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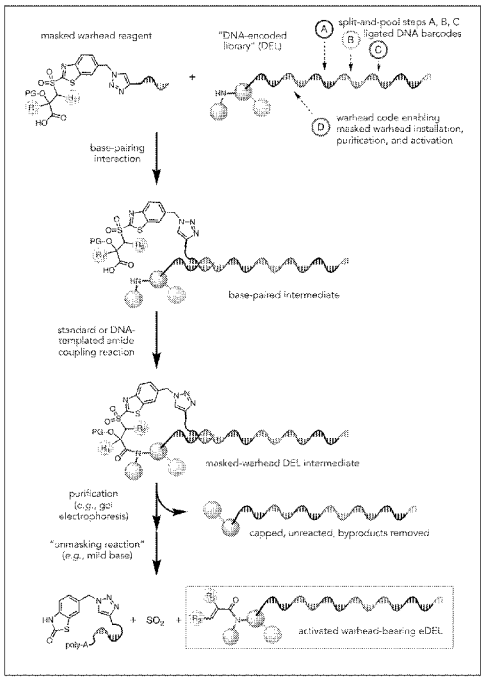
(54) Title: DNA-ENCODED AND AFFINITY-TAGGED MASKED-WARHEAD COMPOUNDS AND USE THEREOF IN ASSEMBLING LIBRARIES OF SMALL MOLECULES ENABLED FOR LATE-STAGE PURIFICATION AND MULTIPLEXED SCREENING OF COVALENT LIGANDS

FIG. 1C



(57) Abstract: The present disclosure relates to DNA-encoded and affinity-tagged masked warhead installation compounds, including substituted and unsubstituted acrylamide warheads, which can be purified from unreacted library intermediates and byproducts. The warhead compounds are used in the synthesis/assembly of electrophilic warhead DNA-Encoded Libraries (eDEL), enabled for late-stage purification and multiplexed screening of covalent ligands.

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DNA-ENCODED AND AFFINITY-TAGGED MASKED-WARHEAD COMPOUNDS AND USE THEREOF IN ASSEMBLING LIBRARIES OF SMALL MOLECULES ENABLED FOR LATE-STAGE PURIFICATION AND MULTIPLEXED SCREENING OF COVALENT LIGANDS

RELATED APPLICATIONS

[1] This application claims the benefit of priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No: 63/272,317, filed October 27, 2021, and U.S. Provisional Application No: 63/323,825, filed March 25, 2022, each of which is incorporated herein by reference in its entirety.

BACKGROUND

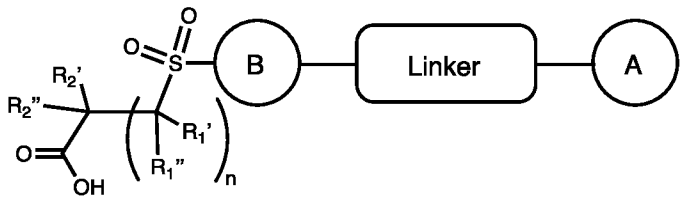
[2] Covalent mechanisms have the potential to massively expand the fraction of the proteome targeted by small-molecules, and by consequence expand the scope of biomedical problems that can be intervened. However, only a small fraction of all known and unknown pockets having reactive residues has ever been explored with covalent ligands. Across academia and the pharmaceutical sector, there is a scarcity of structurally diverse screening collections having appropriately validated warheads, and a lack of technologies to identify covalently targetable pockets in allosteric and non-catalytic domains.

[3] The desirable balance of k_{inact} / K_i parameters of highly selective covalent inhibitors that make them valuable commodities also obfuscates their discovery using conventional screening infrastructure and small-molecule collections devised for reversible ligands including DNA-encoded libraries (DEL), High-Throughput Screening (HTS), and Fragment-Based Screening (FBS) (Goodnow *et al.*, Nat. Rev. Drug Discov. *16(2)*:131–147 (2017); Volochnyuk *et al.*, Drug Discov. Today *24(2)*:390–402 (2019); Lu *et al.*, RSC Chem. Biol. *2(2)*:354–367 (2021); Parker *et al.*, Cell *168(3)*:27-41.e29 (2017)). Complementary methods such as activity-based proteomics can rank-order the reactivity of protein residues using warhead scouting ligands, but pose significant hurdles for screening collections larger than a few thousand compounds (Hacker *et al.*, Nat. Chem. *9(12)*:1181–1190 (2017); Kuljanin et al., Nat. Biotechnol. *39(5)*:630–641 (2021)). Implementing warhead reagents (*e.g.*, reactive olefins, acryloyl halides or equivalent synthons) on established DEL collections has been hindered by two levels of incompatibility: first, at the level of the combinatorial chemistry routes optimized for the

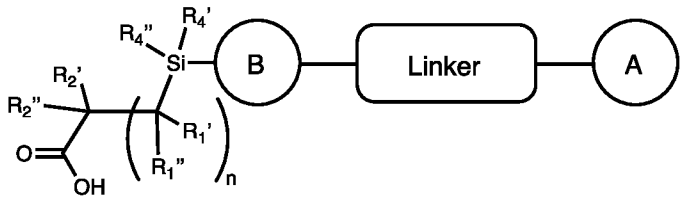
presence of DNA and water; and secondly, at the level of the *in vitro* selection workflows (*e.g.*, one-round enrichment and background noise) (Guilinger *et al.*, Bioorg. Med. Chem. *42*:116223 (2021); Zimmermann *et al.*, Chem.- Eur. J. *23(34)*: 8152–8155 (2017); Zambaldo *et al.*, MedChemComm *7(7)*:1340–1351 (2016); Kuai *et al.*, SLAS Discov. Adv. Sci. Drug Discov. *23(5)*:405–416 (2018); Zhu *et al.*, SLAS Discov. Adv. Sci. Drug Discov. *24(2)*:169–174 (2019); Cochrane *et al.*, ACS Comb. Sci. *21(5)*:425–435 (2019)). An additional hurdle previously observed when implementing warheads is that conventional DEL preparations generate inseparable mixtures (Clark *et al.*, *Nat. Chem. Biol.* *5*(9):647–654 (2009); Shi *et al.*, *RSC Adv.* *11*(4):2359–2376 (2021)). Lack of purity can confound hit identification and screening outcomes because identical DNA barcodes are connected to unreacted intermediates and byproducts (Zambaldo *et al.*, MedChemComm *7(7)*:1340–1351 (2016); Kuai *et al.*, SLAS Discov. Adv. Sci. Drug Discov. *23(5)*:405–416 (2018); Zhu *et al.*, SLAS Discov. Adv. Sci. Drug Discov. *24(2)*:169–174 (2019)).

SUMMARY

[4] A first aspect of the present disclosure is directed to compounds represented by formula (I) or (II), or a pharmaceutical salt or stereoisomer thereof:



(I) or



(II), wherein

(A), (Linker), (B), R_1', R_1'', R_2', R_2'', R_4', R_4'' and n are defined herein.

[5] In another aspect of the present disclosure, methods of making the compounds are provided.

[6] Another aspect of the present disclosure is directed to methods of creating an electrophilic warhead-bearing DNA-Encoded Library (eDEL) comprising:

coupling the compound of formula I or II with a DNA-Encoded Library (DEL) to

generate a stable masked-warhead bearing DEL intermediate library (mwDEL);

purifying the mwDEL intermediate library from unreacted and byproduct entities;

unmasking the mwDEL intermediate to generate a warhead-bearing eDEL; and

purifying the warhead-bearing eDEL.

[7] In some embodiments, the stable masked-warhead bearing mwDEL intermediate comprises an arylsulfone comprising  .

[8] Another aspect of the present disclosure is directed to an eDEL, which is generated from the methods described herein.

[9] In some embodiments, the eDEL is used in an in vitro selection assay followed by DNA sequencing. In some embodiments, the in vitro selection assay is used for screening protein ligands. In some embodiments, the eDEL is used for screening ligands that covalently modify a residue of a protein (*e.g.*, the thiol group of Cysteine, the amino group of Lysine, the imidazole group of Histidine, the hydroxyl groups of Serine, Threonine or Tyrosine, *etc.*).

[10] Disclosed are masked-warhead installation reagents that, upon unmasking afford acrylamide-class warheads attached to DEL (FIG. 1A-FIG. 1B), which altogether solve the myriad incompatibilities that have hindered the assembly, purification and screening of warhead-bearing DELs and covalent ligands in the past (FIG. 2A). The masked-warhead reagents can be coupled directly on DELs featuring traditional nucleophilic functional groups (*e.g.* primary or secondary amines, anilines, thiols, hydroxyls, phenols, *etc.*). The present disclosure can be implemented to solve the scarcity of warhead-bearing small molecule collections, resulting in structurally diverse libraries amenable for covalent ligand screening comprising both therapeutically validated and unexplored warhead classes (Goodnow *et al.*, Nat. Rev. Drug Discov. *16(2)*:131–147 (2017)).

[11] The presently described methodologies and resultant compositions/products are useful for solving the synthetic and the purification challenges associated with the incompatible circumstances of implementing reactive warheads (*e.g.*, acrylamides) in the context of DNA-encoded combinatorial chemistry (Shi *et al.*, RSC Adv. *11*(4):2359–2376 (2021)), which is a process requiring compatibility with split-and-pooled mixed library formats, in the presence of water and DNA, all the while promoting efficient conversion of warhead attachment onto the vastly different nucleophilic groups typically encountered in a DEL library (e.g., anilines, sterically hindered amines, hydroxyls, heterocyclic nitrogens, etc.) (Clark *et al.*, Nat. Chem. Biol. *5*(9):647–654 (2009)). The masked-warhead reagents and methods of the present

disclosure are useful for installing the described acrylamide-type warheads on a panoply of small-molecule classes that can be generated by combinatorial chemistry with DNA barcodes (Shi *et al.*, RSC Adv. *11*(4):2359–2376 (2021)).

BRIEF DESCRIPTION OF THE DRAWINGS

[12] FIG. 1A illustrates highly desirable acrylamide-class warheads that are challenging to implement on DNA-Encoded Libraries of small molecules.

[13] FIG. 1B illustrates the thought process behind the disclosure of the arylsulfone class of warhead-masking reagents, compared to other reactions that are deemed problematic and/or incompatible to implement warheads on DNA-Encoded Libraries of small molecules.

[14] FIG. 1C is an illustrative example of masked warhead reagents implemented on a generic secondary-amine DNA-encoded library (DEL) synthesized by 3 rounds of split-and-pool combinatorial synthesis (building blocks depicted as blue, green, and red spheres), followed by base pairing and DNA-templated amide bond formation with the masked warhead reagent. This amide coupling generates a stable masked-warhead DEL intermediate (mwDEL) that can be purified from capped-, unreacted-, and byproduct-DEL entities. Finally, an unmasking reaction (*e.g.*, with base or alkaline buffer) liberates sulfur dioxide and removes the arylsulfone to afford the activated warhead-bearing eDEL.

[15] FIG. 2A shows the challenges associated with the implementation of acrylamide-class warheads on DNA-encoded libraries in the presence of DNA and water.

[16] FIG. 2B shows masked warhead reagents offering versatility for the introduction of steric and reactivity-tuning substituents on the arylsulfone intermediate and the unmasked warhead of the eDEL.

[17] FIG. 2C shows masked warhead reagents offering versatility for the use of many classes of arylsulfone moieties, including benzothiazole sulfones, pyridine sulfones, pyrimidine sulfones, alkyl- and phenyl-tetrazole sulfones, *etc*.

DETAILED DESCRIPTION

[18] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in art to which the subject matter herein belongs. As used in the specification and the appended claims, unless specified to the contrary, the following terms have the meaning indicated in order to facilitate the understanding of the

present disclosure.

[19] As used in the description and the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a composition" includes mixtures of two or more such compositions, reference to "an inhibitor" includes mixtures of two or more such inhibitors, and the like.

[20] Unless stated otherwise, the term "about" means within 10% (*e.g.*, within 5%, 2% or 1%) of the particular value modified by the term "about."

[21] The transitional term "comprising," which is synonymous with "including," "containing," or "characterized by," is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. By contrast, the transitional phrase "consisting of" excludes any element, step, or ingredient not specified in the claim. The transitional phrase "consisting essentially of" limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention.

[22] With respect to the disclosed compounds, and to the extent the following terms are used herein to further describe them, the following definitions apply.

