/15	11.4
5.	5.2 mg/ 0.8mL	Aqu.NBu ₄ HCO ₃ (0.075M) 160 µL in 7:3 ACN:H ₂ O)	150 °C/15	9.5
6.	8 mg/ 0.6 mL	NBu ₄ OH (6.5 uL) (1:1 ACN:H ₂ O) 1mL	150 °C/20	2.8

Table 3: Optimization of sydnone displacement with radiolabeled fluoride



Isolated RCY (d.c.) = 34 ± 7 (n = 17)

Isolated RCY (n.d.c.) = 21 ± 3 (n = 17)

15

Entry	Qty. Bissyd/ DMSO (mL)	Base (Qty)	Temp(°C)/ Time(min)	d.c. RCY HPLC [%]	n.d.c RCY HPLC [%]

7.	7.2 mg/0.8mL	NEt ₄ HCO ₃ (10 mg) (9:1 ACN:H ₂ O)	150°C/15	26	17
8.	4.2 mg/0.8 mL	NEt ₄ HCO ₃ (10 mg) (9:1 ACN:H ₂ O)	150°C/15	48	26.7
9.	7mg/0.6 mL	NEt ₄ HCO ₃ (12 mg) (8:2 ACN:H ₂ O)	150 °C/10	32	17
10.	7 mg/0.6 mL	NEt ₄ HCO ₃ (11 mg) (8:2 ACN:H ₂ O)	150 °C/15	37	20.1
11.	7 mg/0.45 mL	NEt ₄ HCO ₃ (11 mg) (8:2 ACN:H ₂ O)	150°C/15	35	21.3
12.	6 mg/ 0.6mL	NEt ₄ HCO ₃ (10 mg) (8:2) (ACN:H ₂ O)	150°C/15	22.0	18.5
13.	5 mg/0. 5mL	NEt ₄ HCO ₃ (11 mg) (8:2) (ACN:H ₂ O)	150°C/15	32	22
14.	5 mg/ 0.45mL	NEt ₄ HCO ₃ (10 mg) (8:2 ACN:H ₂ O)	150°C/15	38.4	19.7
15.	4.7 mg/ 0.45mL	NEt ₄ HCO ₃ (11 mg) (8:2 ACN:H ₂ O)	150°C/15	29	16.4
16.	4.5 mg/ 0.45mL	NEt ₄ HCO ₃ (11 mg) (8:2) (ACN:H ₂ O)	150°C/15	28	22.2
17.	4.5mg/0.45mL	NEt ₄ HCO ₃ (10.4 mg) (8:2) (ACN:H ₂ O)	150°C/15#	42	23.1
18.	4.0 mg/0.5mL	NEt ₄ HCO ₃ (10.5 mg) (8:2) (ACN:H ₂ O)	150°C/8#	38	21
19.	3.5 mg/ 0.5mL	NEt ₄ HCO ₃ (10.02 mg) (8:2) (ACN:H ₂ O)	150°C/12#	25	16
20.	3.6 mg/ 0.5mL	NEt ₄ HCO ₃ (10.5 mg) (8:2) (ACN:H ₂ O)	150°C/12#	37	26
21.	2 mg/ 0.45mL	NEt ₄ HCO ₃ (10.5 mg) (8:2) (ACN:H ₂ O)	150°C/15#	26	17.1
22.	1.0 mg/0.45mL	NEt ₄ HCO ₃ (10.4 mg) (8:2) (ACN:H ₂ O)	150°C/15#	44	21.1
23.	1.0 mg/0.5mL	NEt ₄ HCO ₃ (9.8 mg) (8:2) (ACN:H ₂ O)	150°C/12#	34	18.4
24.	1.0 mg/0.5 mL	NEt ₄ HCO ₃ (9.8 mg) (8:2) (ACN:H ₂ O)	150 °C/15	32	22

Table 4: Integrated radio-thin layer chromatography (TLC) scan results

Run	Radio TLC yield (%)
1	61
2	63
3	53
4	60
5	58
6	52
mean	58
st. dev.	4

The molar radioactivity of 4- ^{18}F fluorophenyl sydnone (21a, Figure 15) was determined by the following method. A sample of known volume of purified 21a was injected into the analytical HPLC. The absorbance (mAU*s) of UV peak corresponding to the radiofluorinated product 21a was measured. The UV area was used to calculate the concentration of the product based on linear regression analysis of 4- ^{19}F fluorophenyl sydnone. A calibration curve was generated from a standard solution (5 $\mu\text{g/mL}$) by measuring the UV absorbance at different concentrations. The activity injected (Ci/mL) divided by concentration of the product measured from linear regression ($\mu\text{mol/mL}$) produced the molar specific activity in Ci/ μmol or GBq/ μmol .

Table 5: Molar radioactivity calibration curve data for 4- ^{18}F fluorophenyl sydnone

Volume Injected (μL)	Concentration ($\mu\text{g/mL}$)	Mass injected (μg)	Moles injected (μmol)	Absorbance (mAu*s)
2	5	0.01	5.55E-05	3
5	5	0.025	1.39E-04	7.5
10	5	0.05	2.78E-04	13.87
25	5	0.125	6.94E-04	28.54
50	5	0.25	1.39E-03	54.85
100	5	0.5	2.78E-03	114.56
150	5	0.75	4.16E-03	165.12

Table 6: Molar radioactivity of 4- ^{18}F fluorophenyl sydnone 21a

Volume Injected (μL) ^a	Activity Injected (μCi) ^a	Abs. (mAu*s)	Moles from Curve (μmol)	Molar radioactivity (Ci/ μmol)	Molar radioactivity (GBq/ μmol)
100	400	11.9	2.59E-04	1.54E+00	57.16
50	450	18.4	4.23E-04	1.06E+00	39.36
50	350	14	3.12E-04	1.12E+00	41.52

^a Value at time of injection

DIBAC (1 mM) in PBS:DMSO (1:1) (200 μL) was added to purified 4- ^{18}F fluorophenyl sydnone (2 mCi – 5 mCi) and stirred at 50 °C for 8 min. The crude reaction mixture was injected into analytical HPLC which showed >99.5% conversion to the click product (Figure 15). The identity of the product was confirmed by co-injecting compound (23) with a cold (non-radiolabeled) click adduct.

The results of the experiments are now described.

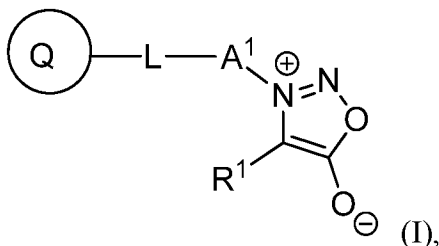
The desired compound 4- ^{18}F fluorophenyl sydnone **21a** (6-10 mCi) was synthesized via the phenyl bissydnone precursor in a fully automated process using an ELIXYS radiosynthesis module, purified, and isolated within 62 min from the end of bombardment in $20 \pm 3\%$ non-decay-corrected radiochemical yield (RCY) (n=18) with a specific activity of $1.3 \text{ Ci } \mu\text{mol}^{-1}$. Purified **21a** was stirred with 1 mM DIBAC **22** in 1:1 DMSO:PBS, a concentration typically reported for ^{18}F peptide labeling (Chem. Commun, 2016, 52, 6083; Nuclear Medicine and Biology, 2013, 40, 223). Clean conversion in $>99\%$ to the 3+2 cycloadduct **23** was observed after 8 mins, determined by radio-HPLC analysis of the crude product (Figure 15). Recently, Taran reported a closely related ultra fast click chemistry reaction with 4-fluorosydones and subsequent radiofluorination of a sydnone- Pd^{II} complex after initial preparation of ^{18}F Selectfluor bis(triflate), to afford ^{18}F -labeled 4-fluorosydnone in 7.5% RCY (Liu, et al., Angew. Chem. Int. Ed., 2016, 55, 12073-12077). The methods described herein provide significant practical advantages over previously reported methods, including synthetic ease, high RCY and full automation.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

What is claimed is:

1. A compound of formula (I):



wherein in formula (I):

A^1 is selected from the group consisting of alkyl, heteroalkyl, alkylcycloalkyl, heterocyclyl, alkylheterocyclyl, aryl, alkylaryl, heteroaryl, and alkylheteroaryl, wherein the alkyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl group and is optionally substituted with 0-5 R^2 groups

R^1 and each occurrence of R^2 are independently selected from the group consisting of H, $-C_1-C_6$ alkyl, $-C_1-C_6$ fluoroalkyl, $-C_1-C_6$ heteroalkyl, F, ^{18}F , Cl, Br, I, -CN, $-\text{NO}_2$, $-\text{OR}^3$, $-\text{SR}^3$, $-\text{S}(=\text{O})\text{R}^3$, $-\text{S}(=\text{O})_2\text{R}^3$, $-\text{NHS}(=\text{O})_2\text{R}^3$, $-\text{C}(=\text{O})\text{R}^3$, $-\text{OC}(=\text{O})\text{R}^3$, $-\text{CO}_2\text{R}^3$, $-\text{OCO}_2\text{R}^3$, $-\text{CH}(\text{R}^3)_2$, $-\text{N}(\text{R}^3)_2$, $-\text{C}(=\text{O})\text{N}(\text{R}^3)_2$, $-\text{OC}(=\text{O})\text{N}(\text{R}^3)_2$, $-\text{NHC}(=\text{O})\text{NH}(\text{R}^3)$, $-\text{NHC}(=\text{O})\text{R}^3$, $-\text{NHC}(=\text{O})\text{OR}^3$, $-\text{C}(\text{OH})(\text{R}^3)_2$, and $-\text{C}(\text{NH}_2)(\text{R}^3)_2$;

each occurrence of R^3 is independently selected from the group consisting of H and $\text{C}_1\text{-C}_6$ alkyl;

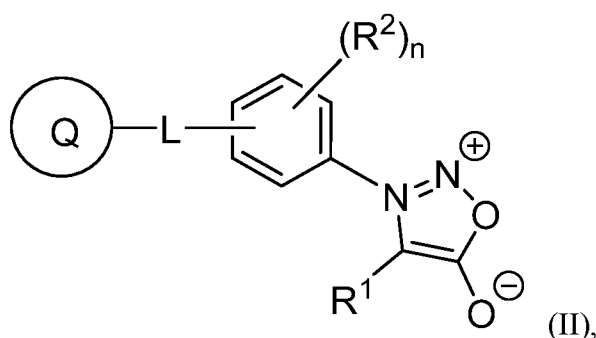
L is a linker; and

Q is an imaging moiety.

a salt thereof, and any combinations thereof.

2. The compound of claim 1, wherein A^1 is a phenyl group, wherein the phenyl group is optionally substituted.

3. The compound of claim 1, wherein L is selected from the group consisting of a bond, an alkyl group, an amide, a poly(alkyl ether), and any combinations thereof.
4. The compound of claim 1, wherein Q is ^{18}F .
5. The compound of claim 1, wherein Q is a fluorescent dye.
6. The compound of claim 5, wherein the fluorescent dye is BODIPY630.
7. The compound of claim 2, wherein the compound of formula (I) is a compound of formula (II):



wherein in formula (II):

n is an integer between 0 and 4;

R^1 and each occurrence of R^2 are each independently selected from the group consisting of H, $-\text{C}_1\text{-C}_6$ alkyl, $-\text{C}_1\text{-C}_6$ fluoroalkyl, $-\text{C}_1\text{-C}_6$ heteroalkyl, F, ^{18}F , Cl, Br, I, -CN, $-\text{NO}_2$, $-\text{OR}^3$, $-\text{SR}^3$, $-\text{S}(=\text{O})\text{R}^3$, $-\text{S}(=\text{O})_2\text{R}^3$, $-\text{NHS}(=\text{O})_2\text{R}^3$, $-\text{C}(=\text{O})\text{R}^3$, $-\text{OC}(=\text{O})\text{R}^3$, $-\text{CO}_2\text{R}^3$, $-\text{OCO}_2\text{R}^3$, $-\text{CH}(\text{R}^3)_2$, $-\text{N}(\text{R}^3)_2$, $-\text{C}(=\text{O})\text{N}(\text{R}^3)_2$, $-\text{OC}(=\text{O})\text{N}(\text{R}^3)_2$, $-\text{NHC}(=\text{O})\text{NH}(\text{R}^3)$, $-\text{NHC}(=\text{O})\text{R}^3$, $-\text{NHC}(=\text{O})\text{OR}^3$, $-\text{C}(\text{OH})(\text{R}^3)_2$, and $-\text{C}(\text{NH}_2)(\text{R}^3)_2$;