[23] As used herein, the term "alkyl" refers to a saturated linear or branched-chain monovalent hydrocarbon radical. In one embodiment, the alkyl radical is a C_1-C_{18} group. In other embodiments, the alkyl radical is a C_0 -C_6, C_0-C_5, C_0-C_3, C_1-C_{12}, C_1-C_8, C_1-C_6, C_1-C_5, C_1-C_4 or C_1-C_3 group (wherein C_0 alkyl refers to a bond). Examples of alkyl groups include methyl, ethyl, 1-propyl, 2-propyl, i-propyl, 1-butyl, 2-methyl-1-propyl, 2-butyl, 2-methyl-2-propyl, 1-pentyl, n-pentyl, 2-pentyl, 3-pentyl, 2-methyl-2-butyl, 3-methyl-2-butyl, 3-methyl-1-butyl, 2-methyl-1-butyl, 1-hexyl, 2-hexyl, 3-hexyl, 2-methyl-2-pentyl, 3-methyl-2-pentyl, 4-methyl-2-pentyl, 3-methyl-3-pentyl, 2-methyl-3-pentyl, 2,3-dimethyl-2-butyl, 3,3-dimethyl-2-butyl, heptyl, octyl, nonyl, decyl, undecyl and dodecyl. In some embodiments, an alkyl group is a C_1-C_3 alkyl group.

[24] As used herein, the term "alkylene" refers to a straight or branched divalent hydrocarbon chain linking the rest of the molecule to a radical group, consisting solely of carbon and hydrogen, containing no unsaturation and having from one to 12 carbon atoms, for example, methylene, ethylene, propylene, n-butylene, and the like. The alkylene chain may be attached to the rest of the molecule through a single bond and to the radical group through a single bond. In some embodiments, the alkylene group contains one to 8 carbon atoms (C_1-C_8

alkylene). In other embodiments, an alkylene group contains one to 5 carbon atoms (C_1-C_5 alkylene). In other embodiments, an alkylene group contains one to 4 carbon atoms (C_1-C_4 alkylene). In other embodiments, an alkylene contains one to three carbon atoms (C_1-C_3 alkylene). In other embodiments, an alkylene group contains one to two carbon atoms (C_1-C_2 alkylene). In other embodiments, an alkylene group contains one carbon atom (C_1 alkylene).

[25] As used herein, the term "alkenyl" refers to a linear or branched-chain monovalent hydrocarbon radical with at least one carbon-carbon double bond. An alkenyl includes radicals having "cis" and "trans" orientations, or alternatively, "E" and "Z" orientations. In one example, the alkenyl radical is a C_2-C_{18} group. In other embodiments, the alkenyl radical is a C_2-C_{12}, C_2-C_{10}, C_2-C_8, C_2-C_6 or C_2-C_3 group. Examples include ethenyl or vinyl, prop-1-enyl, prop-2-enyl, 2-methylprop-1-enyl, but-1-enyl, but-2-enyl, but-3-enyl, buta-1,3-dienyl, 2-methylbuta-1,3-diene, hex-1-enyl, hex-2-enyl, hex-3-enyl, hex-4-enyl and hexa-1,3-dienyl.

[26] The terms "alkoxyl" or "alkoxy" as used herein refer to an alkyl group, as defined above, having an oxygen radical attached thereto. Representative alkoxyl groups include methoxy, ethoxy, propyloxy, tert-butoxy and the like. An "ether" is two hydrocarbyl groups covalently linked by an oxygen. Accordingly, the substituent of an alkyl that renders that alkyl an ether is or resembles an alkoxyl, such as can be represented by one of -O-alkyl, -O-alkenyl, and -O-alkynyl.

[27] As used herein, the term "alkoxylene" refers to a saturated monovalent aliphatic radicals of the general formula (-O-C_mH_{2m}-) where m represents an integer (*e.g.*, 1, 2, 3, 4, 5, 6, or 7) and is inclusive of both straight-chain and branched-chain radicals. The alkoxylene chain may be attached to the rest of the molecule through a single bond and to the radical group through a single bond. In some embodiments, the alkoxylene group contains one to 3 carbon atoms (-O-C_1-C_3 alkoxylene). In other embodiments, an alkoxylene group contains one to 5 carbon atoms (-O-C_1-C_5 alkoxylene).

[28] As used herein, the term "cyclic group" broadly refers to any group that used alone or as part of a larger moiety, contains a saturated, partially saturated or aromatic ring system *e.g.*, carbocyclic (cycloalkyl, cycloalkenyl), heterocyclic (heterocycloalkyl, heterocycloalkenyl), aryl and heteroaryl groups. Cyclic groups may have one or more (*e.g.*, fused) ring systems. Thus, for example, a cyclic group can contain one or more carbocyclic, heterocyclic, aryl or heteroaryl groups.

[29] As used herein, the term "carbocyclic" (also "carbocyclyl") refers to a group that used

alone or as part of a larger moiety, contains a saturated, partially unsaturated, or aromatic ring system having 3 to 20 carbon atoms, that is alone or part of a larger moiety (*e.g.*, an alkcarbocyclic group). The term carbocyclyl includes mono-, bi-, tri-, fused, bridged, and spiro-ring systems, and combinations thereof. In one embodiment, carbocyclyl includes 3 to 15 carbon atoms (C_3-C_{15}). In one embodiment, carbocyclyl includes 3 to 12 carbon atoms (C_3-C_{12}). In another embodiment, carbocyclyl includes C_3-C_8, C_3-C_{10} or C_5-C_{10}. In another embodiment, carbocyclyl, as a monocycle, includes C_3-C_8, C_3-C_6 or C_5-C_6. In some embodiments, carbocyclyl, as a bicycle, includes C_7-C_{12}. In another embodiment, carbocyclyl, as a spiro system, includes C_5-C_{12}. Representative examples of monocyclic carbocyclyls include cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, perdeuteriocyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, 1-cyclohex-3-enyl, cyclohexadienyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, cycloundecyl, phenyl, and cyclododecyl; bicyclic carbocyclyls having 7 to 12 ring atoms include [4,3], [4,4], [4,5], [5,5], [5,6] or [6,6] ring systems, such as for example bicyclo[2.2.1]heptane, bicyclo[2.2.2]octane, naphthalene, and bicyclo[3.2.2]nonane. Representative examples of spiro carbocyclyls include spiro[2.2]pentane, spiro[2.3]hexane, spiro[2.4]heptane, spiro[2.5]octane and spiro[4.5]decane. The term carbocyclyl includes aryl ring systems as defined herein. The term carbocycyl also includes cycloalkyl rings (*e.g.*, saturated or partially unsaturated mono-, bi-, or spiro-carbocycles). The term carbocyclic group also includes a carbocyclic ring fused to one or more (*e.g.*, 1, 2 or 3) different cyclic groups (*e.g.*, aryl or heterocyclic rings), where the radical or point of attachment is on the carbocyclic ring.

[30] Thus, the term carbocyclic also embraces carbocyclylalkyl groups which as used herein refer to a group of the formula –R^c-carbocyclyl where R^c is an alkylene chain. The term carbocyclic also embraces carbocyclylalkoxy groups which as used herein refer to a group bonded through an oxygen atom of the formula –O–R^c-carbocyclyl where R^c is an alkylene chain.

[31] As used herein, the term "aryl" used alone or as part of a larger moiety (*e.g.*, "aralkyl", wherein the terminal carbon atom on the alkyl group is the point of attachment, *e.g.*, a benzyl group), "aralkoxy" wherein the oxygen atom is the point of attachment, or "aroxyalkyl" wherein the point of attachment is on the aryl group) refers to a group that includes monocyclic, bicyclic or tricyclic, carbon ring system, that includes fused rings, wherein at least one ring in

the system is aromatic. In some embodiments, the aralkoxy group is a benzoxy group. The term "aryl" may be used interchangeably with the term "aryl ring". In one embodiment, aryl includes groups having 6-18 carbon atoms. In another embodiment, aryl includes groups having 6-10 carbon atoms. Examples of aryl groups include phenyl, naphthyl, anthracyl, biphenyl, phenanthrenyl, naphthacenyl, 1,2,3,4-tetrahydronaphthalenyl, 1H-indenyl, 2,3-dihydro-1H-indenyl, naphthyridinyl, and the like, which may be substituted or independently substituted by one or more substituents described herein. A particular aryl is phenyl. In some embodiments, an aryl group includes an aryl ring fused to one or more (*e.g.*, 1, 2 or 3) different cyclic groups (*e.g.*, carbocyclic rings or heterocyclic rings), where the radical or point of attachment is on the aryl ring. The structure of any aryl group that is capable of having double bonds positioned differently is considered so as to embrace any and all such resonance structures.

[32] Thus, the term aryl embraces aralkyl groups (*e.g.*, benzyl) which as disclosed above refer to a group of the formula $–R^c$-aryl where R^c is an alkylene chain such as methylene or ethylene. In some embodiments, the aralkyl group is an optionally substituted benzyl group. The term aryl also embraces aralkoxy groups which as used herein refer to a group bonded through an oxygen atom of the formula $–O—R^c–$aryl where R^c is an alkylene chain such as methylene or ethylene.

[33] As used herein, the term "heterocyclyl" refers to a "carbocyclyl" that used alone or as part of a larger moiety, contains a saturated, partially unsaturated or aromatic ring system, wherein one or more (*e.g.*, 1, 2, 3, or 4) carbon atoms have been replaced with a heteroatom (*e.g.*, O, N, N(O), S, S(O), or $S(O)_2$). The term heterocyclyl includes mono-, bi-, tri-, fused, bridged, and spiro-ring systems, and combinations thereof. In some embodiments, a heterocyclyl refers to a 3 to 15 membered heterocyclyl ring system. In some embodiments, a heterocyclyl refers to a 3 to 12 membered heterocyclyl ring system. In some embodiments, a heterocyclyl refers to a saturated ring system, such as a 3 to 12 membered saturated heterocyclyl ring system. In some embodiments, a heterocyclyl refers to a heteroaryl ring system, such as a 5 to 14 membered heteroaryl ring system. The term heterocyclyl also includes C_3-C_8 heterocycloalkyl, which is a saturated or partially unsaturated mono-, bi-, or spiro-ring system containing 3-8 carbons and one or more (1, 2, 3 or 4) heteroatoms.

[34] In some embodiments, a heterocyclyl group includes 3-12 ring atoms and includes monocycles, bicycles, tricycles and spiro ring systems, wherein the ring atoms are carbon, and one to 5 ring atoms is a heteroatom such as nitrogen, sulfur or oxygen. In some embodiments,

heterocyclyl includes 3- to 7-membered monocycles having one or more heteroatoms selected from nitrogen, sulfur and oxygen. In some embodiments, heterocyclyl includes 4- to 6-membered monocycles having one or more heteroatoms selected from nitrogen, sulfur and oxygen. In some embodiments, heterocyclyl includes 3-membered monocycles. In some embodiments, heterocyclyl includes 4-membered monocycles. In some embodiments, heterocyclyl includes 5-6 membered monocycles. In some embodiments, the heterocyclyl group includes 0 to 3 double bonds. In any of the foregoing embodiments, heterocyclyl includes 1, 2, 3 or 4 heteroatoms. Any nitrogen or sulfur heteroatom may optionally be oxidized (*e.g.*, NO, SO, SO_2), and any nitrogen heteroatom may optionally be quaternized (*e.g.*, $[NR_4]^+Cl^-$, $[NR_4]^+OH^-$). Representative examples of heterocyclyls include oxiranyl, aziridinyl, thiiranyl, azetidinyl, oxetanyl, thietanyl, 1,2-dithietanyl, 1,3-dithietanyl, pyrrolidinyl, dihydro-1H-pyrrolyl, dihydrofuranyl, tetrahydropyranyl, dihydrothienyl, tetrahydrothienyl, imidazolidinyl, piperidinyl, piperazinyl, morpholinyl, thiomorpholinyl, 1,1-dioxo-thiomorpholinyl, dihydropyranyl, tetrahydropyranyl, hexahydrothiopyranyl, hexahydropyrimidinyl, oxazinanyl, thiazinanyl, thioxanyl, homopiperazinyl, homopiperidinyl, azepanyl, oxepanyl, thiepanyl, oxazepinyl, oxazepanyl, diazepanyl, 1,4-diazepanyl, diazepinyl, thiazepinyl, thiazepanyl, tetrahydrothiopyranyl, oxazolidinyl, thiazolidinyl, isothiazolidinyl, 1,1-dioxoisothiazolidinonyl, oxazolidinonyl, imidazolidinonyl, 4,5,6,7-tetrahydro[2H]indazolyl, tetrahydrobenzoimidazolyl, 4,5,6,7-tetrahydrobenzo[d]imidazolyl, 1,6-dihydroimidazol[4,5-d]pyrrolo[2,3-b]pyridinyl, thiazinyl, thiophenyl, oxazinyl, thiadiazinyl, oxadiazinyl, dithiazinyl, dioxazinyl, oxathiazinyl, thiatriazinyl, oxatriazinyl, dithiadiazinyl, imidazolinyl, dihydropyrimidyl, tetrahydropyrimidyl, 1-pyrrolinyl, 2-pyrrolinyl, 3-pyrrolinyl, indolinyl, thiapyranyl, 2H-pyranyl, 4H-pyranyl, dioxanyl, 1,3-dioxolanyl, pyrazolinyl, pyrazolidinyl, dithianyl, dithiolanyl, pyrimidinonyl, pyrimidindionyl, pyrimidin-2,4-dionyl, piperazinonyl, piperazindionyl, pyrazolidinylimidazolinyl, 3-azabicyclo[3.1.0]hexanyl, 3,6-diazabicyclo[3.1.1]heptanyl, 6-azabicyclo[3.1.1]heptanyl, 3-azabicyclo[3.1.1]heptanyl, 3-azabicyclo[4.1.0]heptanyl, azabicyclo[2.2.2]hexanyl, 2-azabicyclo[3.2.1]octanyl, 8-azabicyclo[3.2.1]octanyl, 2-azabicyclo[2.2.2]octanyl, 8-azabicyclo[2.2.2]octanyl, 7-oxabicyclo[2.2.1]heptane, azaspiro[3.5]nonanyl, azaspiro[2.5]octanyl, azaspiro[4.5]decanyl, 1-azaspiro[4.5]decan-2-only, azaspiro[5.5]undecanyl, tetrahydroindolyl, octahydroindolyl, tetrahydroisoindolyl, tetrahydroindazolyl, 1,1-dioxohexahydrothiopyranyl. Examples of 5-membered heterocyclyls containing a sulfur or oxygen atom and one to three nitrogen atoms