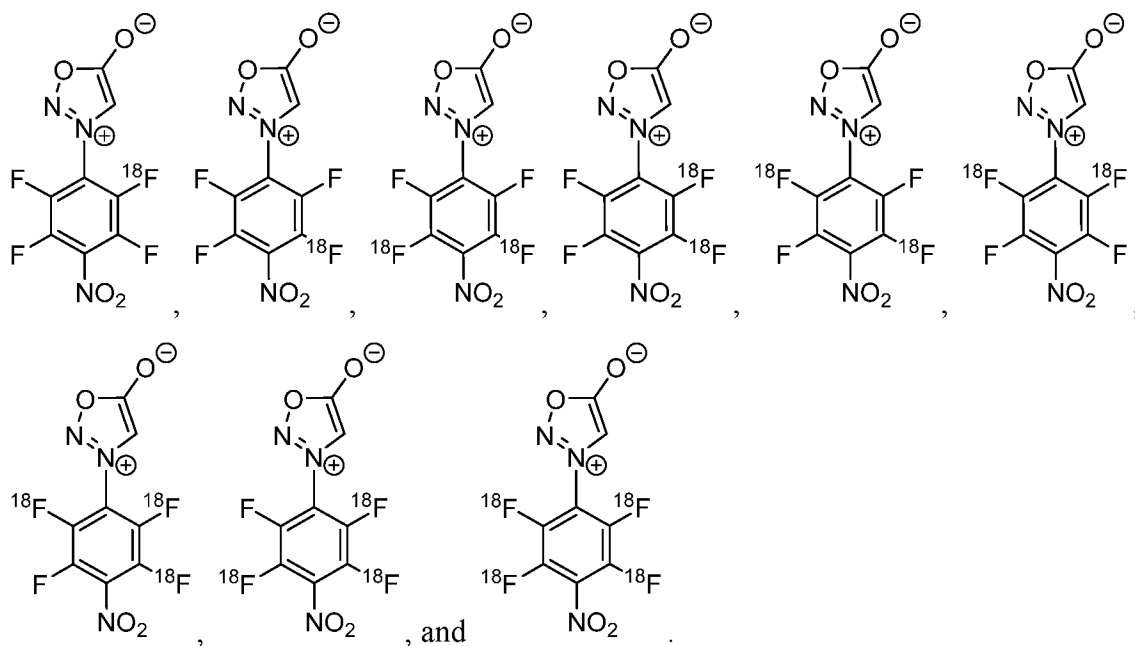
each occurrence of R^3 is independently selected from the group consisting of H and $\text{C}_1\text{-C}_6$ alkyl;

L is a linker; and

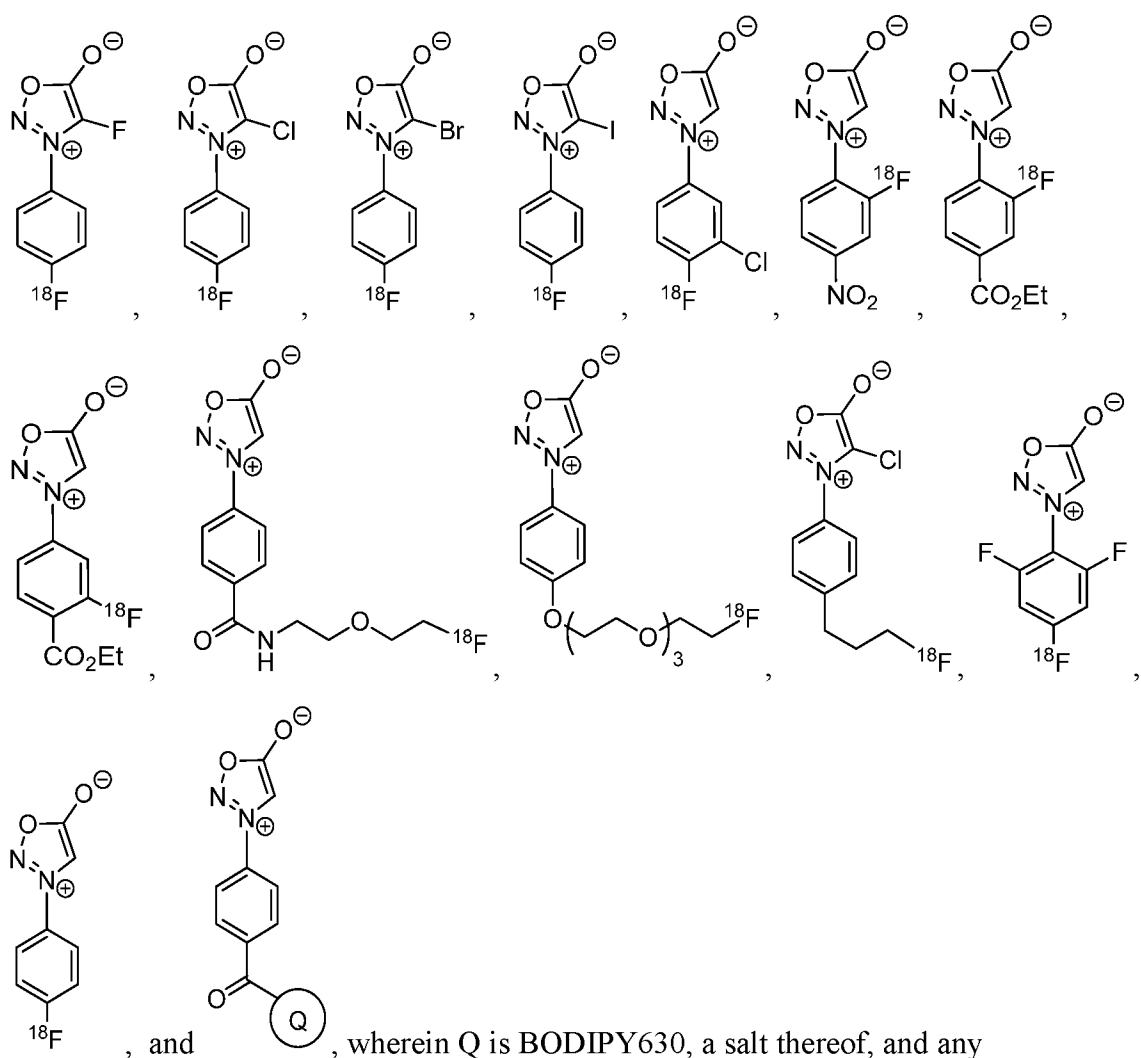
Q is an imaging moiety.

8. The compound of claim 7, wherein n is at least 1 and at least one R^2 is F.

9. The compound of claim 1, wherein the compound is selected from the group consisting of:



10. The compound of claim 1, wherein the compound is selected from the group consisting of:

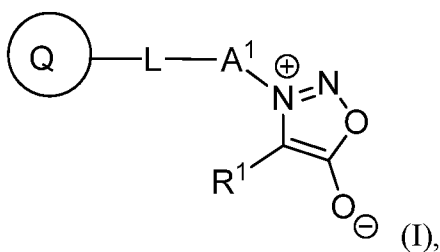


combinations thereof.

11. A method for labeling a biomolecule comprising the steps of:
covalently attaching or conjugating a dipolarophile to a biomolecule to form a
biomolecule-dipolarophile conjugate; and

contacting the biomolecule-dipolarophile conjugate with a compound, wherein the compound undergoes a cycloaddition reaction with the dipolarophile to provide a labeled biomolecule.

12. The method of claim 11, wherein the compound is at least one compound of formula (I):



wherein in formula (I):

A^1 is selected from the group consisting of alkyl, heteroalkyl, alkylcycloalkyl, heterocyclyl, alkylheterocyclyl, aryl, alkylaryl, heteroaryl, and alkylheteroaryl, wherein the alkyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl group and is optionally substituted with 0-5 R^2 groups

R^1 and each occurrence of R^2 are independently selected from the group consisting of H, $-C_1-C_6$ alkyl, $-C_1-C_6$ fluoroalkyl, $-C_1-C_6$ heteroalkyl, F, ^{18}F , Cl, Br, I, -CN, $-\text{NO}_2$, $-\text{OR}^3$, $-\text{SR}^3$, $-\text{S}(=\text{O})\text{R}^3$, $-\text{S}(=\text{O})_2\text{R}^3$, $-\text{NHS}(=\text{O})_2\text{R}^3$, $-\text{C}(=\text{O})\text{R}^3$, $-\text{OC}(=\text{O})\text{R}^3$, $-\text{CO}_2\text{R}^3$, $-\text{OCO}_2\text{R}^3$, $-\text{CH}(\text{R}^3)_2$, $-\text{N}(\text{R}^3)_2$, $-\text{C}(=\text{O})\text{N}(\text{R}^3)_2$, $-\text{OC}(=\text{O})\text{N}(\text{R}^3)_2$, -NHC(=O)NH(R^3), -NHC(=O) R^3 , -NHC(=O)OR 3 , $-\text{C}(\text{OH})(\text{R}^3)_2$, and $-\text{C}(\text{NH}_2)(\text{R}^3)_2$;

each occurrence of R^3 is independently selected from the group consisting of H and C_1-C_6 alkyl;

L is a linker; and

Q is an imaging moiety.

a salt thereof, and any combinations thereof.

13. The method of claim 12, wherein A^1 is a phenyl group, wherein the phenyl group is optionally substituted.

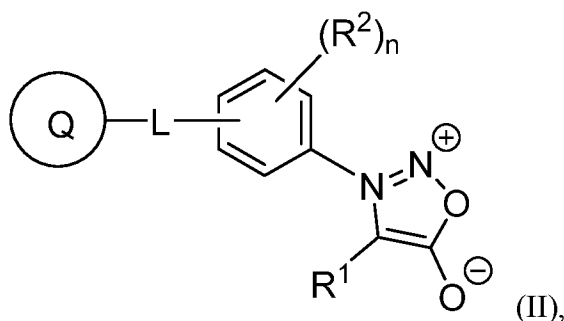
14. The method of claim 12, wherein L is selected from the group consisting of a bond, an alkyl group, an amide, a poly(alkyl ether) group, and any combinations thereof.

15. The method of claim 12, wherein Q is ^{18}F .

16. The method of claim 12, wherein Q is a fluorescent dye.

17. The method of claim 16, wherein the fluorescent dye is BODIPY630.

18. The method of claim 13, wherein the compound of formula (I) is a compound of formula (II):



wherein in formula (II):

n is an integer between 0 and 4;

R^1 and each occurrence of R^2 are each independently selected from the group consisting of H, $-C_1-C_6$ alkyl, $-C_1-C_6$ fluoroalkyl, $-C_1-C_6$ heteroalkyl, F, ^{18}F , Cl, Br, I, -CN, $-\text{NO}_2$, $-\text{OR}^3$, $-\text{SR}^3$, $-\text{S}(=\text{O})\text{R}^3$, $-\text{S}(=\text{O})_2\text{R}^3$, $-\text{NHS}(=\text{O})_2\text{R}^3$, $-\text{C}(=\text{O})\text{R}^3$, $-\text{OC}(=\text{O})\text{R}^3$, $-\text{CO}_2\text{R}^3$, $-\text{OCO}_2\text{R}^3$, $-\text{CH}(\text{R}^3)_2$, $-\text{N}(\text{R}^3)_2$, $-\text{C}(=\text{O})\text{N}(\text{R}^3)_2$, $-\text{OC}(=\text{O})\text{N}(\text{R}^3)_2$, $-\text{NHC}(=\text{O})\text{NH}(\text{R}^3)$, $-\text{NHC}(=\text{O})\text{R}^3$, $-\text{NHC}(=\text{O})\text{OR}^3$, $-\text{C}(\text{OH})(\text{R}^3)_2$, and $-\text{C}(\text{NH}_2)(\text{R}^3)_2$;

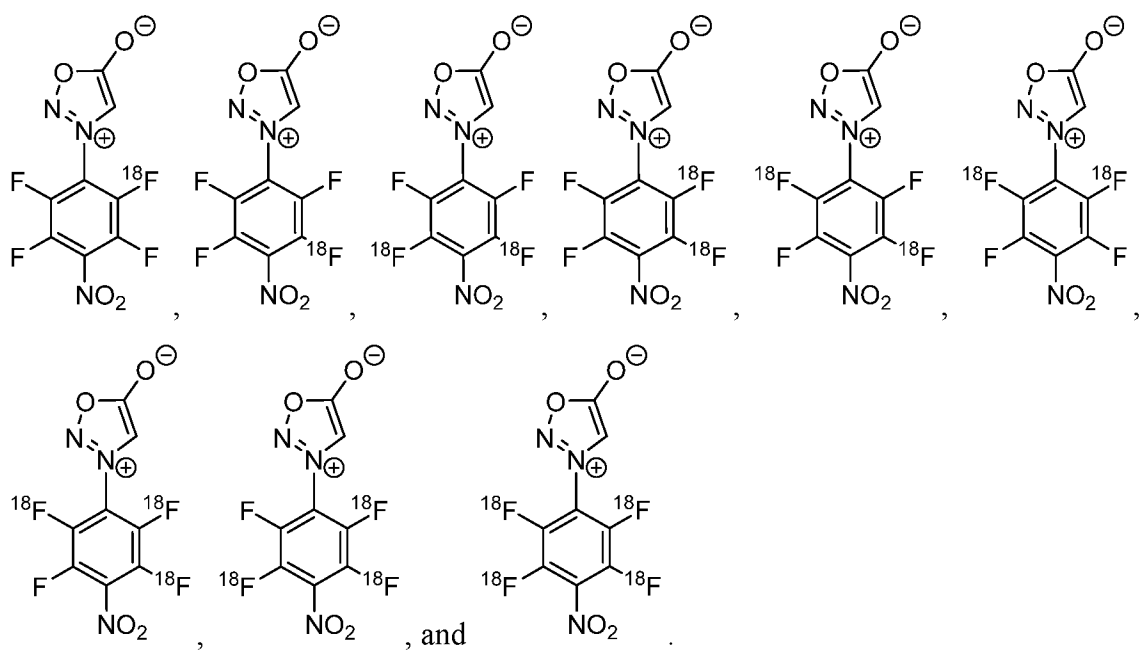
each occurrence of R^3 is independently selected from the group consisting of H and $\text{C}_1\text{-C}_6$ alkyl;

L is a linker; and

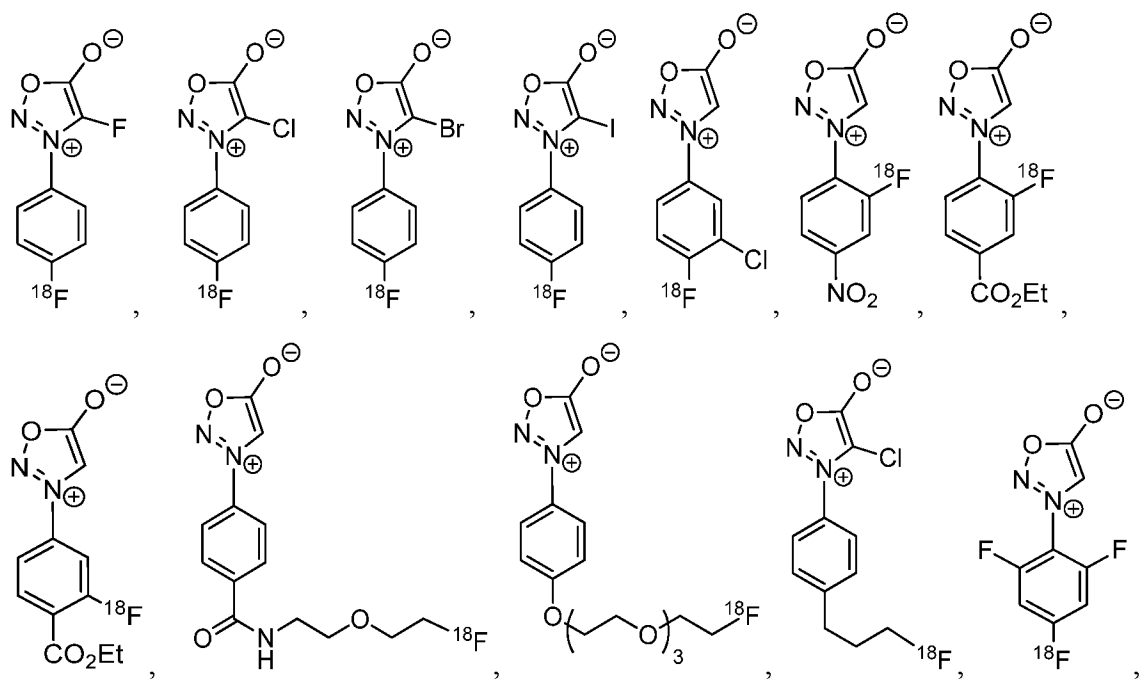
Q is an imaging moiety.

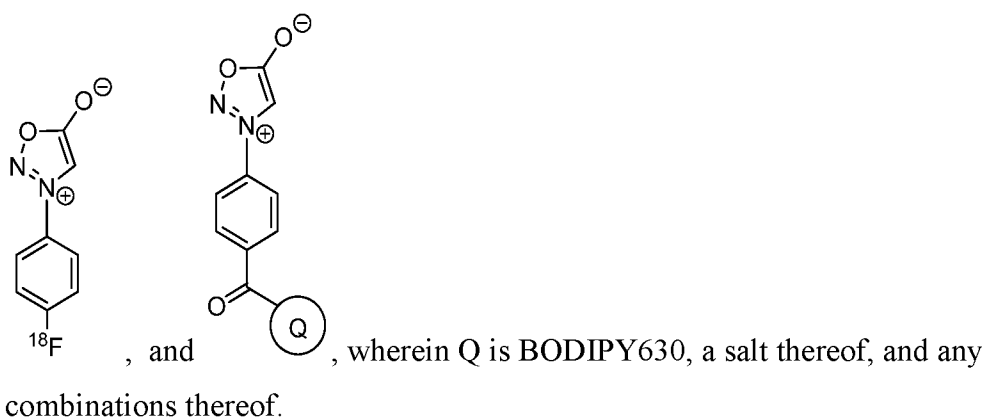
19. The method of claim 18, wherein n is at least 1 and at least one R^2 is F.

20. The method of claim 12, wherein the compound is selected from the group consisting of:



21. The method of claim 12, wherein the compound is selected from the group consisting of:



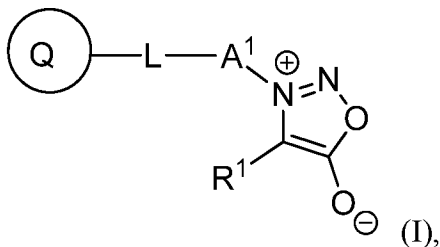


22. The method of claim 11, wherein the dipolarophile is dibenzocyclooctyne (DBCO).

23. The method of claim 11, wherein the dipolarophile is biarylazacyclooctynone (BARAC).

24. A method for imaging a biomolecule comprising the steps of:
 providing a sample comprising a biomolecule;
 covalently attaching or conjugating a dipolarophile to a biomolecule to form a biomolecule-dipolarophile conjugate;
 contacting the biomolecule-dipolarophile conjugate with a compound, wherein the compound undergoes a cycloaddition reaction with the dipolarophile to provide a labeled biomolecule;
 and detecting the labeled biomolecule, thereby imaging the biomolecule.

25. The method of claim 24, wherein the compound is at least one compound of formula (I):



wherein in formula (I):

A^1 is selected from the group consisting of alkyl, heteroalkyl, alkylcycloalkyl, heterocyclyl, alkylheterocyclyl, aryl, alkylaryl, heteroaryl, and alkylheteroaryl, wherein the alkyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl group is optionally substituted with 0-5 R^2 groups

R^1 and each occurrence of R^2 are independently selected from the group consisting of H, $-C_1-C_6$ alkyl, $-C_1-C_6$ fluoroalkyl, $-C_1-C_6$ heteroalkyl, F, ^{18}F , Cl, Br, I, -CN, $-NO_2$, $-OR^3$, $-SR^3$, $-S(=O)R^3$, $-S(=O)_2R^3$, $-NHS(=O)_2R^3$, $-C(=O)R^3$, $-OC(=O)R^3$, $-CO_2R^3$, $-OCO_2R^3$, $-CH(R^3)_2$, $-N(R^3)_2$, $-C(=O)N(R^3)_2$, $-OC(=O)N(R^3)_2$, $-NHC(=O)NH(R^3)$, $-NHC(=O)R^3$, $-NHC(=O)OR^3$, $-C(OH)(R^3)_2$, and $-C(NH_2)(R^3)_2$;

each occurrence of R^3 is independently selected from the group consisting of H and C_1-C_6 alkyl;

L is a linker; and

Q is an imaging moiety.

a salt thereof, and any combinations thereof.

26. The method of claim 25, wherein A^1 is a phenyl group, wherein the phenyl group is optionally substituted.

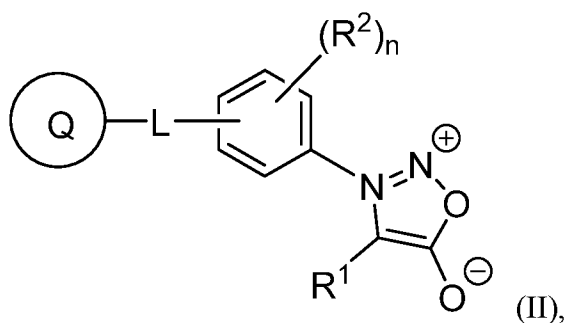
27. The method of claim 25, wherein L is selected from the group consisting of a bond, an alkyl group, an amide, a poly(alkyl ether) group, and any combinations thereof.

28. The method of claim 25, wherein Q is ^{18}F .

29. The method of claim 25, wherein Q is a fluorescent dye.

30. The method of claim 29, wherein the fluorescent dye is BODIPY630.

31. The method of claim 25, wherein the compound of formula (I) is a compound of formula (II):



wherein in formula (II):

n is an integer between 0 and 4;

R^1 and each occurrence of R^2 are each independently selected from the group consisting of H, $-C_1-C_6$ alkyl, $-C_1-C_6$ fluoroalkyl, $-C_1-C_6$ heteroalkyl, F, ^{18}F , Cl, Br, I, -CN, $-\text{NO}_2$, $-\text{OR}^3$, $-\text{SR}^3$, $-\text{S}(=\text{O})\text{R}^3$, $-\text{S}(=\text{O})_2\text{R}^3$, $-\text{NHS}(=\text{O})_2\text{R}^3$, $-\text{C}(=\text{O})\text{R}^3$, $-\text{OC}(=\text{O})\text{R}^3$, $-\text{CO}_2\text{R}^3$, $-\text{OCO}_2\text{R}^3$, $-\text{CH}(\text{R}^3)_2$, $-\text{N}(\text{R}^3)_2$, $-\text{C}(=\text{O})\text{N}(\text{R}^3)_2$, $-\text{OC}(=\text{O})\text{N}(\text{R}^3)_2$, $-\text{NHC}(=\text{O})\text{NH}(\text{R}^3)$, $-\text{NHC}(=\text{O})\text{R}^3$, $-\text{NHC}(=\text{O})\text{OR}^3$, $-\text{C}(\text{OH})(\text{R}^3)_2$, and $-\text{C}(\text{NH}_2)(\text{R}^3)_2$;

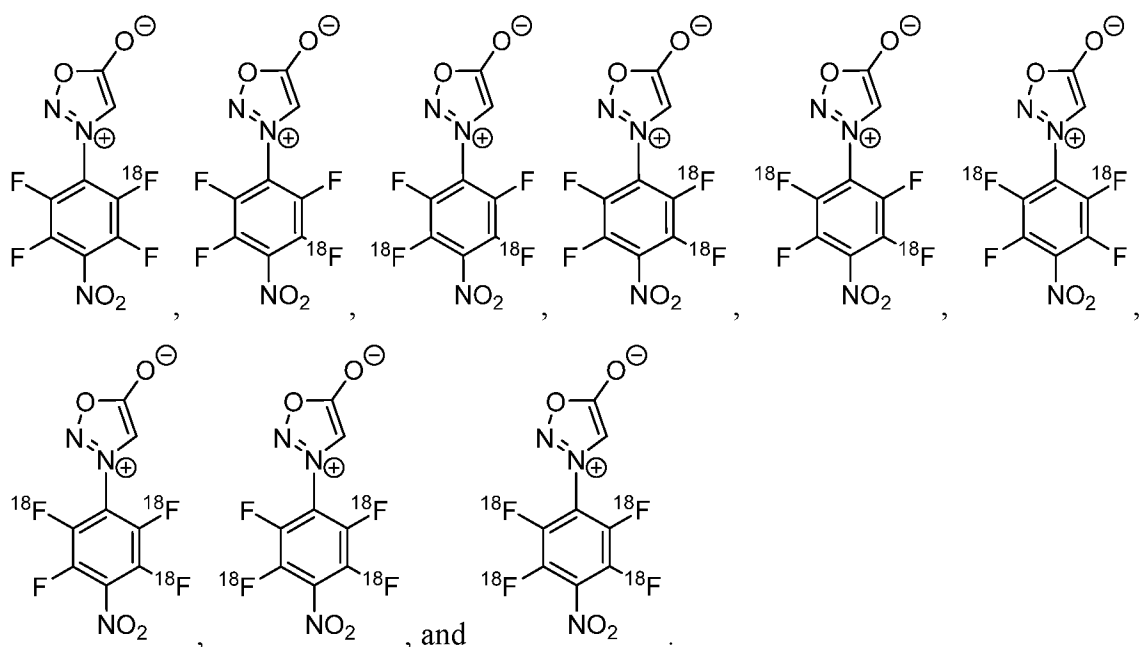
each occurrence of R^3 is independently selected from the group consisting of H and C_1-C_6 alkyl;

L is a linker; and

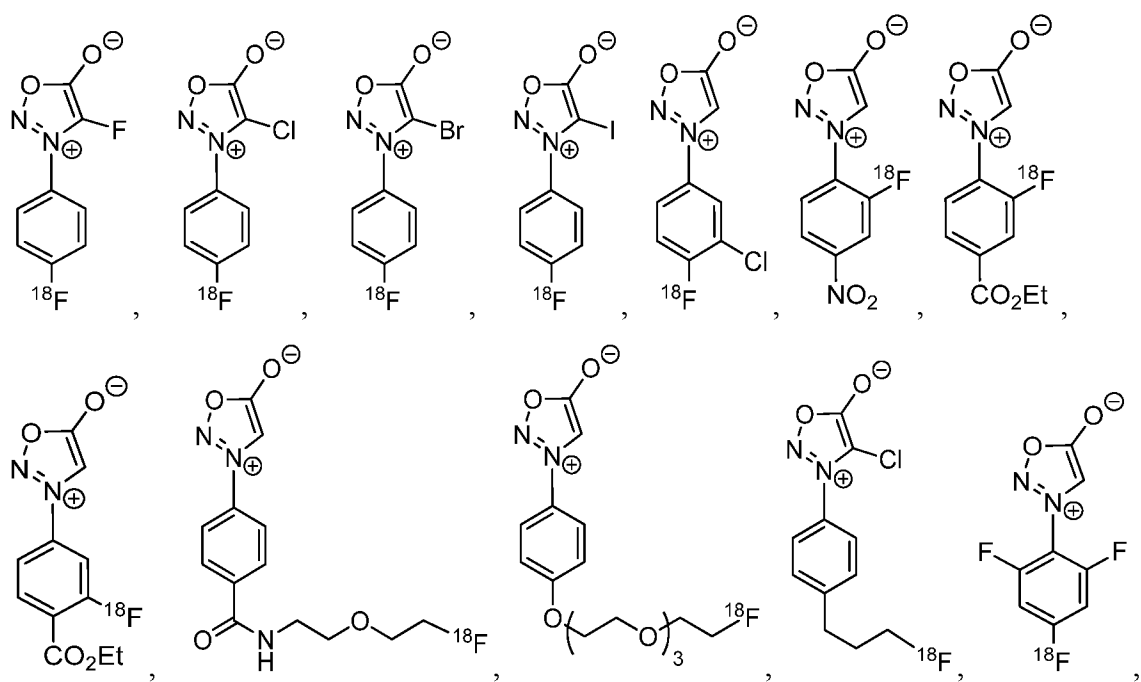
Q is an imaging moiety.