are thiazolyl, including thiazol-2-yl and thiazol-2-yl N-oxide, thiadiazolyl, including 1,3,4-thiadiazol-5-yl and 1,2,4-thiadiazol-5-yl, oxazolyl, for example oxazol-2-yl, and oxadiazolyl, such as 1,3,4-oxadiazol-5-yl, and 1,2,4-oxadiazol-5-yl. Example 5-membered ring heterocyclyls containing 2 to 4 nitrogen atoms include imidazolyl, such as imidazol-2-yl; triazolyl, such as 1,3,4-triazol-5-yl; 1,2,3-triazol-5-yl, 1,2,4-triazol-5-yl, and tetrazolyl, such as 1H-tetrazol-5-yl. Representative examples of benzo-fused 5-membered heterocyclyls are benzoxazol-2-yl, benzthiazol-2-yl and benzimidazol-2-yl. Example 6-membered heterocyclyls contain one to three nitrogen atoms and optionally a sulfur or oxygen atom, for example pyridyl, such as pyrid-2-yl, pyrid-3-yl, and pyrid-4-yl; pyrimidyl, such as pyrimid-2-yl and pyrimid-4-yl; triazinyl, such as 1,3,4-triazin-2-yl and 1,3,5-triazin-4-yl; pyridazinyl, in particular pyridazin-3-yl, and pyrazinyl. The pyridine N-oxides and pyridazine N-oxides and the pyridyl, pyrimid-2-yl, pyrimid-4-yl, pyridazinyl and the 1,3,4-triazin-2-yl groups, are yet other examples of heterocyclyl groups. In some embodiments, a heterocyclic group includes a heterocyclic ring fused to one or more (*e.g.*, 1, 2 or 3) different cyclic groups (*e.g.*, carbocyclic rings or heterocyclic rings), where the radical or point of attachment is on the heterocyclic ring, and in some embodiments wherein the point of attachment is a heteroatom contained in the heterocyclic ring.

[35] Thus, the term heterocyclic embraces N-heterocyclyl groups which as used herein refer to a heterocyclyl group containing at least one nitrogen and where the point of attachment of the heterocyclyl group to the rest of the molecule is through a nitrogen atom in the heterocyclyl group. Representative examples of N-heterocyclyl groups include 1-morpholinyl, 1-piperidinyl, 1-piperazinyl, 1-pyrrolidinyl, pyrazolidinyl, imidazolinyl and imidazolidinyl. The term heterocyclic also embraces C-heterocyclyl groups which as used herein refer to a heterocyclyl group containing at least one heteroatom and where the point of attachment of the heterocyclyl group to the rest of the molecule is through a carbon atom in the heterocyclyl group. Representative examples of C-heterocyclyl radicals include 2-morpholinyl, 2- or 3- or 4-piperidinyl, 2-piperazinyl, and 2- or 3-pyrrolidinyl. The term heterocyclic also embraces heterocyclylalkyl groups which as disclosed above refer to a group of the –R^c-heterocyclyl where R^c is an alkylene chain. The term heterocyclic also embraces heterocyclylalkoxy groups which as used herein refer to a radical bonded through an oxygen atom of the formula –O–R^c-heterocyclyl where R^c is an alkylene chain.

[36] As used herein, the term "heteroaryl" used alone or as part of a larger moiety (*e.g.*, "heteroarylalkyl" (also "heteroaralkyl"), or "heteroarylalkoxy" (also "heteroaralkoxy"), refers to a monocyclic, bicyclic or tricyclic ring system having 5 to 14 ring atoms, wherein at least one ring is aromatic and contains at least one heteroatom. In one embodiment, heteroaryl includes 5-6 membered monocyclic aromatic groups where one or more ring atoms is nitrogen, sulfur or oxygen. Representative examples of heteroaryl groups include thienyl, furyl, imidazolyl, pyrazolyl, thiazolyl, isothiazolyl, oxazolyl, isoxazolyl, triazolyl, thiadiazolyl, oxadiazolyl, tetrazolyl, thiatriazolyl, oxatriazolyl, pyridyl, pyrimidyl, imidazopyridyl, pyrazinyl, pyridazinyl, triazinyl, tetrazinyl, tetrazolo[1,5-b]pyridazinyl, purinyl, deazapurinyl, benzoxazolyl, benzofuryl, benzothiazolyl, benzothiadiazolyl, benzotriazolyl, benzoimidazolyl, indolyl, 1,3-thiazol-2-yl, 1,3,4-triazol-5-yl, 1,3-oxazol-2-yl, 1,3,4-oxadiazol-5-yl, 1,2,4-oxadiazol-5-yl, 1,3,4-thiadiazol-5-yl, 1H-tetrazol-5-yl, 1,2,3-triazol-5-yl, and pyrid-2-yl N-oxide. The term "heteroaryl" also includes groups in which a heteroaryl is fused to one or more cyclic (*e.g.*, carbocyclyl, or heterocyclyl) rings, where the radical or point of attachment is on the heteroaryl ring. Nonlimiting examples include indolyl, indolizinyl, isoindolyl, benzothienyl, benzothiophenyl, methylenedioxyphenyl, benzofuranyl, dibenzofuranyl, indazolyl, benzimidazolyl, benzodioxazolyl, benzthiazolyl, quinolyl, isoquinolyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, 4H-quinolizinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl and pyrido[2,3-b]-1,4-oxazin-3(4H)-one. A heteroaryl group may be mono-, bi- or tri-cyclic. In some embodiments, a heteroaryl group includes a heteroaryl ring fused to one or more (*e.g.*, 1, 2 or 3) different cyclic groups (*e.g.*, carbocyclic rings or heterocyclic rings), where the radical or point of attachment is on the heteroaryl ring, and in some embodiments wherein the point of attachment is a heteroatom contained in the heterocyclic ring. The structure of any heteroaryl group that is capable of having double bonds positioned differently is considered to embrace any and all such resonance structures.

[37] Thus, the term heteroaryl embraces N-heteroaryl groups which as used herein refer to a heteroaryl group as defined above containing at least one nitrogen and where the point of attachment of the heteroaryl group to the rest of the molecule is through a nitrogen atom in the heteroaryl group. The term heteroaryl also embraces C-heteroaryl groups which as used herein refer to a heteroaryl group as defined above and where the point of attachment of the heteroaryl group to the rest of the molecule is through a carbon atom in the heteroaryl group. The term

heteroaryl also embraces heteroarylalkyl groups which as disclosed above refer to a group of the formula –R^c-heteroaryl, wherein R^c is an alkylene chain as defined above. The term heteroaryl also embraces heteroaralkoxy (or heteroarylalkoxy) groups which as used herein refer to a group bonded through an oxygen atom of the formula –O–R^c-heteroaryl, where R^c is an alkylene group as defined above.

[38] Unless stated otherwise, and to the extent not further defined for any particular group(s), any of the groups described herein may be substituted or unsubstituted. As used herein, the term "substituted" broadly refers to all permissible substituents with the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, *i.e.*, a compound that does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. Representative substituents include halogens, hydroxyl groups, and any other organic groupings containing any number of carbon atoms, *e.g.*, 1-14 carbon atoms, and which may include one or more (*e.g.*, 1, 2, 3, or 4) heteroatoms such as oxygen, sulfur, and nitrogen grouped in a linear, branched, or cyclic structural format.

[39] To the extent not disclosed otherwise for any particular group(s), representative examples of substituents may thus include alkyl, substituted alkyl (*e.g.*, C_1-C_6, C_1-C_5, C_1-C_4, C_1-C_3, C_1-C_2, C_1), alkoxy (*e.g.*, C_1-C_6, C_1-C_5, C_1-C_4, C_1-C_3, C_1-C_2, C_1), substituted alkoxy (*e.g.*, C_1-C_6, C_1-C_5, C_1-C_4, C_1-C_3, C_1-C_2, C_1), haloalkyl (*e.g.*, CF_3), alkenyl (*e.g.*, C_2-C_6, C_2-C_5, C_2-C_4, C_2-C_3, C_2), substituted alkenyl (*e.g.*, C_2-C_6, C_2-C_5, C_2-C_4, C_2-C_3, C_2), alkynyl (*e.g.*, C_2-C_6, C_2-C_5, C_2-C_4, C_2-C_3, C_2), substituted alkynyl (*e.g.*, C_2-C_6, C_2-C_5, C_2-C_4, C_2-C_3, C_2), cyclic (*e.g.*, C_3-C_{12}, C_5-C_6), substituted cyclic (*e.g.*, C_3-C_{12}, C_5-C_6), carbocyclic (*e.g.*, C_3-C_{12}, C_5-C_6), substituted carbocyclic (*e.g.*, C_3-C_{12}, C_5-C_6), heterocyclic (*e.g.*, C_3-C_{12}, C_5-C_6), substituted heterocyclic (*e.g.*, C_3-C_{12}, C_5-C_6), aryl (*e.g.*, benzyl and phenyl), substituted aryl (*e.g.*, substituted benzyl or phenyl), heteroaryl (*e.g.*, pyridyl or pyrimidyl), substituted heteroaryl (*e.g.*, substituted pyridyl or pyrimidyl), aralkyl (*e.g.*, benzyl), substituted aralkyl (*e.g.*, substituted benzyl), halo, hydroxyl, aryloxy (*e.g.*, C_6-C_{12}, C_6), substituted aryloxy (*e.g.*, C_6-C_{12}, C_6), alkylthio (*e.g.*, C_1-C_6), substituted alkylthio (*e.g.*, C_1-C_6), arylthio (*e.g.*, C_6-C_{12}, C_6), substituted arylthio (*e.g.*, C_6-C_{12}, C_6), cyano, carbonyl, substituted carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, thio, substituted thio, sulfinyl, substituted sulfinyl, sulfonyl, substituted sulfonyl, sulfinamide, substituted

sulfinamide, sulfonamide, substituted sulfonamide, urea, substituted urea, carbamate, substituted carbamate, amino acid, and peptide groups.