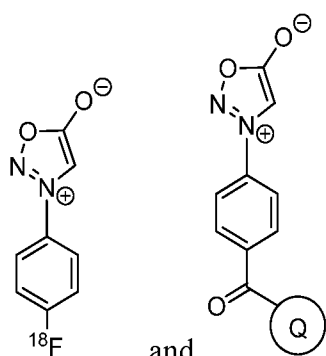
32. The method of claim 31, wherein n is at least 1 and at least one R^2 is F.

33. The method of claim 25, wherein the compound is selected from the group consisting of:



34. The method of claim 25, wherein the compound is selected from the group consisting of:





, and , wherein Q is BODIPY630, a salt thereof, and any combinations thereof.

35. The method of claim 24, wherein the dipolarophile is dibenzocyclooctyne (DBCO).

36. The method of claim 24, wherein the dipolarophile is biarylazacyclooctynone (BARAC).

37. The method of claim 24, wherein the biomolecule is imaged using positron emission tomography (PET).

38. The method of claim 24, wherein the biomolecule is imaged in vivo.

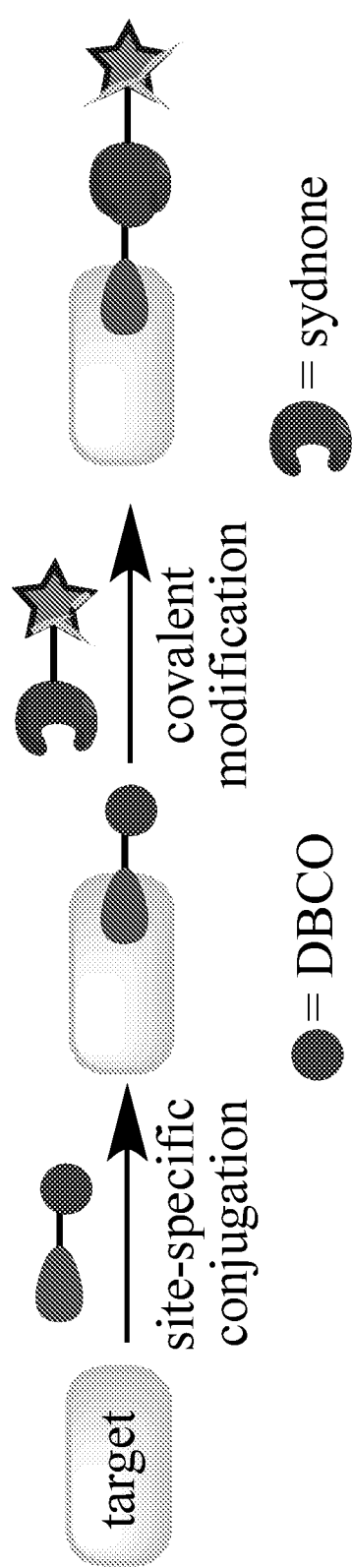


Figure 1

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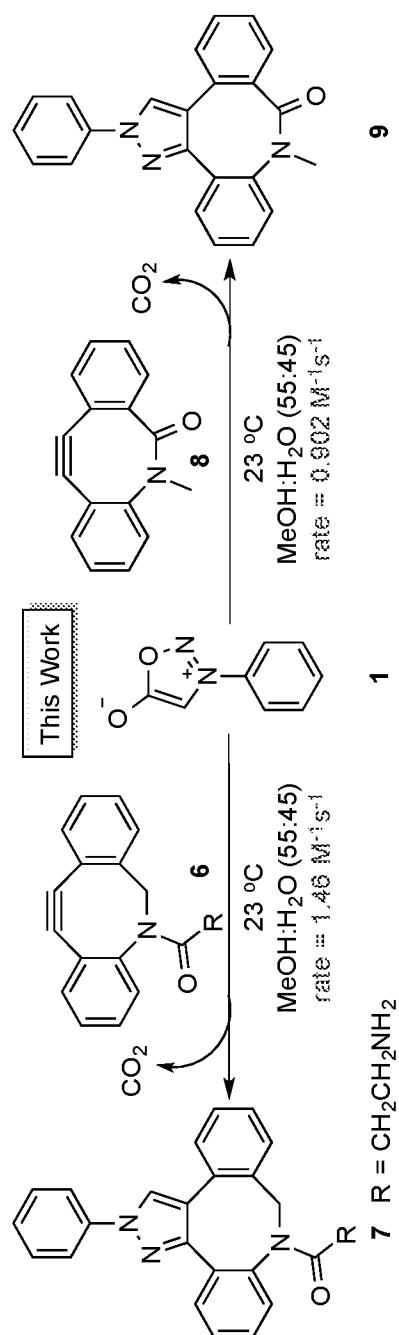
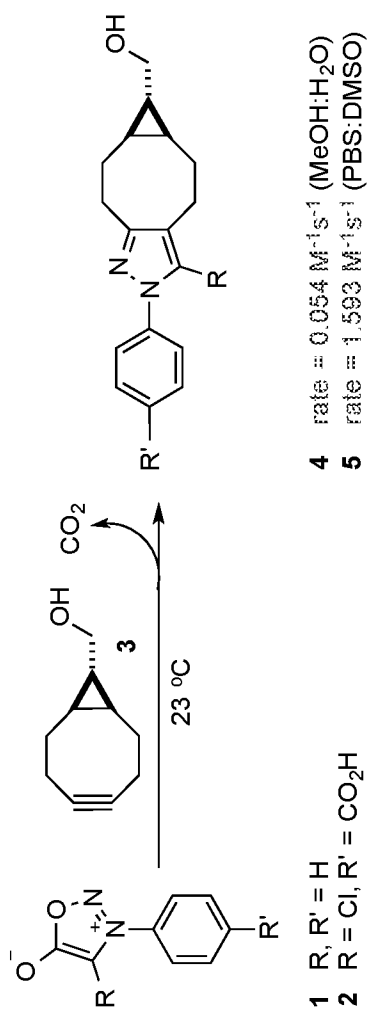


Figure 2

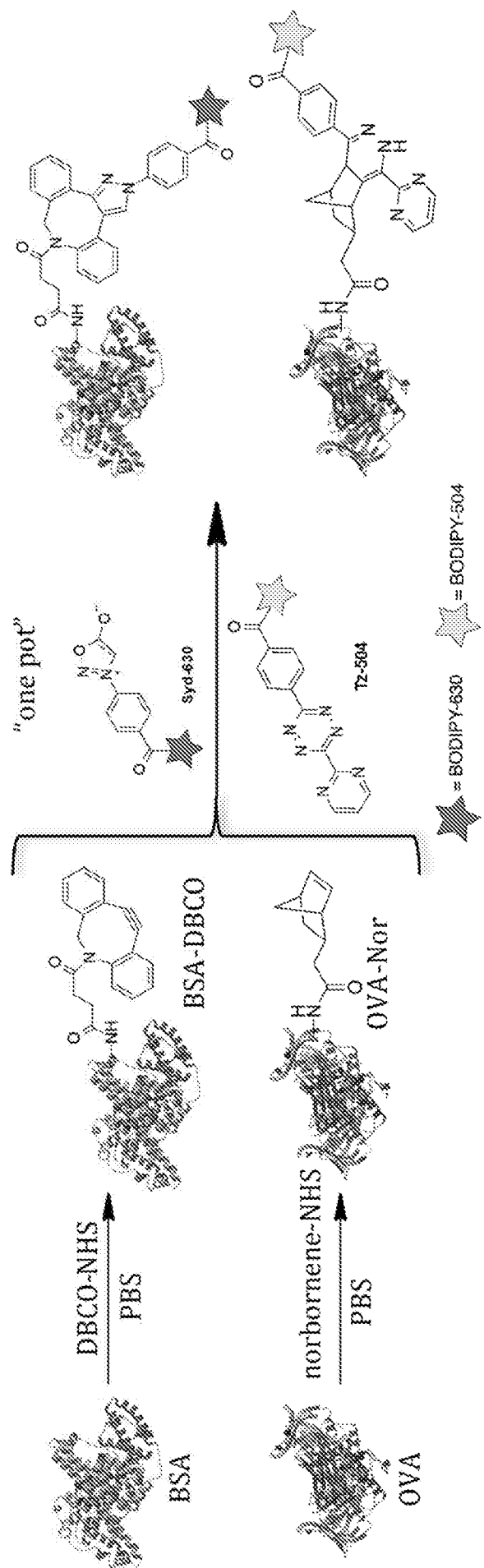


Figure 3A

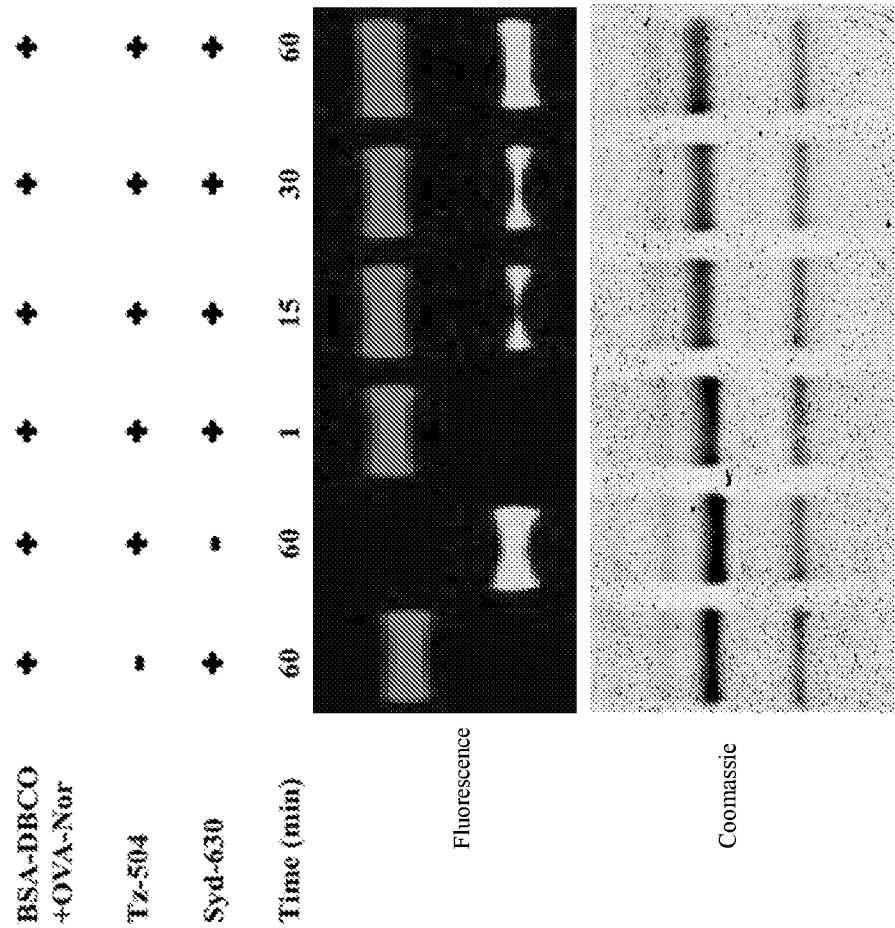


Figure 3B

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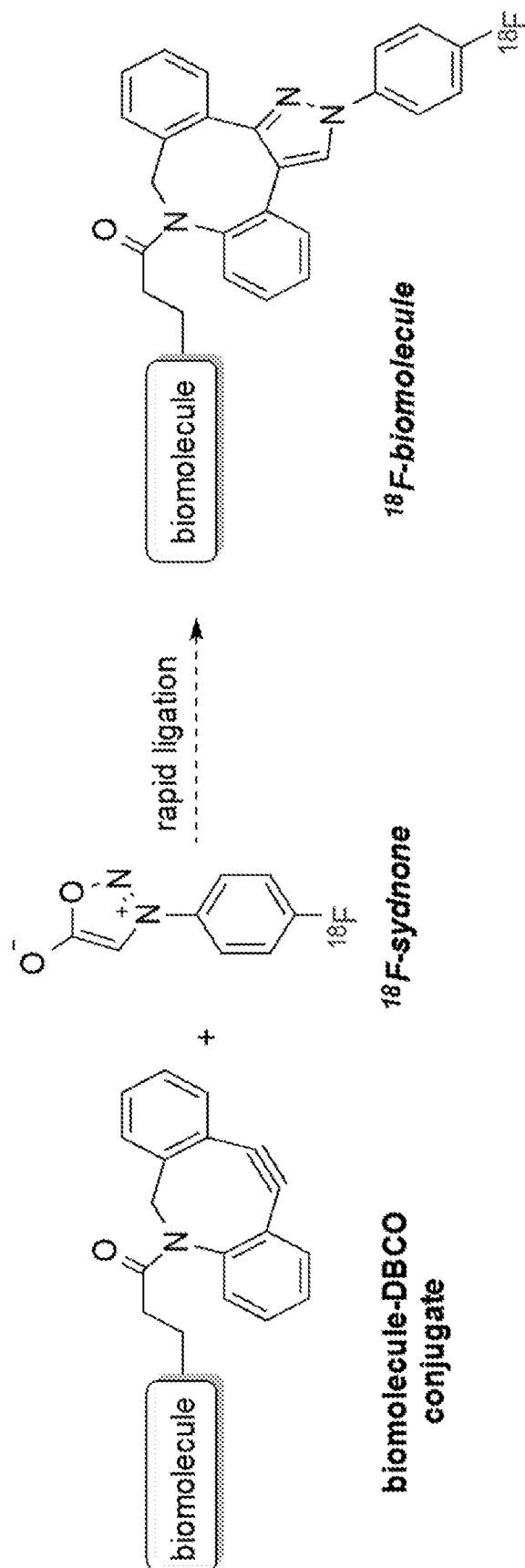


Figure 4

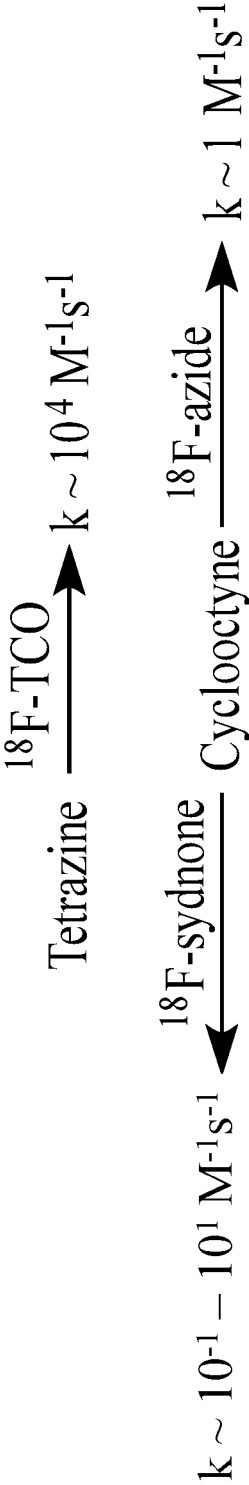
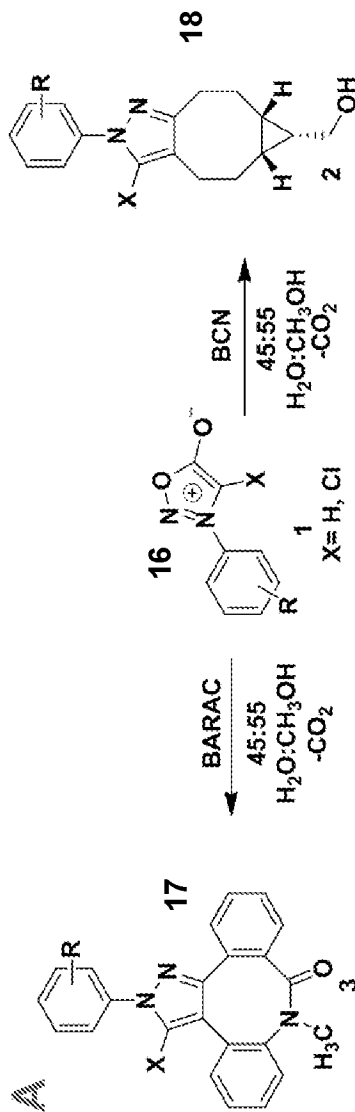


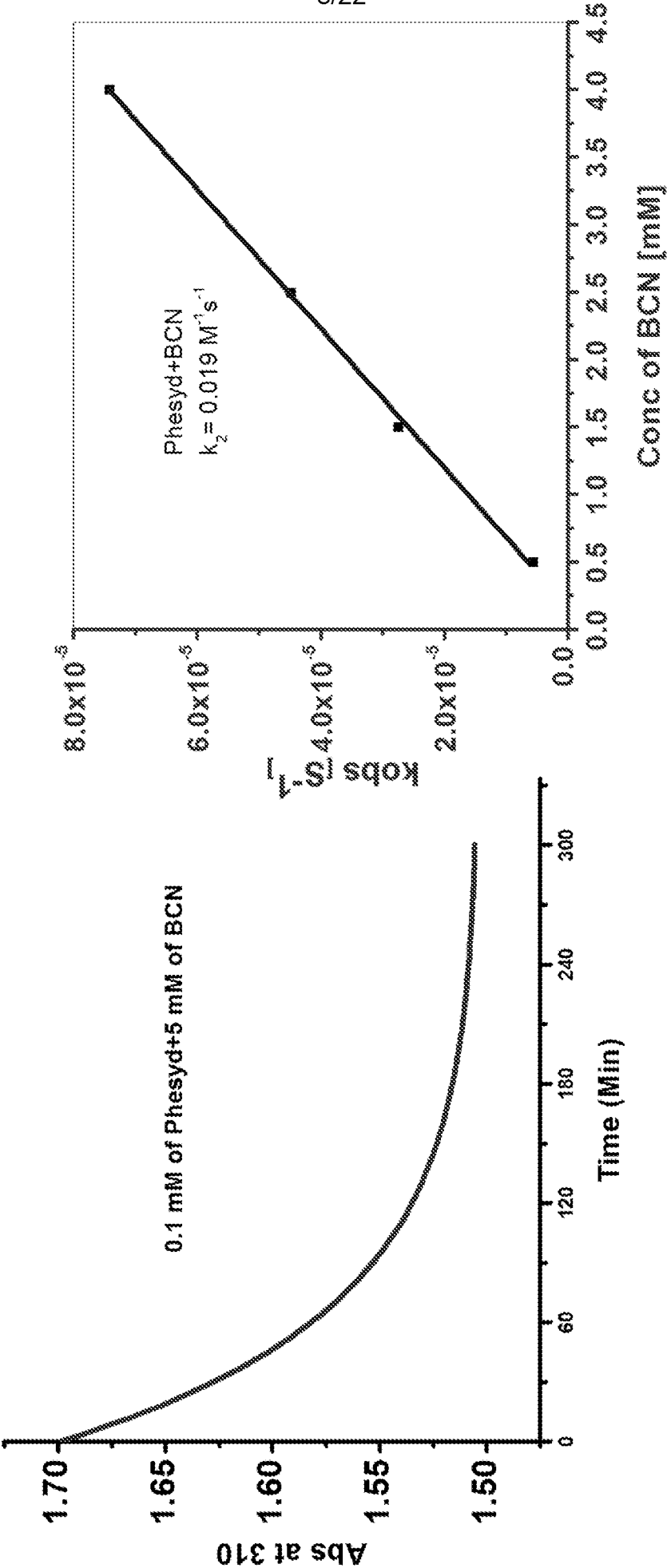
Figure 5

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Figures 6A

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Figures 6B

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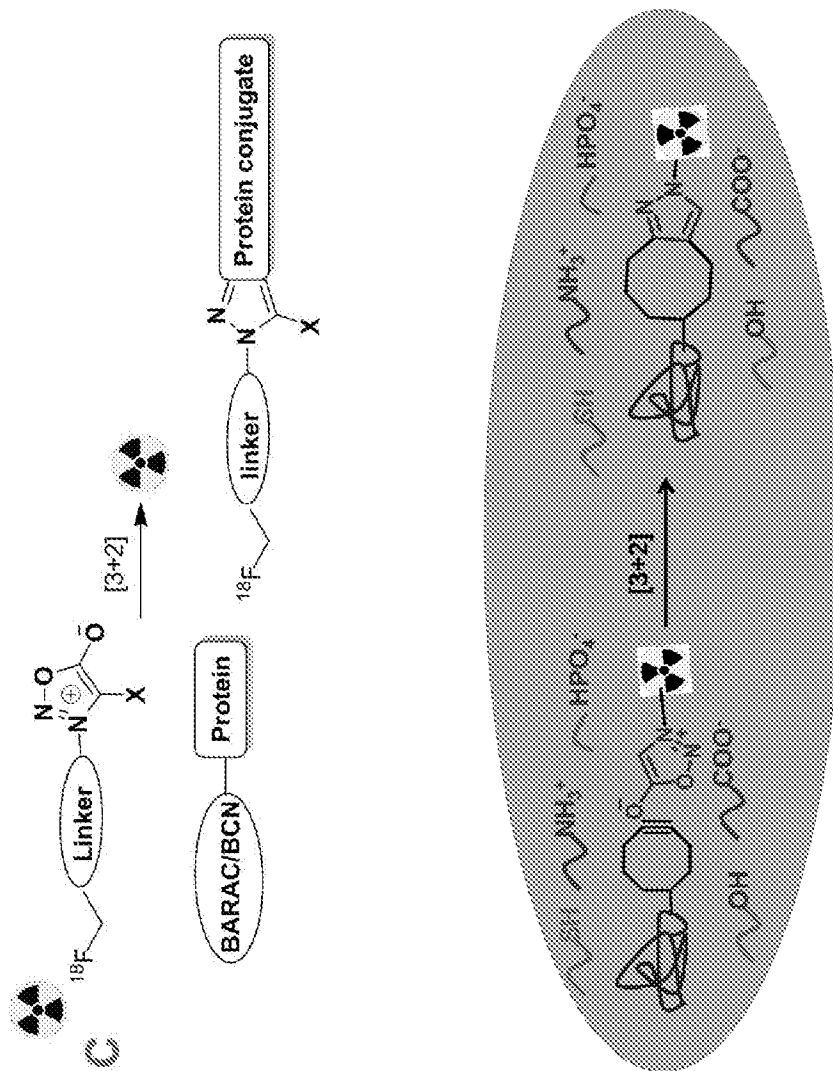


Figure 6C

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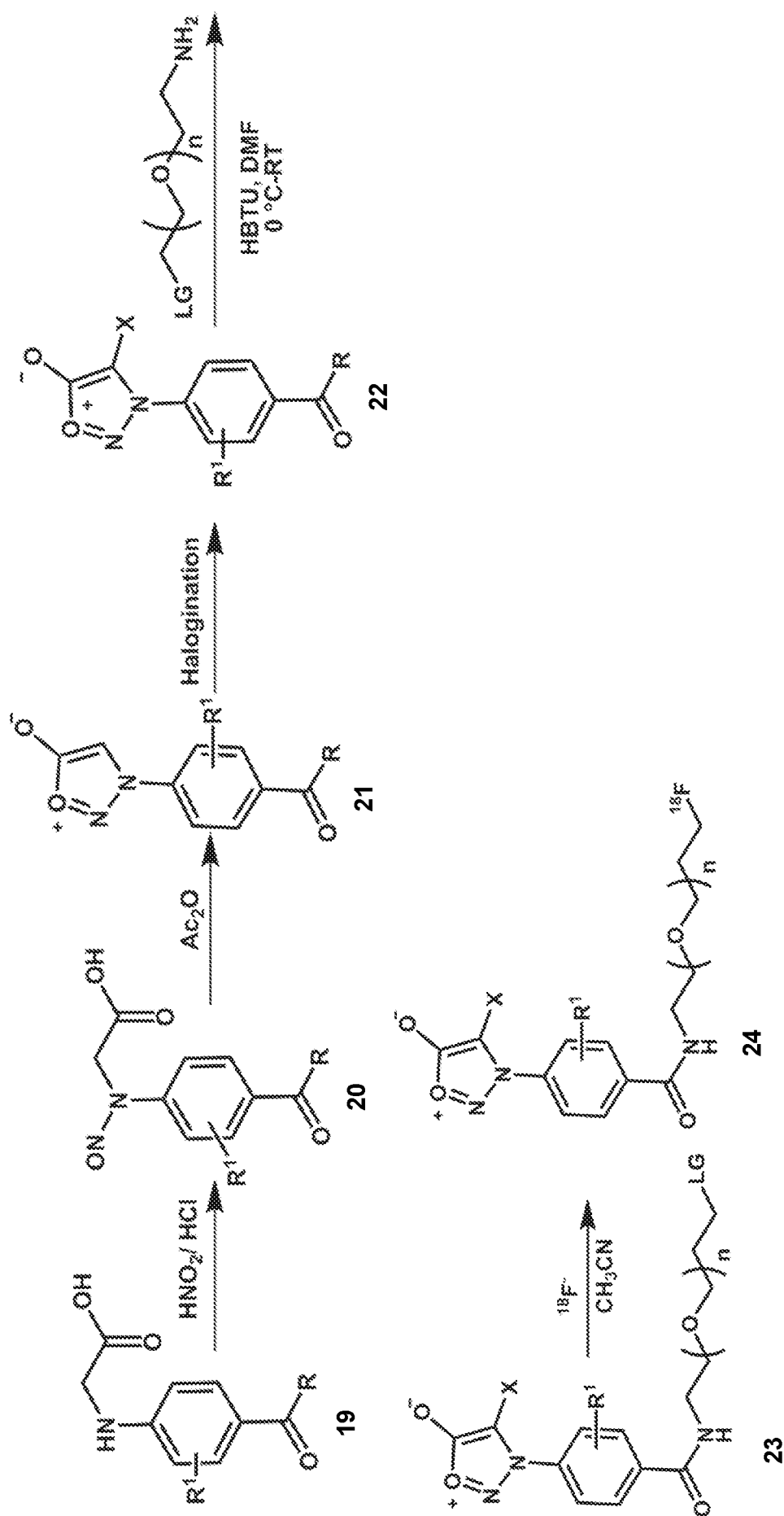