[40] The substituent present on an oxygen atom is an oxygen protecting group (also referred to herein as an "hydroxyl protecting group" or "–PG"). Oxygen protecting groups include, but are not limited to, $-R^{aa}$, $-N(R^{bb})_2$, $-C(=O)SR^{aa}$, $-C(=O)R^{aa}$, $-CO_2R^{aa}$, $-C(=O)N(R^{bb})_2$, $-C(=NR^{bb})R^{aa}$, $-C(=NR^{bb})OR^{aa}$, $-C(=NR^{bb})N(R^{bb})_2$, $-S(=O)R^{aa}$, $-SO_2R^{aa}$, $-Si(R^{aa})_3$, $-P(R^{cc})_2$, $-P(R^{cc})_3{}^+X^-$, $-P(OR^{cc})_2$, $-P(OR^{cc})_3{}^+X^-$, $-P(=O)(R^{aa})_2$, $-P(=O)(OR^{cc})_2$, and $-P(=O)(N(R^{bb})_2)_2$, wherein X^-, R^{aa}, R^{bb}, and R^{cc}, wherein:

each instance of R^{aa} is, independently, selected from C_{1-10} alkyl, C_{1-10} haloalkyl, C_{1-10} perhaloalkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, heteroC_{1-10} alkyl, heteroC_{2-10}alkenyl, heteroC_{2-10}alkynyl, C_{3-10} carbocyclyl, 3-14 membered heterocyclyl, C_{6-14} aryl, and 5-14 membered heteroaryl, or two R^{aa} groups are joined to form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups;

each instance of R^{bb} is, independently, selected from hydrogen, $-OH$, $-OR^{aa}$, $-N(R^{cc})_2$, $-CN$, $-C(=O)R^{aa}$, $-C(=O)N(R^{cc})_2$, $-CO_2R^{aa}$, $-SO_2R^{aa}$, $-C(=NR^{cc})OR^{aa}$, $-C(=NR^{cc})N(R^{cc})_2$, $-SO_2N(R^{cc})_2$, $-SO_2R^{cc}$, $-SO_2OR^{cc}$, $-SOR^{aa}$, $-C(=S)N(R^{cc})_2$, $-C(=O)SR^{cc}$, $-C(=S)SR^{cc}$, $-P(=O)(R^{aa})_2$, $-P(=O)(OR^{cc})_2$, $-P(=O)(N(R^{cc})_2)_2$, C_{1-10} alkyl, C_{1-10} haloalkyl, C_{1-10} perhaloalkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, heteroC_{1-10}alkyl, heteroC_{2-10}alkenyl, heteroC_{2-10}alkynyl, C_{3-10} carbocyclyl, 3-14 membered heterocyclyl, C_{6-14} aryl, and 5-14 membered heteroaryl, or two R^{bb} groups are joined to form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups; wherein X^- is a counterion;

each instance of R^{cc} is, independently, selected from hydrogen, C_{1-10} alkyl, C_{1-10} haloalkyl, C_{1-10} perhaloalkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, heteroC_{1-10} alkyl, heteroC_{2-10} alkenyl, heteroC_{2-10} alkynyl, C_{3-10} carbocyclyl, 3-14 membered heterocyclyl, C_{6-14} aryl, and 5-14 membered heteroaryl, or two R^{cc} groups are joined to form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups;

each instance of R^{dd} is, independently, selected from halogen, $-CN$, $-NO_2$, $-N_3$, $-SO_2H$, $-SO_3H$, $-OH$, $-OR^{ee}$, $-ON(R^{ff})_2$, $-N(R^{ff})_2$, $-N(R^{ff})_3^+X^-$, $-N(OR^{ee})R^{ff}$, $-SH$, $-SR^{ee}$, $-SSR^{ee}$, $-C(=O)R^{ee}$, $-CO_2H$, $-CO_2R^{ee}$, $-OC(=O)R^{ee}$, $-OCO_2R^{ee}$, $-C(=O)N(R^{ff})_2$, $-OC(=O)N(R^{ff})_2$, $-NR^{ff}C(=O)R^{ee}$, $-NR^{ff}CO_2R^{ee}$, $-NR^{ff}C(=O)N(R^{ff})_2$, $-C(=NR^{ff})OR^{ee}$, $-OC(=NR^{ff})R^{ee}$, $-OC(=NR^{ff})OR^{ee}$, $-C(=NR^{ff})N(R^{ff})_2$, $-OC(=NR^{ff})N(R^{ff})_2$, $-NR^{ff}C(=NR^{ff})N(R^{ff})_2$, $-NR^{ff}SO_2R^{ee}$, $-SO_2N(R^{ff})_2$, $-SO_2R^{ee}$, $-SO_2OR^{ee}$, $-OSO_2R^{ee}$, $-S(=O)R^{ee}$, $-Si(R^{ee})_3$, $-OSi(R^{ee})_3$, $-C(=S)N(R^{ff})_2$, $-C(=O)SR^{ee}$, $-C(=S)SR^{ee}$, $-SC(=S)SR^{ee}$, $-P(=O)(OR^{ee})_2$, $-P(=O)(R^{ee})_2$, $-OP(=O)(R^{ee})_2$, $-OP(=O)(OR^{ee})_2$, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} perhaloalkyl,C_{2-6} alkenyl, C_{2-6} alkynyl, heteroC_{1-6}alkyl, heteroC_{2-6}alkenyl, heteroC_{2-6}alkynyl, C_{3-10} carbocyclyl, 3-10 membered heterocyclyl, C_{6-10} aryl, 5-10 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{gg} groups, or two geminal R^{dd} substituents can be joined to form =O or =S; wherein X^- is a counterion;

each instance of R^{ee} is, independently, selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} perhaloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, heteroC_{1-6} alkyl, heteroC_{2-6}alkenyl, heteroC_{2-6} alkynyl, C_{3-10} carbocyclyl, C_{6-10} aryl, 3-10 membered heterocyclyl, and 3-10 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{gg} groups;

each instance of R^{ff} is, independently, selected from hydrogen, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} perhaloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, heteroC_{1-6}alkyl, heteroC_{2-6}alkenyl, heteroC_{2-6}alkynyl, C_{3-10} carbocyclyl, 3-10 membered heterocyclyl, C_{6-10} aryl, and 5-10 membered heteroaryl, or two R^{ff} groups are joined to form a 3-10 membered heterocyclyl or 5-10 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{gg} groups; and

each instance of R^{gg} is, independently, halogen, $-CN$, $-NO_2$, $-N_3$, $-SO_2H$, $-SO_3H$, $-OH$, $-OC_{1-6}$ alkyl, $-ON(C_{1-6}$ alkyl$)_2$, $-N(C_{1-6}$ alkyl$)_2$, $-N(C_{1-6}$ alkyl$)_3^+X^-$, $-NH(C_{1-6}$ alkyl$)_2^+X^-$, $-NH_2(C_{1-6}$ alkyl$)^+X^-$, $-NH_3^+X^-$, $-N(OC_{1-6}$ alkyl$)(C_{1-6}$ alkyl$)$, $-N(OH)(C_{1-6}$ alkyl$)$, $-NH(OH)$, $-SH$, $-SC_{1-6}$ alkyl, $-SS(C_{1-6}$ alkyl$)$, $-C(=O)(C_{1-6}$ alkyl$)$, $-CO_2H$, $-CO_2(C_{1-6}$ alkyl$)$, $-OC(=O)(C_{1-6}$ alkyl$)$, $-OCO_2(C_{1-6}$ alkyl$)$, $-C(=O)NH_2$, $-C(=O)N(C_{1-6}$ alkyl$)_2$, $-OC(=O)NH(C_{1-6}$ alkyl$)$, $-NHC(=O)($ C_{1-6} alkyl$)$, $-N(C_{1-6}$ alkyl$)C(=O)($ C_{1-6} alkyl$)$, $-NHCO_2(C_{1-6}$ alkyl$)$, $-NHC(=O)N(C_{1-6}$ alkyl$)_2$, $-NHC(=O)NH(C_{1-6}$ alkyl$)$, $-NHC(=O)NH_2$,

−C(=NH)O(C_{1-6} alkyl), −OC(=NH)(C_{1-6} alkyl), −OC(=NH)OC_{1-6} alkyl, −C(=NH)N(C_{1-6} alkyl)$_2$, −C(=NH)NH(C_{1-6} alkyl), −C(=NH)NH_2, −OC(=NH)N(C_{1-6} alkyl)$_2$, −OC(NH)NH(C_{1-6} alkyl), −OC(NH)NH_2, −NHC(NH)N(C_{1-6} alkyl)$_2$, −NHC(=NH)NH_2, −$NHSO_2$(C_{1-6} alkyl), −SO_2N(C_{1-6} alkyl)$_2$, −SO_2NH(C_{1-6} alkyl), −SO_2NH_2, −SO_2C_{1-6} alkyl, −SO_2OC_{1-6} alkyl, −OSO_2C_{1-6} alkyl, −SOC_{1-6} alkyl, −Si(C_{1-6} alkyl)$_3$, −OSi(C_{1-6} alkyl)$_3$ −C(=S)N(C_{1-6} alkyl)$_2$, C(=S)NH(C_{1-6} alkyl), C(=S)NH_2, −C(=O)S(C_{1-6} alkyl), −C(=S)SC_{1-6} alkyl, −SC(=S)SC_{1-6} alkyl, −P(=O)(OC_{1-6} alkyl)$_2$, −P(=O)(C_{1-6} alkyl)$_2$, −OP(=O)(C_{1-6} alkyl)$_2$, −OP(=O)(OC_{1-6} alkyl)$_2$, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} perhaloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, heteroC_{1-6}alkyl, heteroC_{2-6}alkenyl, heteroC_{2-6}alkynyl, C_{3-10} carbocyclyl, C_{6-10} aryl, 3-10 membered heterocyclyl, 5-10 membered heteroaryl; or two geminal R^{gg} substituents can be joined to form =O or =S; wherein X^- is a counterion. Oxygen protecting groups are well known in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, incorporated herein by reference.

[41] Exemplary oxygen protecting groups include, but are not limited to, methyl, methoxylmethyl (MOM), methylthiomethyl (MTM), t-butylthiomethyl, (phenyldimethylsilyl)methoxymethyl (SMOM), benzyloxymethyl (BOM), p-methoxybenzyloxymethyl (PMBM), (4-methoxyphenoxy)methyl (p-AOM), guaiacolmethyl (GUM), t-butoxymethyl, 4-pentenyloxymethyl (POM), siloxymethyl, 2-methoxyethoxymethyl (MEM), 2,2,2-trichloroethoxymethyl, bis(2-chloroethoxy)methyl, 2-(trimethylsilyl)ethoxymethyl (SEMOR), tetrahydropyranyl (THP), 3-bromotetrahydropyranyl, tetrahydrothiopyranyl, 1-methoxycyclohexyl, 4-methoxytetrahydropyranyl (MTHP), 4-methoxytetrahydrothiopyranyl, 4-methoxytetrahydrothiopyranyl S,S-dioxide, 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl (CTMP), 1,4-dioxan-2-yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3a,4,5,6,7,7a-octahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2-yl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 1-methyl-1-methoxyethyl, 1-methyl-1-benzyloxyethyl, 1-methyl-1-benzyloxy-2-fluoroethyl, 2,2,2-trichloroethyl, 2-trimethylsilylethyl, 2-(phenylselenyl)ethyl, t-butyl, allyl, p-chlorophenyl, p-methoxyphenyl, 2,4-dinitrophenyl, benzyl (Bn), p-methoxybenzyl, 3,4-dimethoxybenzyl, o-nitrobenzyl, p-nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, p-phenylbenzyl, 2-picolyl, 4-picolyl, 3-methyl-2-picolyl N-oxido, diphenylmethyl, p,p'-dinitrobenzhydryl, 5-dibenzosuberyl, triphenylmethyl, α-naphthyldiphenylmethyl, p-methoxyphenyldiphenylmethyl, di(p-methoxyphenyl)phenylmethyl, tri(p-

methoxyphenyl)methyl, 4-(4'-bromophenacyloxyphenyl)diphenylmethyl, 4,4′,4″-tris(4,5-dichlorophthalimidophenyl)methyl, 4,4′,4″-tris(levulinoyloxyphenyl)methyl, 4,4′,4″-tris(benzoyloxyphenyl)methyl, 3-(imidazol-1-yl)bis(4′,4″-dimethoxyphenyl)methyl, 1,1-bis(4-methoxyphenyl)-1′-pyrenylmethyl, 9-anthryl, 9-(9-phenyl)xanthenyl, 9-(9-phenyl-10-oxo)anthryl, 1,3-benzodithiolan-2-yl, benzisothiazolyl S,S-dioxido, trimethylsilyl (TMS), triethylsilyl (TES), triisopropylsilyl (TIPS), dimethylisopropylsilyl (IPDMS), diethylisopropylsilyl (DEIPS), dimethylthexylsilyl, t-butyldimethylsilyl (TBDMS), t-butyldiphenylsilyl (TBDPS), tribenzylsilyl, tri-p-xylylsilyl, triphenylsilyl, diphenylmethylsilyl (DPMS), t-butylmethoxyphenylsilyl (TBMPS), formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, p-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate (levulinate), 4,4-(ethylenedithio)pentanoate (levulinoyldithioacetal), pivaloate, adamantoate, crotonate, 4-methoxycrotonate, benzoate, p-phenylbenzoate, 2,4,6-trimethylbenzoate (mesitoate), methyl carbonate, 9-fluorenylmethyl carbonate (Fmoc), ethyl carbonate, 2,2,2-trichloroethyl carbonate (Troc), 2-(trimethylsilyl)ethyl carbonate (TMSEC), 2-(phenylsulfonyl) ethyl carbonate (Psec), 2-(triphenylphosphonio) ethyl carbonate (Peoc), isobutyl carbonate, vinyl carbonate, allyl carbonate, t-butyl carbonate (BOC or Boc), p-nitrophenyl carbonate, benzyl carbonate, p-methoxybenzyl carbonate, 3,4-dimethoxybenzyl carbonate, o-nitrobenzyl carbonate, p-nitrobenzyl carbonate, S-benzyl thiocarbonate, 4-ethoxy-1-napththyl carbonate, methyl dithiocarbonate, 2-iodobenzoate, 4-azidobutyrate, 4-nitro-4-methylpentanoate, o-(dibromomethyl)benzoate, 2-formylbenzenesulfonate, 2-(methylthiomethoxy)ethyl, 4-(methylthiomethoxy)butyrate, 2-(methylthiomethoxymethyl)benzoate, 2,6-dichloro-4-methylphenoxyacetate, 2,6-dichloro-4-(1,1,3,3-tetramethylbutyl)phenoxyacetate, 2,4-bis(1,1-dimethylpropyl)phenoxyacetate, chlorodiphenylacetate, isobutyrate, monosuccinoate, (E)-2-methyl-2-butenoate, o-(methoxyacyl)benzoate, α-naphthoate, nitrate, alkyl N,N,N',N'-tetramethylphosphorodiamidate, alkyl N-phenylcarbamate, borate, dimethylphosphinothioyl, alkyl 2,4-dinitrophenylsulfenate, sulfate, methanesulfonate (mesylate), benzylsulfonate, and tosylate (Ts).

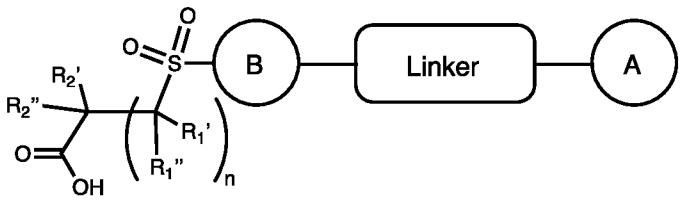
[42] A "counterion" or "anionic counterion" is a negatively charged group associated with a positively charged group in order to maintain electronic neutrality. An anionic counterion may be monovalent (i.e., including one formal negative charge). An anionic counterion may

also be multivalent (i.e., including more than one formal negative charge), such as divalent or trivalent. Exemplary counterions include halide ions (*e.g.*, F^-, Cl^-, Br^-, I^-), NO_3^-, ClO_4^-, OH^-, $H_2PO_4^-$, HCO_3^-, HSO_4^-, sulfonate ions (*e.g.*, methansulfonate, trifluoromethanesulfonate, *p*–toluenesulfonate, benzenesulfonate, 10–camphor sulfonate, naphthalene–2–sulfonate, naphthalene–1–sulfonic acid–5–sulfonate, ethan–1–sulfonic acid–2–sulfonate, and the like), carboxylate ions (*e.g.*, acetate, propanoate, benzoate, glycerate, lactate, tartrate, glycolate, gluconate, and the like), BF_4^-, PF_4^-, PF_6^-, AsF_6^-, SbF_6^-, $B[3,5\text{-}(CF_3)_2C_6H_3]_4]^-$, $B(C_6F_5)_4^-$, BPh_4^-, $Al(OC(CF_3)_3)_4^-$, and carborane anions (*e.g.*, $CB_{11}H_{12}^-$ or $(HCB_{11}Me_5Br_6)^-$). Exemplary counterions which may be multivalent include CO_3^{2-}, HPO_4^{2-}, PO_4^{3-}, $B_4O_7^{2-}$, SO_4^{2-}, $S_2O_3^{2-}$, carboxylate anions (*e.g.*, tartrate, citrate, fumarate, maleate, malate, malonate, gluconate, succinate, glutarate, adipate, pimelate, suberate, azelate, sebacate, salicylate, phthalates, aspartate, glutamate, and the like), and carboranes.

[43] The term "Click chemistry" has been applied to a collection of reliable and self-directed organic reactions (Kolb et al., Angew. Chem. Int. Ed., *40*:2004-2021 (2001); Fantoni et al., Chem. Rev. *121*(12):7122–7154 (2021)).

[44] The term "binding" as it relates to interaction between targeted protein/s and a member of the eDEL collection, including the warhead, typically refering to an inter-molecular or a covalent interaction that is preferential (also referred to herein as "selective") in that binding of the member of the eDEL collection, including the warhead, with other proteins present in the cell is substantially less and, in some cases, may be functionally insignificant.

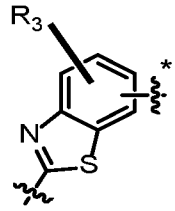
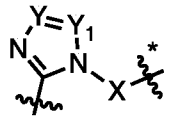
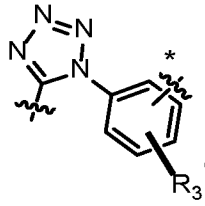
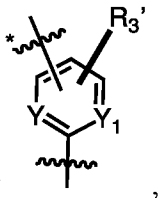
[45] Broadly, the compounds of the present disclosure are represented by formula (I) or a pharmaceutical salt or stereoisomer thereof:



(I), wherein

(A) is an oligonucleotide tag represented by  or an affinity tag;

Linker is a linker that covalently attaches (A) to (B);

Ⓑ is  ,  ,  , or  , wherein

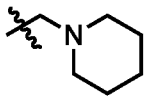
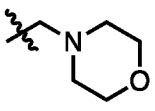
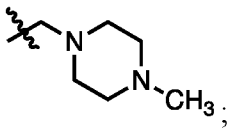
 is the connection to the sulfone group,

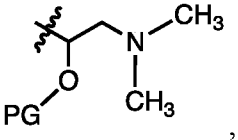
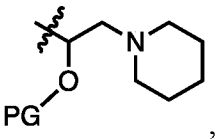
 is the connection to the linker,

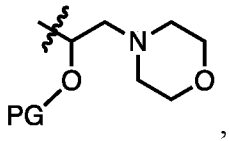
R_3' is H, halogen, amino, hydroxyl, (C_1-C_6) alkyl, (C_1-C_6) hydroxyalkyl, (C_1-C_6) aminoalkyl, (C_3-C_6) carbocyclyl, 4- to 6-membered heterocyclyl, (C_1-C_6) alkyl-(C_3-C_6) carbocyclyl, or (C_1-C_6) alkyl-4- to 6-membered heterocyclyl, wherein said alkyl, hydroxyalkyl, aminoalkyl, carbocyclyl, or heterocyclyl is further optionally substituted by one or more, identical or different R_{1a} groups, wherein each R_{1a} is independently (C_1-C_6) alkyl, (C_1-C_6) alkoxy, (C_1-C_6) alkyl-(C_1-C_3) alkoxy, halogen, amino, hydroxyl, (C_1-C_6) haloalkyl, NH-(C_1-C_6) alkyl, N((C_1-C_6)alkyl)$_2$, (C_3-C_6) carbocyclyl, or 4- to 6-membered heterocyclyl,

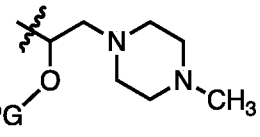
X is (C_1-C_6) alkyl, wherein said alkyl is further optionally substituted by one or more, identical or different R_{1a} groups, wherein each R_{1a} is independently (C_1-C_6) alkyl, (C_1-C_6) alkoxy, (C_1-C_6) alkyl-(C_1-C_3) alkoxy, halogen, amino, hydroxyl, (C_1-C_6) haloalkyl, NH-(C_1-C_6) alkyl, N((C_1-C_6)alkyl)$_2$, (C_3-C_6) carbocyclyl, or 4- to 6-membered heterocyclyl, and

Y and Y_1 are each independently CH or N;

R_1' and R_1'' are each independently H, CH_3, CF_3, halogen, CH_2NMe_2,  ,  , or  ;

R_2' and R_2'' are each independently H, halogen, CF_3, OH, OAc, CH_2OH, $CH(CH_3)OH$, $C(CH_3)_2OH$, CH_2OAc, CH_2OPG,  ,  ,

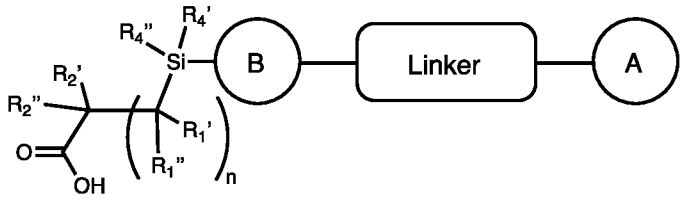


, or


, wherein PG is a protecting group, provided that both R_2' and R_2" are not H, or R_2' and R_2" can be joined to form =O; and

n is 0 or 1.

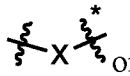
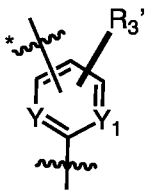
[46] In some embodiments, the compounds of the present disclosure are represented by formula (II) or a pharmaceutical salt or stereoisomer thereof:



(II), wherein

(A) is an oligonucleotide tag represented by  or an affinity tag;

Linker is a linker that covalently attaches (A) to (B);

(B) is  or 

, wherein

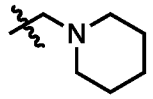
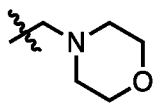
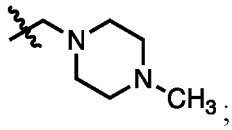
 is the connection to the silyl group,

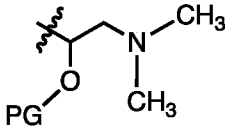
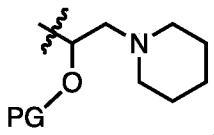
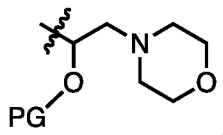
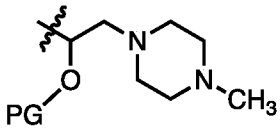
 is the connection to the linker,

R_3' is H, halogen, amino, hydroxyl, (C_1-C_6) alkyl, (C_1-C_6) hydroxyalkyl, (C_1-C_6) aminoalkyl, (C_3-C_6) carbocyclyl, 4- to 6-membered heterocyclyl, (C_1-C_6) alkyl-(C_3-C_6) carbocyclyl, or (C_1-C_6) alkyl-4- to 6-membered heterocyclyl, wherein said alkyl, hydroxyalkyl, aminoalkyl, carbocyclyl, or heterocyclyl is further optionally substituted by one or more, identical or different R_{1a} groups, wherein each R_{1a} is independently (C_1-C_6) alkyl, (C_1-C_6) alkoxy, (C_1-C_6) alkyl-(C_1-C_3) alkoxy, halogen, amino, hydroxyl, (C_1-C_6) haloalkyl, NH-(C_1-C_6) alkyl, N((C_1-C_6)alkyl)$_2$, (C_3-C_6) carbocyclyl, or 4- to 6-membered heterocyclyl,

X is (C_1-C_6) alkyl, wherein said alkyl is further optionally substituted by one or more, identical or different R_{1a} groups, wherein each R_{1a} is independently (C_1-C_6) alkyl, (C_1-C_6) alkoxy, (C_1-C_6) alkyl-(C_1-C_3) alkoxy, halogen, amino, hydroxyl, (C_1-C_6) haloalkyl, NH-(C_1-C_6) alkyl, N((C_1-C_6)alkyl)$_2$, (C_3-C_6) carbocyclyl, or 4- to 6-membered heterocyclyl, and

Y and Y_1 are each independently CH or N;

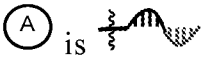
R_1' and R_1'' are each independently H, CH_3, halogen, CH_2NMe_2,  ,  , or  ;

R_2' and R_2'' are each independently H, CF_3, halogen, OH, OAc, CH_2OH, $CH(CH_3)OH$, $C(CH_3)_2OH$, CH_2OAc, CH_2OPG,  ,  ,  , or  wherein PG is a Protecting Group, provided that both R_2' and R_2'' are not H, or R_2' and R_2'' can be joined to form =O;

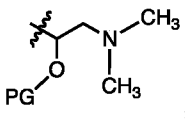
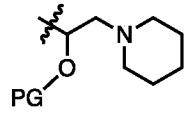
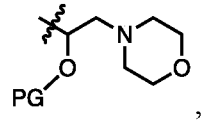
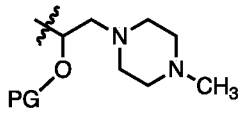
R_4' and R_4'' are each independently alkyl or aryl; and

n is 0 or 1.