Figure 7

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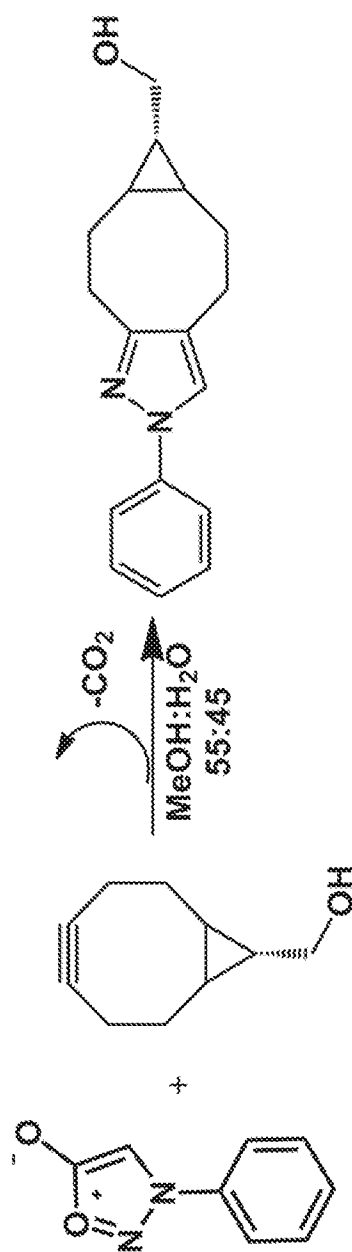


Figure 8

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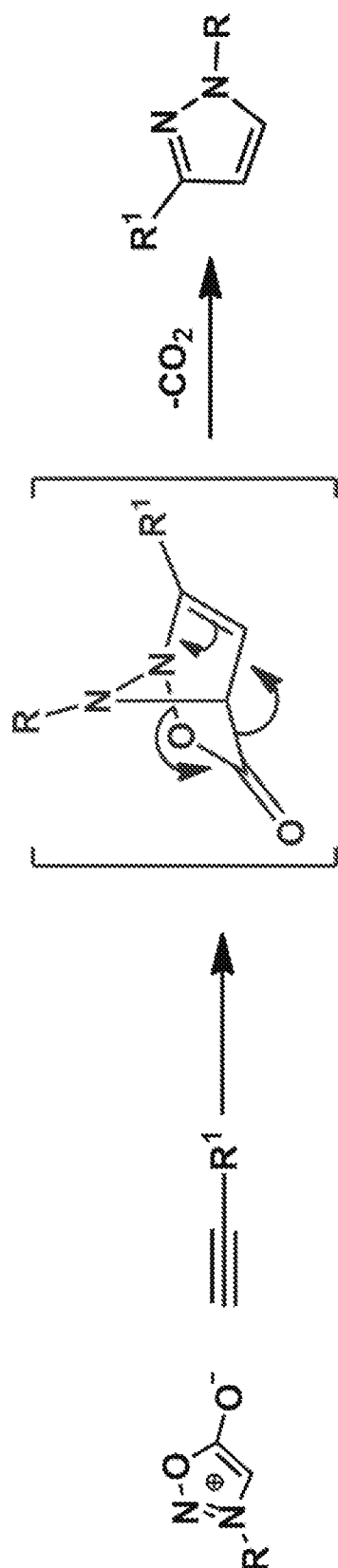


Figure 9

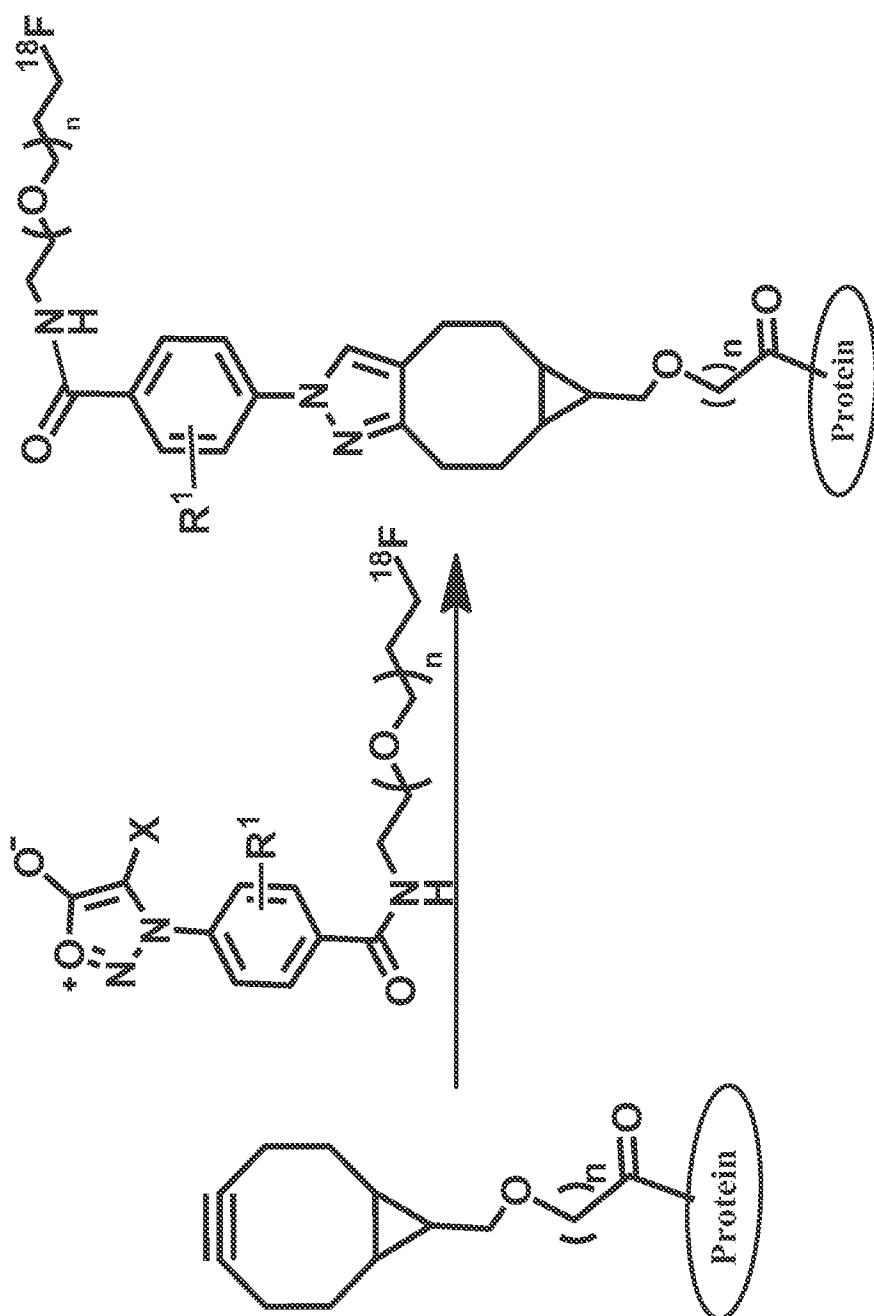


Figure 10

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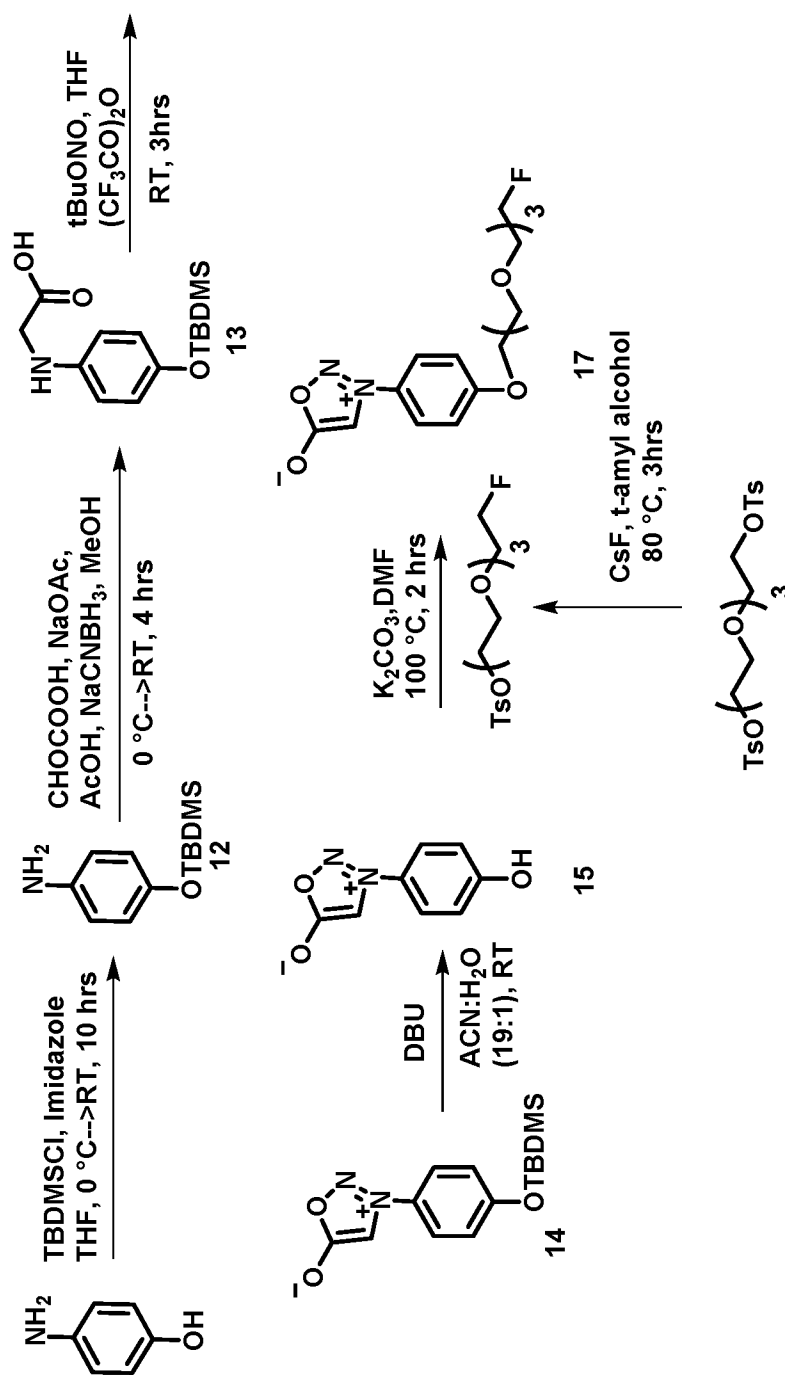


Figure 11

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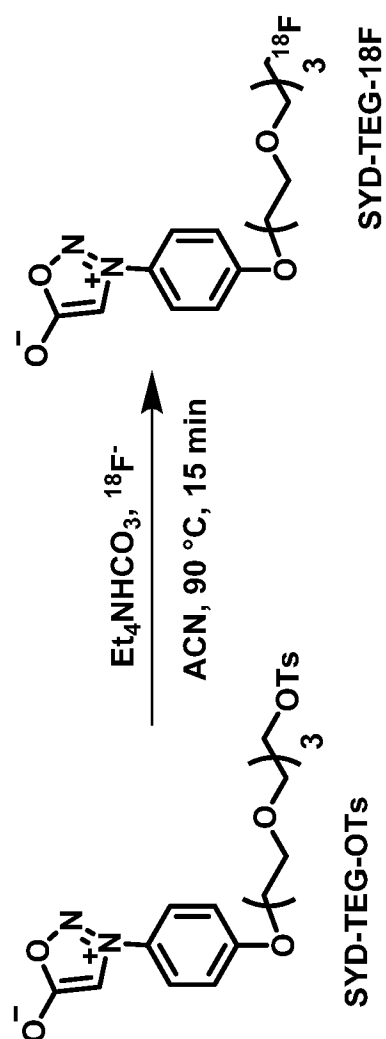