[47] In some embodiments, R_4' and R_4'' are each independently CH_3, CH_2CH_3, CF_3, propyl, isopropyl, butyl, isobutyl, alkyl, or phenyl.

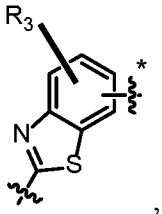
[48] In some embodiments,  is .

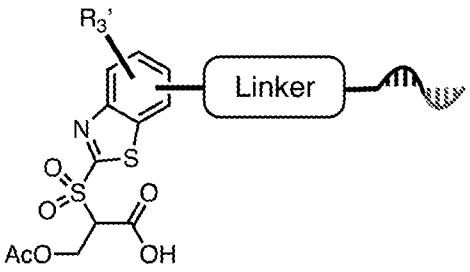
[49] In some embodiments, n is 0.

[50] In some embodiments, R_2' and R_2'' are each independently H, CF_3, halogen, CH_2OAc, CH_2OH, $CH(CH_3)OH$, $C(CH_3)_2OH$, CH_2OPG,  ,  ,  , or  , or R_2' and R_2'' can be joined to form =O, and PG is a protecting group. In some embodiments, PG is an acyl group (*e.g.*, acetyl, benzoyl, chloroacetyl, dichloroacetyl, trichloroacetyl, trifluoroacetyl, methoxyacetyl, o-(dibromomethyl)benzoyl, *etc.*). In some embodiments, PG is a carbonate group (*e.g.*, methyl carbonate, 9-fluorenylmethyl carbonate, trichloroethyl carbonate (Troc), 2-(trimethylsilyl)ethyl carbonate (TMSEC), isobutyl carbonate, methyl dithiocarbonate, p-methoxybenzyl carbonate, *etc.*). In some embodiments, PG is a carbamate group (*e.g.*, methylamine carbamate, alkyl N-phenylcarbamate, *etc.*). In some embodiments, PG is an ether group (*e.g.*, methoxylmethyl (MOM), benzyl, p-

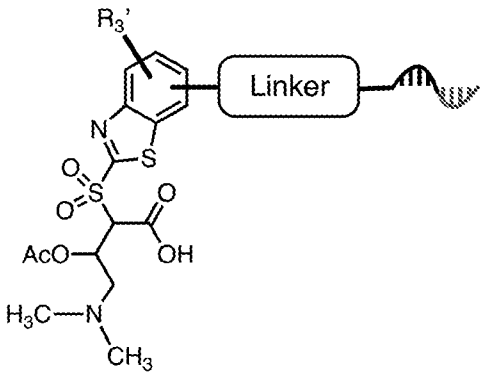
methoxybenzyl, methylthiomethyl ether (MTM), tetrahydropyranyl (THP), *etc.*). In some embodiments, PG is a silyl group (*e.g.*, trimethylsilyl (TMS), triethylsilyl (TES), diethylisopropylsilyl (DEIPS), t-butyldimethylsilyl (TBDMS), diphenylmethylsilyl (DPMS), t-butyldimethylsilyl (TBS), *etc.*).

[51] In some embodiments, R_2' and R_2'' are each independently H or F .

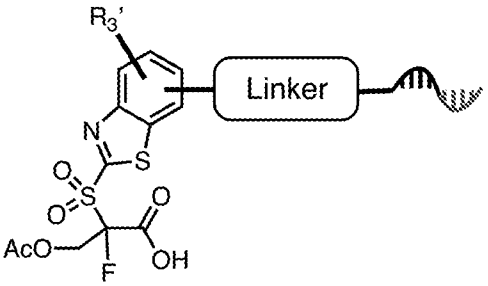
[52] In some embodiments, Ⓑ is  , and the compound of formula I is represented by structure:



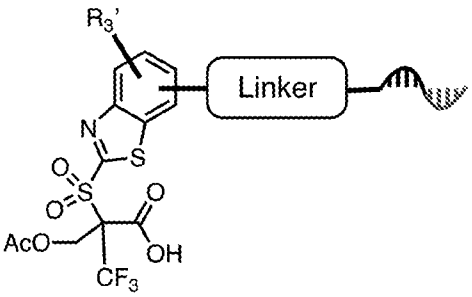
(2);



(12);

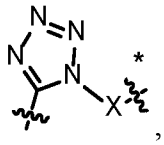


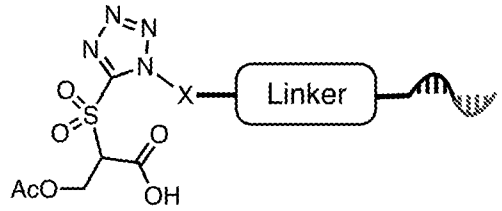
(21); or



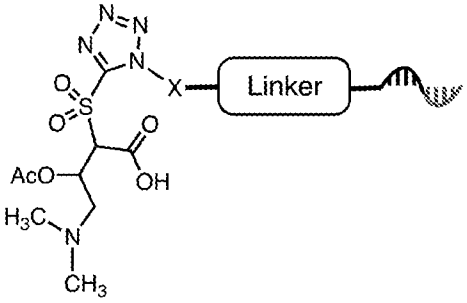
(26),

or a pharmaceutical salt or stereoisomer thereof.

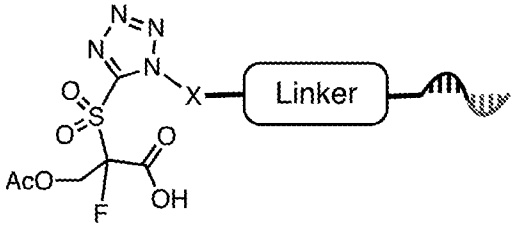
[53] In some embodiments, Ⓑ is  , and the compound of formula I is represented by structure:



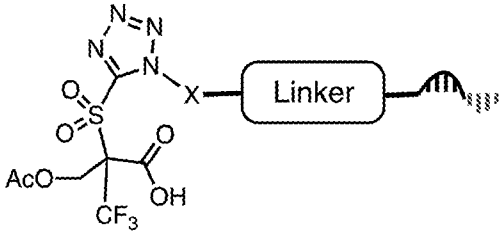
(8);



(18);

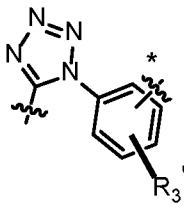


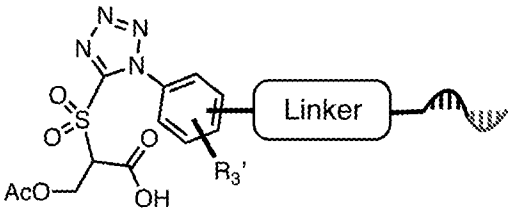
(23); or



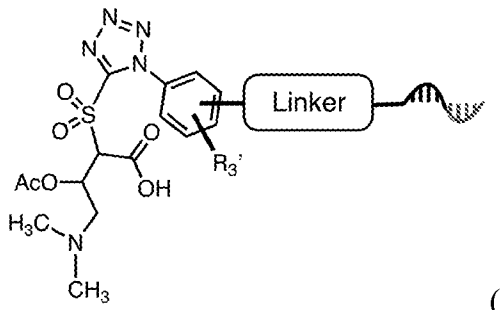
(27),

or a pharmaceutical salt or stereoisomer thereof.

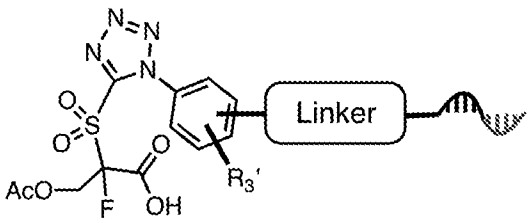
[54] In some embodiments, Ⓑ is  , and the compound of formula I is represented by structure:



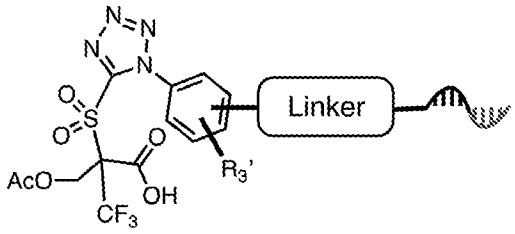
(9);



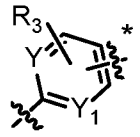
(19);

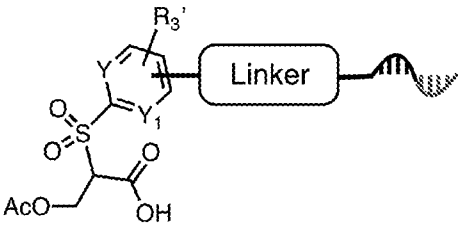


(24); or

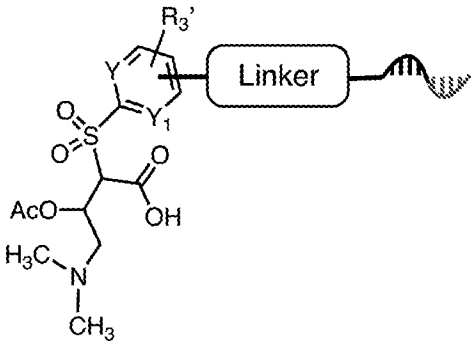


(28), or a pharmaceutical salt or stereoisomer thereof.

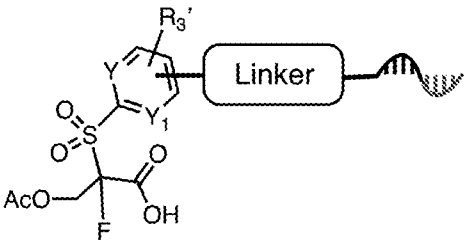
[55] In some embodiments, Ⓑ is  , and the compound of formula I is represented by structure:



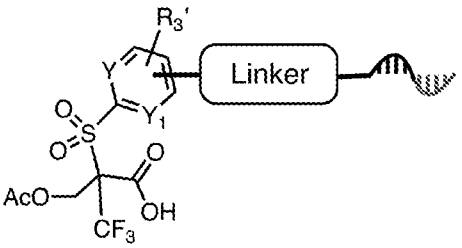
(10);



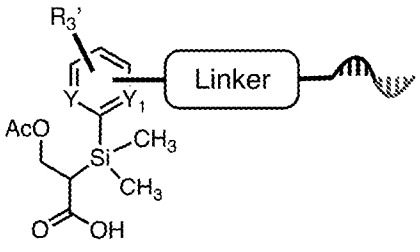
(20);



(25);



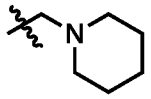
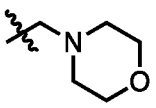
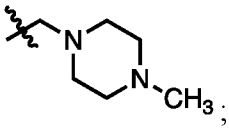
(29); or



(37), or a pharmaceutical salt or stereoisomer thereof.

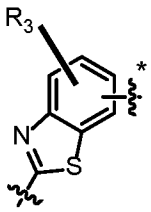
[56] In some embodiments, n is 1.

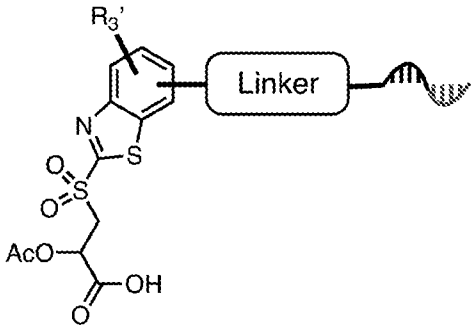
[57] In some embodiments, R_1' and R_1" are each independently H or CH_2NMe_2.

[58] In some embodiments, R_1' and R_1" are each independently H or  ; or  ; or  ;

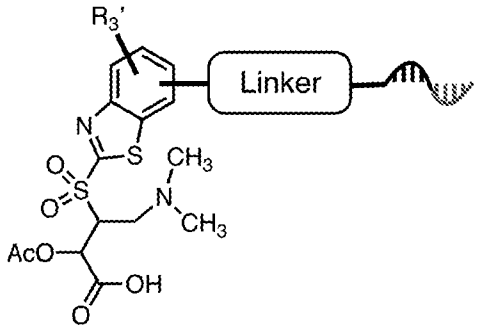
[59] In some embodiments, R_1' and R_1" are both H.

[60] In some embodiments, R_2' and R_2" are each independently H or acetate.