Figure 12

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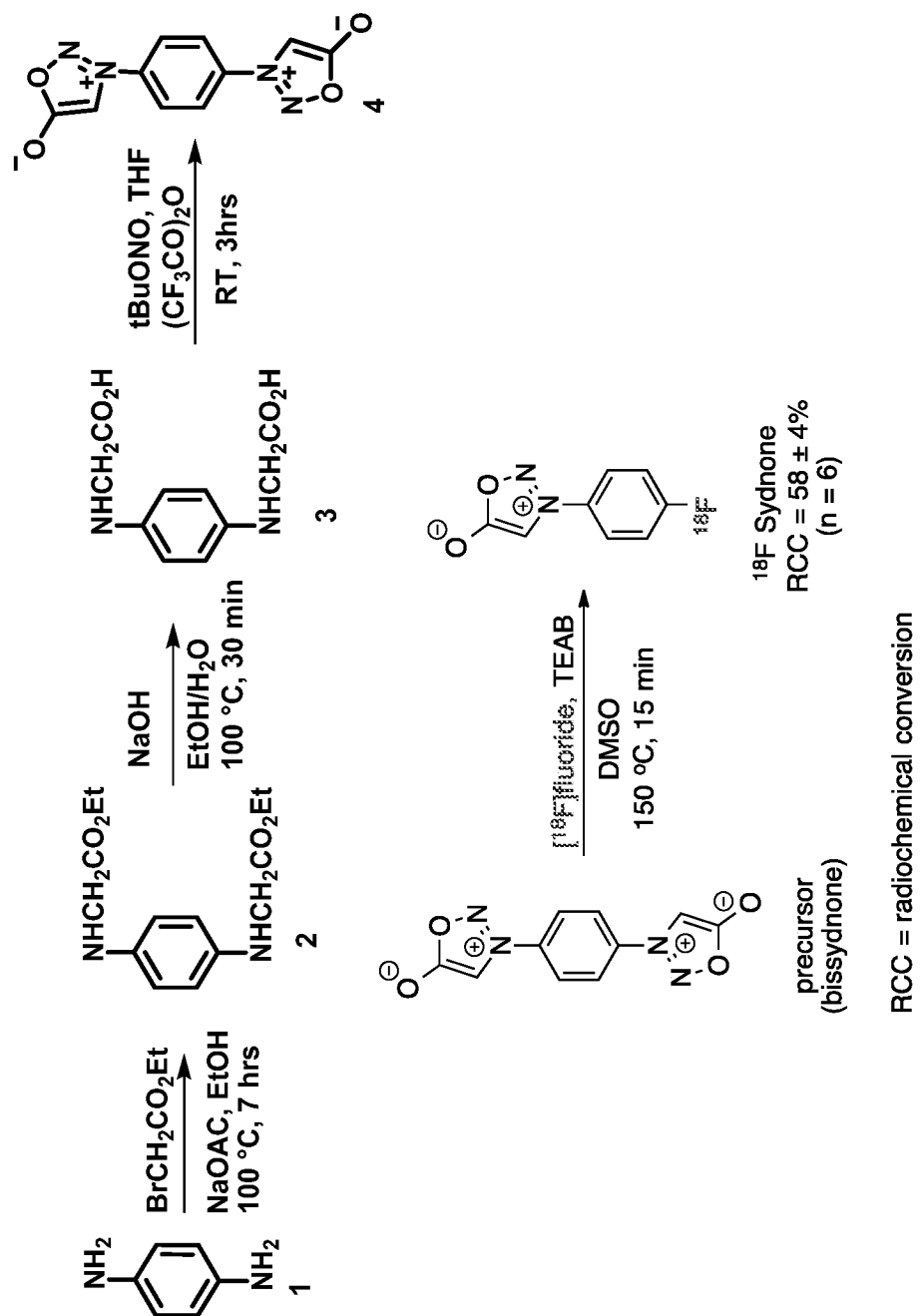


Figure 13

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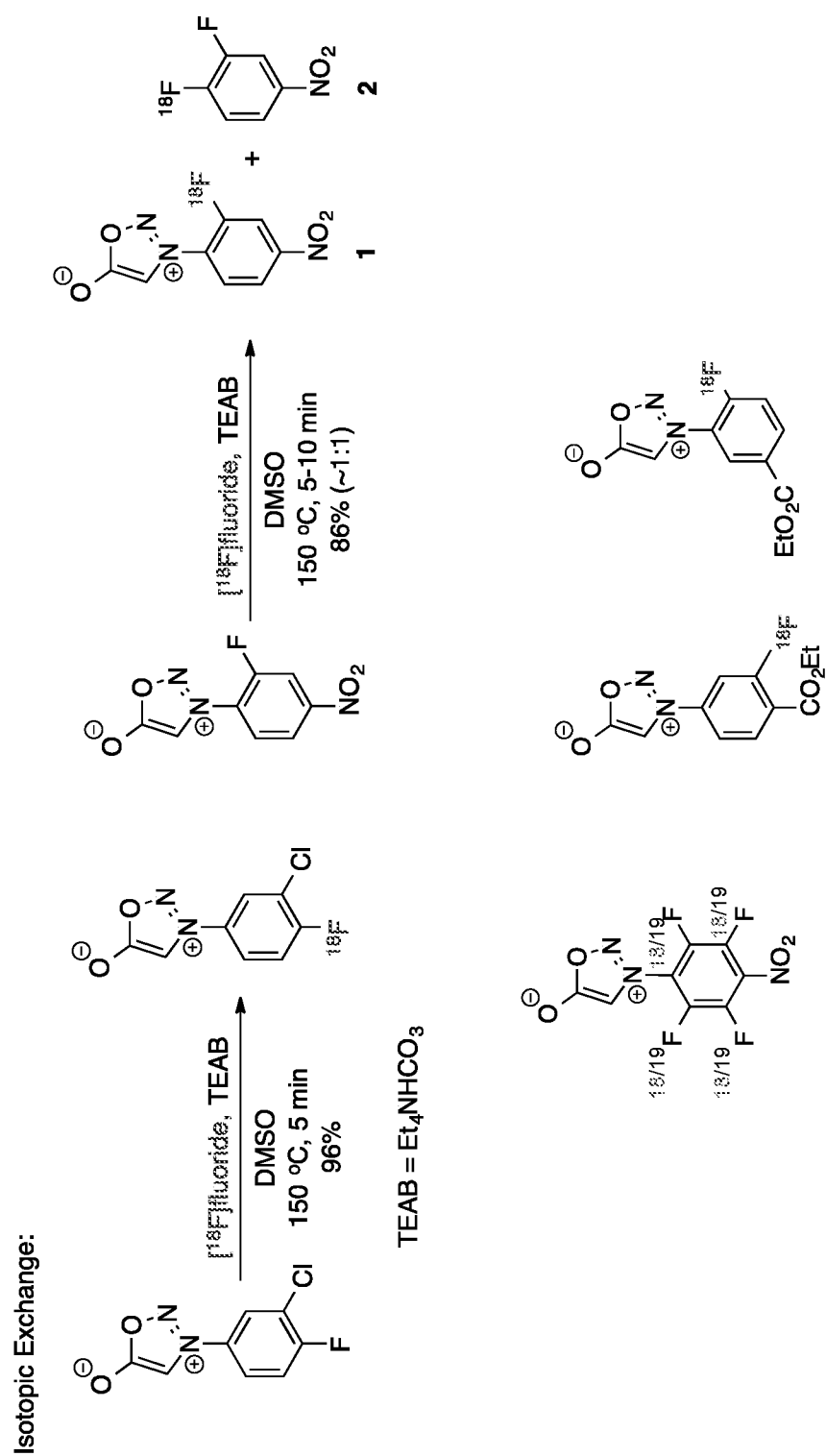


Figure 14

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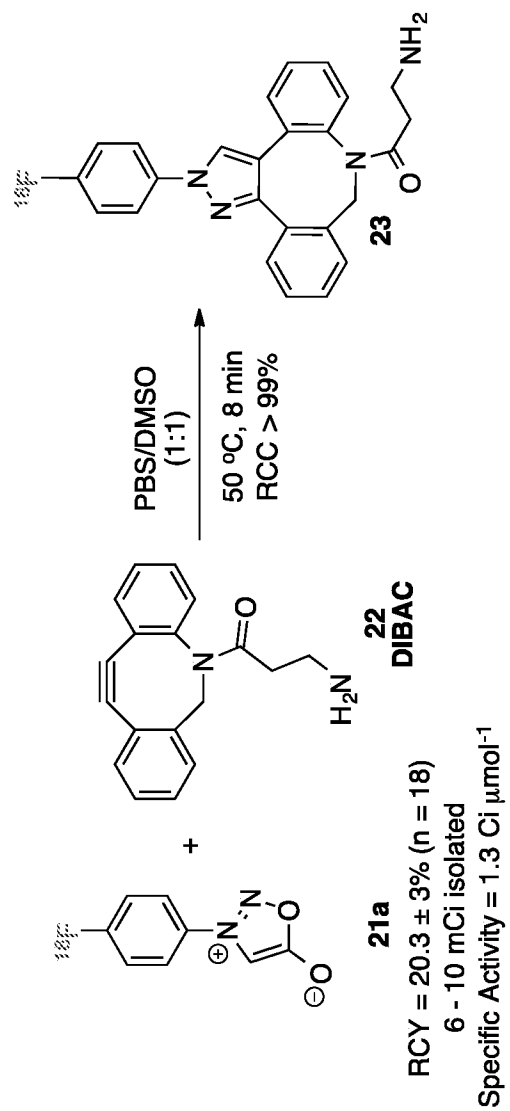