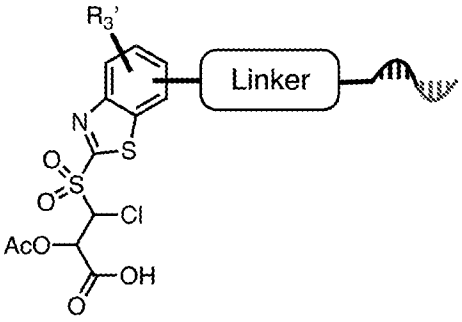
[61] In some embodiments,  is  , and the compound of formula I is represented by structure:



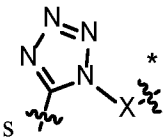
(1); or

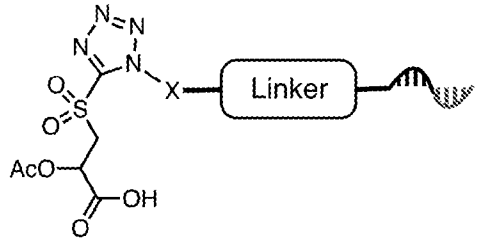


(11); or

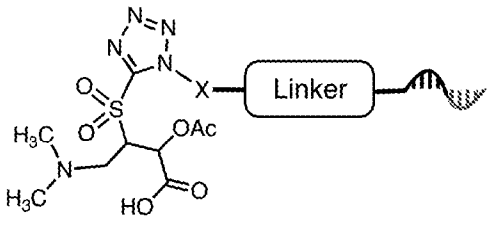


(30), or a pharmaceutical salt or stereoisomer thereof.

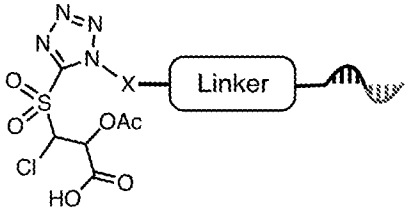
[62] In some embodiments, is  , and the compound of formula I is represented by structure:



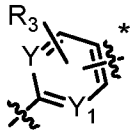
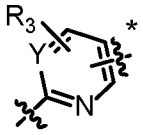
(5);

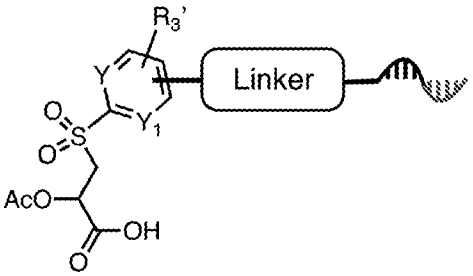


(15);

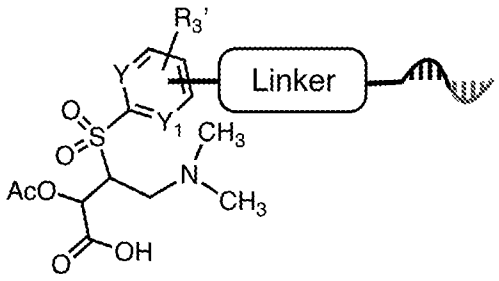


(31), or a pharmaceutical salt or stereoisomer thereof.

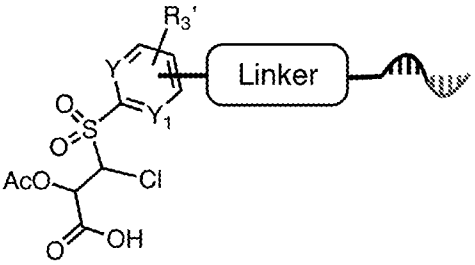
[63] In some embodiments, Ⓑ is  or  , and the compound of formula I is represented by structure:



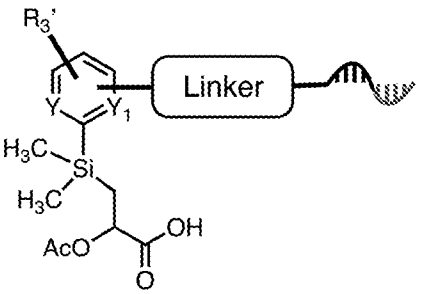
(7);



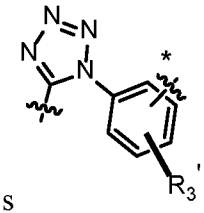
(17);



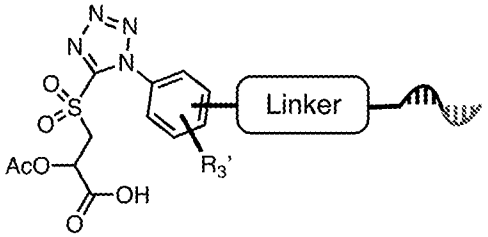
(32);



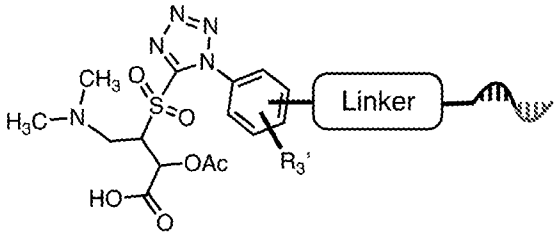
or (36), or a pharmaceutical salt or stereoisomer thereof.



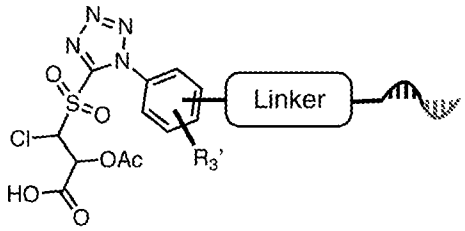
[64] In some embodiments, (B) is , and the compound of formula I is represented by structure:



(6);



(16);



or (33), or a pharmaceutical salt or stereoisomer thereof.

[65] In some embodiments,  is an oligonucleotide tag (*e.g.*, deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) tag) generated by phosphoramidite chemistry (Roy & Caruthers, *Molecules*. 18(11):14268-14284 (2013)), by polymerase enzymes, by enzymatic ligation of oligonucleotides, or by chemical ligation.

[66] In some embodiments,  is an oligonucleotide tag comprising natural or unnatural nucleobases; or oligonucleotides of unnatural backbone structures (*e.g.*, phosphodiester backbone, peptide nucleic acid (PNA) backbone, triazole, phosphorothioate ester backbone, and sugar components including ribose, 2-deoxyribose, threose, glycol, fluoro-arabino, 1,5-anhydrohexitol, *etc.*) (Ochoa & Milam, Molecules. *25*(20):4659 (2020)).

[67] In some embodiments, the oligonucleotide tag is a 5'-O-modified DNA bound to the linker L.

[68] In some embodiments, the oligonucleotide tag is a 3'-O-modified DNA bound to the linker L.

[69] In some embodiments, the oligonucleotide tag is a DNA with a modified nucleobase

bound to the linker L.

[70] In some embodiments, the oligonucleotide tag is single-stranded.

[71] In some embodiments, the oligonucleotide tag is double-stranded.

[72] In some embodiments, the oligonucleotide tag has a length between 4 and 10 nucleotides.

[73] In some embodiments, the oligonucleotide tag has a length between 10 and 20 nucleotides.

[74] In some embodiments, the oligonucleotide tag has a length between 20 and 50 nucleotides.

[75] In some embodiments, the oligonucleotide tag has a length between 50 and 100 nucleotides.

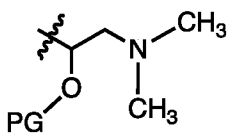
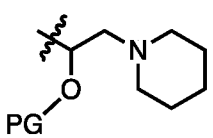
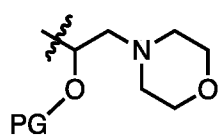
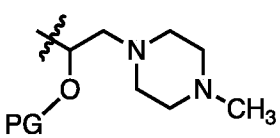
[76] In some embodiments, the oligonucleotide tag has a length between 100 and 200 nucleotides.

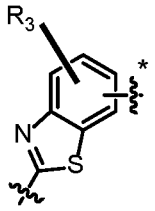
[77] In some embodiments, the oligonucleotide tag has a length between 200 and 500 nucleotides.

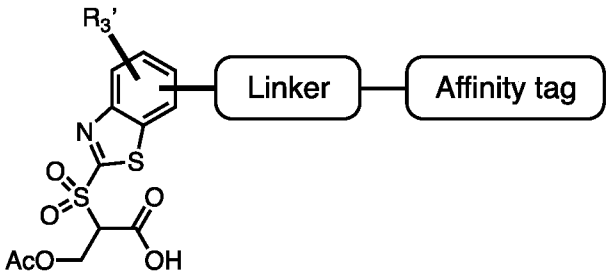
[78] In some embodiments, the oligonucleotide tag has base-pairing complementarity to a DEL barcode as described in Gartner *et al.,* Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10*(7):704–714 (2018), and International Patent Publication No. WO 2019/168654, which is incorporated by reference.

[79] In some embodiments,  is an affinity tag. Exemplary affinity tags that may be used in the disclosed compounds are described in Kimple *et al.*, Curr Protoc Protein Sci. *73*:9.9.1-9.9.23 (2013) and Lotze *et al.*, Mol Biosyst. *12*(6):1731-45 (2016).

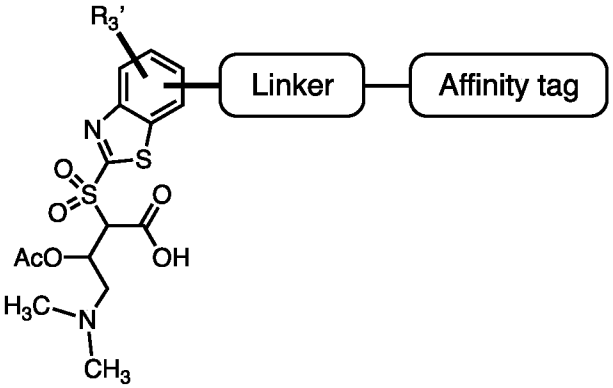
[80] In some embodiments, n is 0.

[81] In some embodiments, wherein n is 0, R_2' and R_2" are each independently H, halogen, fluorine, CF_3, CH_3, CH_2OAc, CH_2OH, $CH(CH_3)OH$, $C(CH_3)_2OH$, CH_2OPG,  ,  ,  ,  , or R_2' and R_2" can be joined to form =O. In some embodiments, the halogen is F.

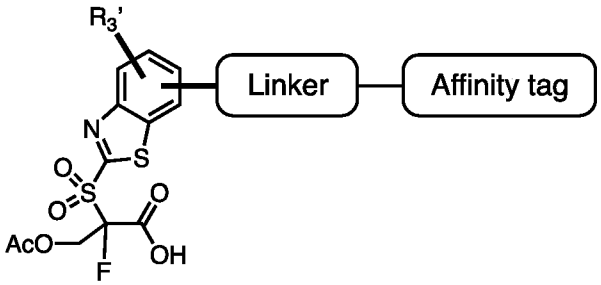
[82] In some embodiments, (B) is  , (A) is an affinity tag, and the compound of formula I is represented by structures:



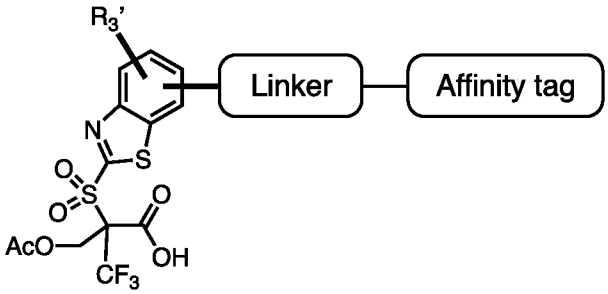
(4);



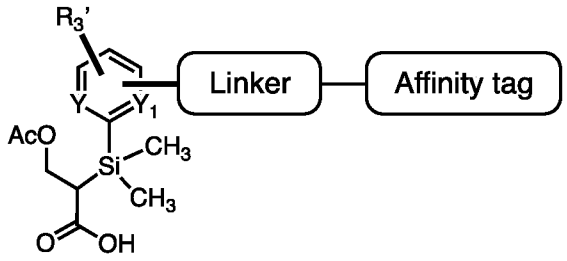
(14);



(22); or



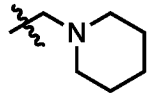
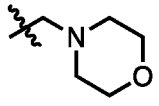
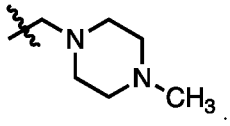
(34); or



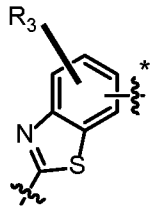
(36), or a pharmaceutical salt or stereoisomer thereof.