Figure 15

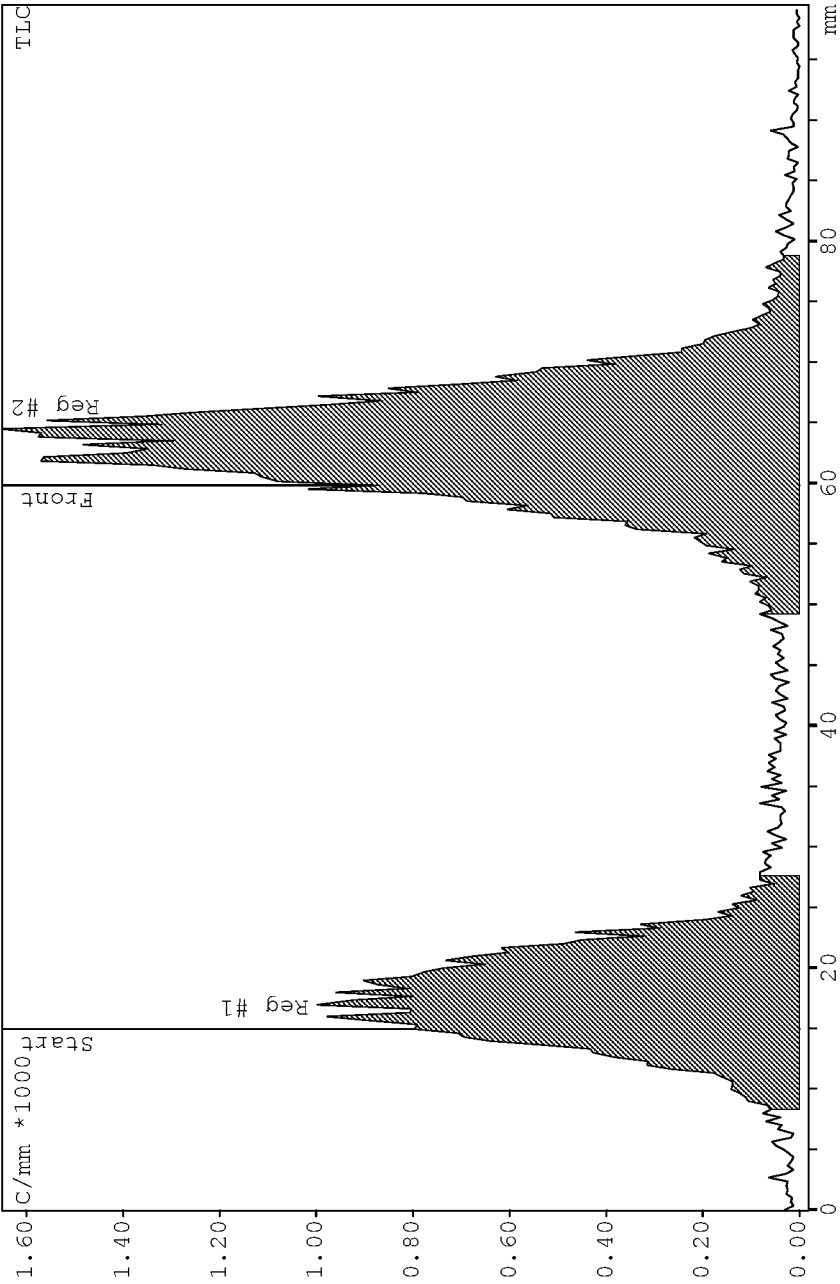


Figure 16

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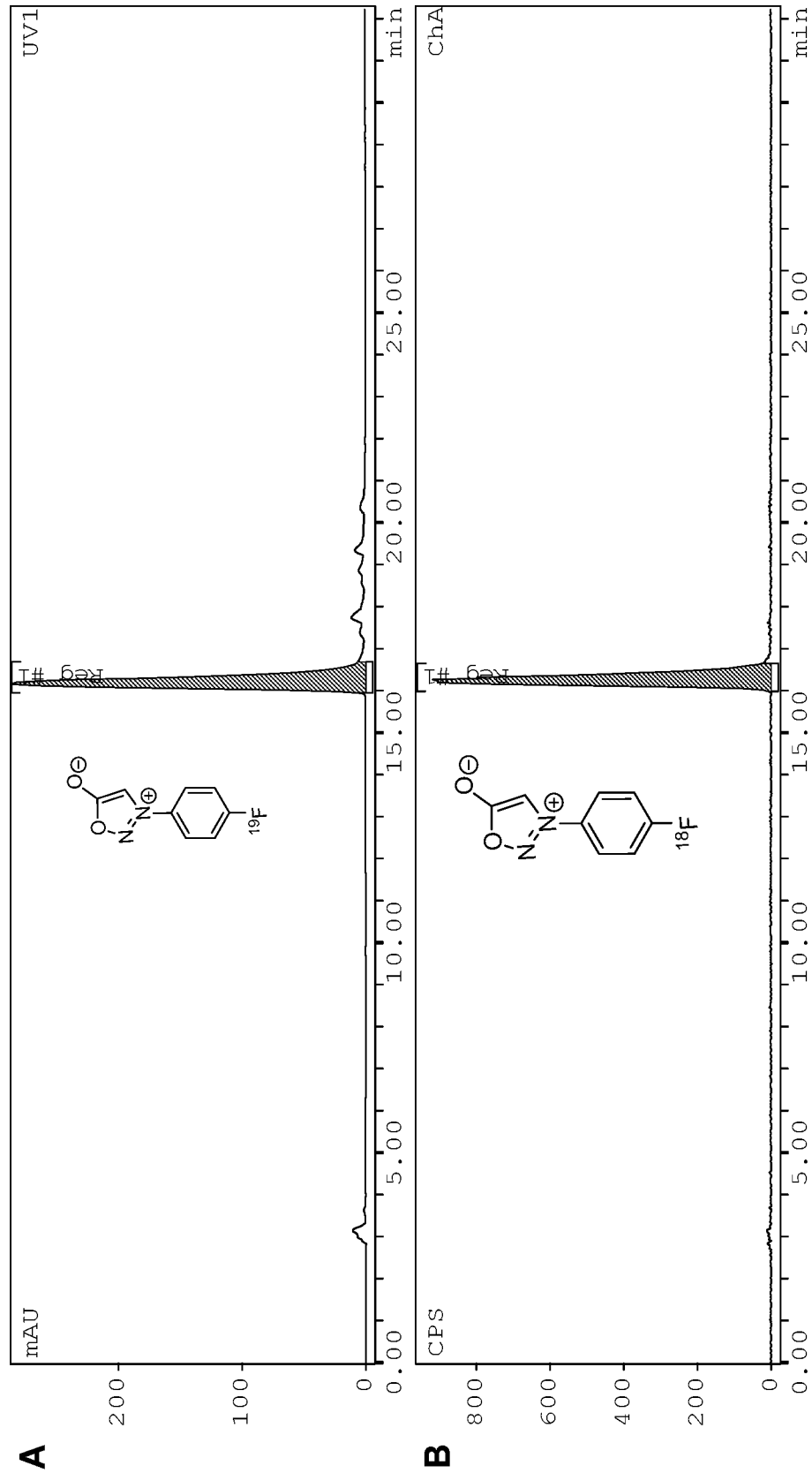


Figure 17

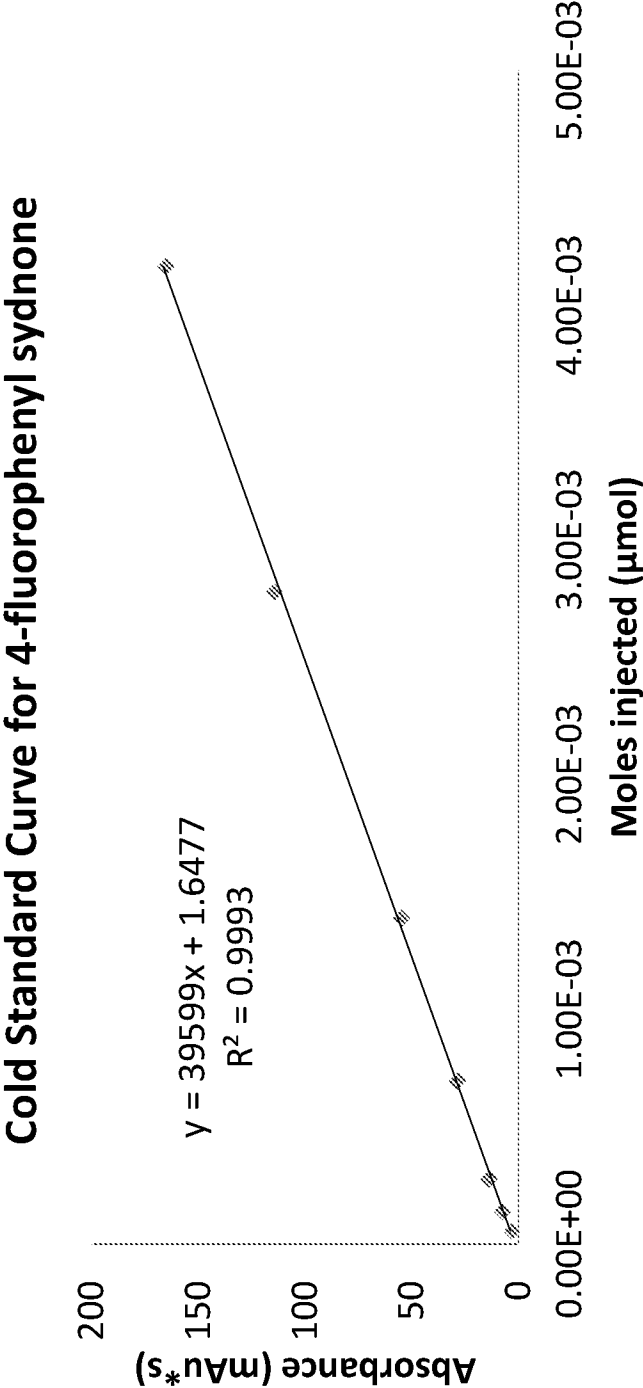


Figure 18

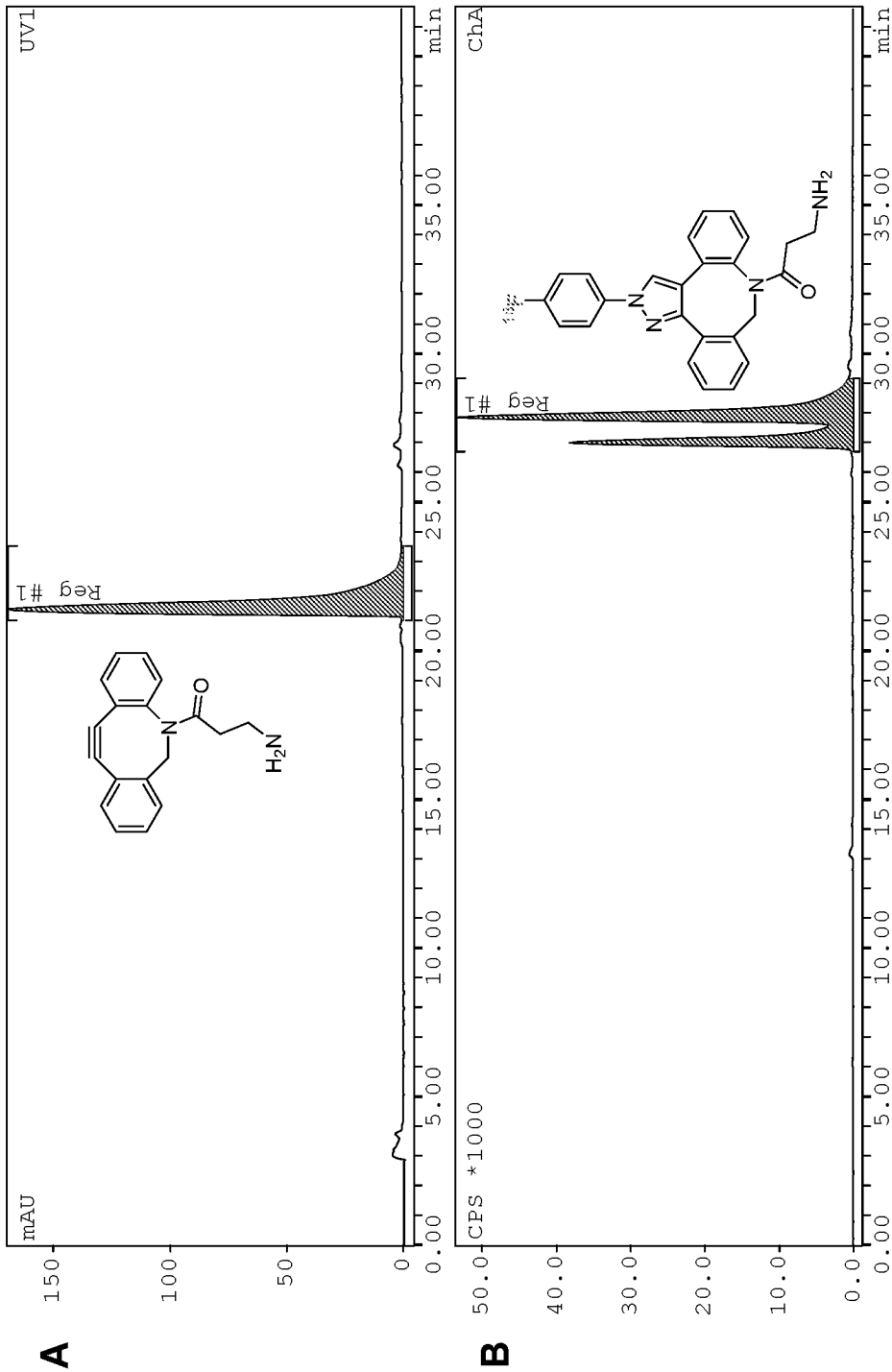


Figure 19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/029791**A. CLASSIFICATION OF SUBJECT MATTER****C07D 271/04(2006.01)i, A61K 51/04(2006.01)i**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07D 271/04; A61K 51/04

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal), STN (Registry, Caplus), Google & Keywords: sydnone, cycloaddition reaction, imaging moiety, 18F, fluorescent dye, BODIPY630**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NARAYANAM, M. K. et al., "Discovery of new mutually orthogonal bioorthogonal cycloaddition pairs through computational screening", Chemical Science, [pub] 11 November 2015, Vol. 7, pages 1257-1261 See pages 1257-1260; and figure 3(a).	1-23
X	WALLACE, S. et al., "Strain-promoted sydnone bicyclo-[6.1.0]-nonyne cycloaddition", Chemical Science, 2014, Vol. 5, pages 1742-1744 See pages 1743, 1744.	1-23
X	PLOUGASTEL, L. et al., "4-Halogeno-sydones for fast strain promoted cycloaddition with bicyclo-[6.1.0]-nonyne", Chemical Communications, 2014, Vol. 50, pages 9376-9378 See page 9377, table 1.	1-23
A	DURUST, Y. et al., "[1,3]-Dipolar cycloaddition of N-aryl sydnones to benzothiophene 1,1-dioxide, 1-cyclopropylprop-2-yn-1-ol and 1-(prop-2-ynyl)-1H-indole", Tetrahedron, 2014, Vol. 70, pages 6012-6019 See page 6013.	1-23

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

07 August 2017 (07.08.2017)

Date of mailing of the international search report

07 August 2017 (07.08.2017)

Name and mailing address of the ISA/KR

International Application Division

Korean Intellectual Property Office

189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea



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Authorized officer

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/029791**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 24-38
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 24-38 pertain to diagnostic methods in vivo and thus relate to a subject matter which this International Searching Authority is not required to search under PCT Article 17(2)(a)(i) and Rule 39.1(iv).
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/029791

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	COMAS-BARCELO, J. et al., "Cu-Promoted sydnone cycloadditions of alkynes: Scope and mechanism studies", Chemistry: A European Journal, 2015, Vol. 21, pages 3257-3263 See page 3260.	1-23

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2017/029791Patent document
cited in search reportPublication
datePatent family
member(s)Publication
date

None