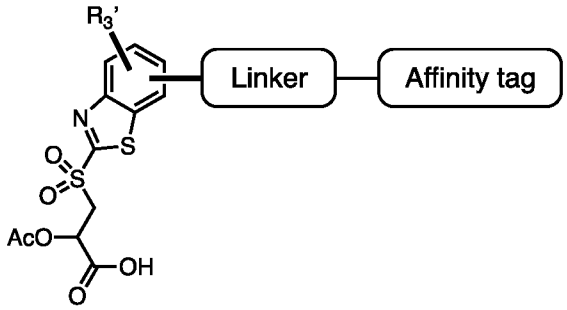
[83] In some embodiments, n is 1.

[84] In some embodiments, R_1' and R_1" are each independently H or CH_2NMe_2.

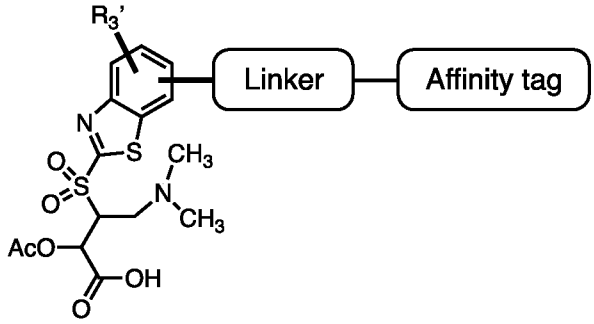
[85] In some embodiments, R_1' and R_1" are each independently H or  ; or  ; or  .

[86] In some embodiments, R_1' and R_1" are both H.

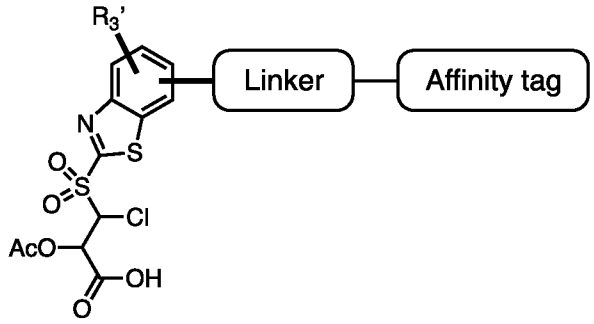
[87] In some embodiments, Ⓑ is  , Ⓐ is an affinity tag, and the compound of formula I is represented by structures:



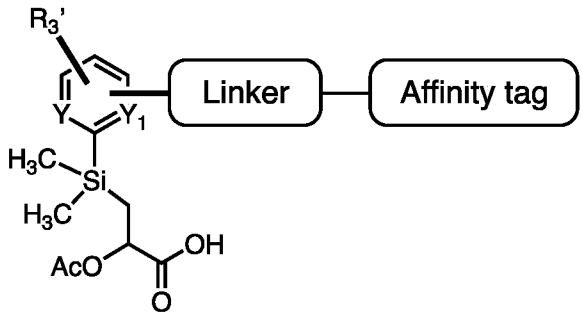
(3);



(13); or



(35); or



(38), or a pharmaceutically acceptable salt or stereoisomer thereof.

[88] In some embodiments, the affinity tag is biotin or a biotin analog.

[89] In some embodiments, the affinity tag is a peptide epitope for antibody-based affinity purification (*e.g.*, Human influenza hemagglutinin (HA) tag, Myc tag, Flag tag, *etc.*).

[90] In some embodiments, the affinity tag is a chloroalkyl group (also known as a Halo-tag).

[91] In some embodiments, the affinity tag is a benzylated nucleobase analog (*e.g.*, a SNAP-tag® or CLIP-tag™).

Linkers

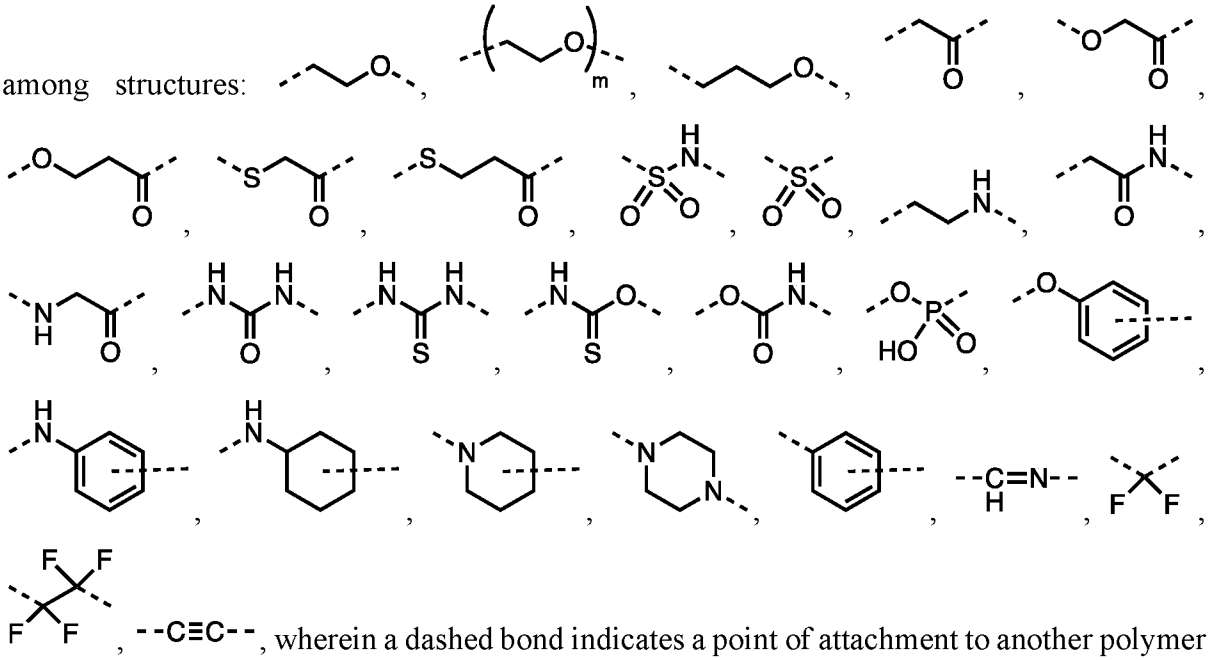
[92] The linker (L) provides a covalent attachment between (A) and (B).

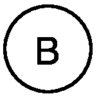
[93] In some embodiments, the linker (L) is a branched or unbranched, linear or cyclic, substituted or unsubstituted, saturated or unsaturated, group having between 2 and 80 carbon atoms, and optionally having one or more heteroatoms selected from O, N, or S. A variety of

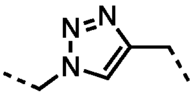
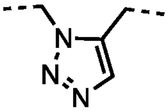
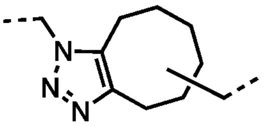
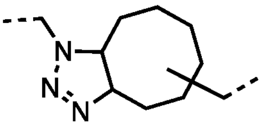
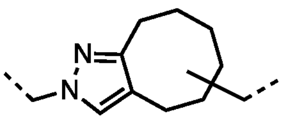
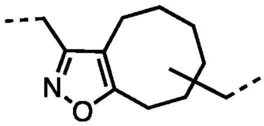
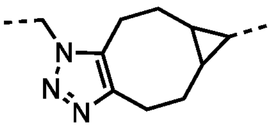
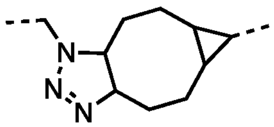
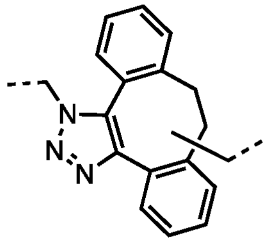
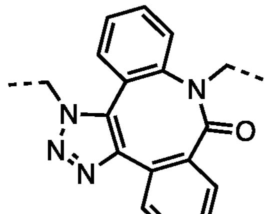
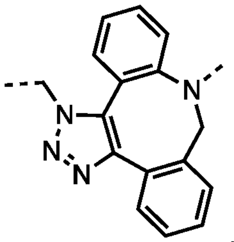
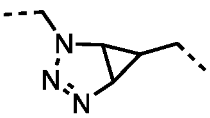
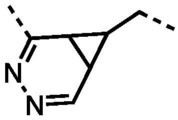
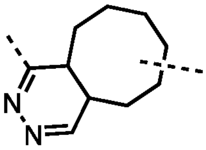
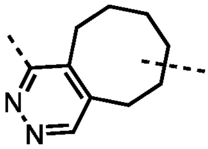
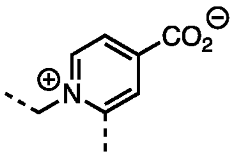
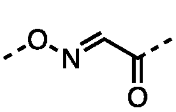
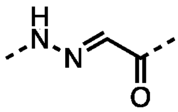
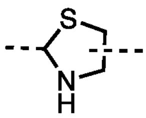
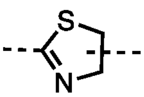
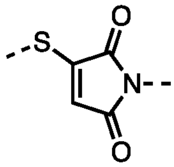
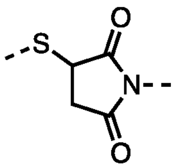
linkers are known to one of skill in the art and may be used in the masked warhead compounds described herein. For example, in certain embodiments, L comprises one or more optionally substituted groups selected from amino acids, polyether chains, aliphatic groups, and any combinations thereof. In certain embodiments, L consists of one or more optionally substituted groups selected from amino acids, polyether chains, aliphatic groups, and any combinations thereof. In certain embodiments, L consists of one or more groups selected from amino acids, polyether chains, aliphatic groups, and any combinations thereof.

[94] In certain embodiments, L is a covalent bond or a bivalent C_{1-30} saturated or unsaturated, straight or branched, hydrocarbon chain, wherein 1-10 methylene units of L are optionally and independently replaced by cyclopropylene, -N(H)-, -N(C_{1-4} alkyl)-, -N(C_{3-5} cycloalkyl)-, -O-, -C(O)-, -S-, -SO-, or -SO_2-. In certain embodiments, L is a covalent bond.

[95] In certain embodiments, the linker is a bivalent C_{1-20} saturated or unsaturated, straight or branched, hydrocarbon chain, wherein 1-7 methylene units of L are optionally and independently replaced by -N(H)-, -N(C_{1-4} alkyl)-, -O-, or -C(O)-. In certain embodiments, L is a bivalent C_{5-15} saturated or unsaturated, straight or branched, hydrocarbon chain, wherein 1-7 methylene units of L are optionally and independently replaced by -N(H)-, -N(C_{1-4} alkyl)-, -O-, or -C(O)-.

[96] In certain embodiments, the linker comprises a polymer defined as L_1 having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 homotypically- or heterotypically-repeating subunits selected among structures:  , wherein a dashed bond indicates a point of attachment to another polymer

unit, to a saturated or unsaturated alkyl chain, to a linear or branched alkyl chain, to a methylene, to an amino acid, to an oligopeptide, to  , to  , or to the rest of the compound, and m is a number between 1 and 12.

[97] In certain embodiments, the linker (L) comprises a substituted "Click chemistry" product exemplified by any one of L_2 structures:  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  or  that may be part of larger structures where H atoms are substituted with fluorine, alkyl, phenyl, and aryl groups, wherein a dashed bond indicates a point of attachment to an L_1 structure, to a saturated or unsaturated alkyl chain, to a linear or branched alkyl chain, to a

methylene, to an amino acid, to a peptide, to  or  .

[98] In certain embodiments, the linker L is described by the formulas L_1–L_2, L_1–L_2–L_1', L_1–L_1'–L_2, wherein L_1 and L_1' are comprised of the same or of different polymer sub-unit arrangements.

[99] In certain embodiments, L comprises a polyethylene glycol chain ranging in size from about 1 to about 12 ethylene glycol units, from about 1 to about 10 ethylene glycol units, from about 2 to about 6 ethylene glycol units, from about 2 to about 5 ethylene glycol units, or from about 2 to about 4 ethylene glycol units.

[100] In certain embodiments, L is optionally substituted (poly)ethyleneglycol having between 1 and about 100 ethylene glycol units, between about 1 and about 50 ethylene glycol units, between 1 and about 25 ethylene glycol units, between about 1 and about 10 ethylene glycol units, between 1 and about 8 ethylene glycol units, between 1 and about 6 ethylene glycol units, between 2 and about 4 ethylene glycol units, or optionally substituted alkyl groups interdispersed with optionally substituted, O, N, S, P or Si atoms. In certain embodiments, L is substituted with an aryl, phenyl, benzyl, alkyl, alkylene, or heterocycle group.

[101] In certain embodiments, L is $-(A^L)_q-$, wherein:

q is an integer greater than or equal to 1 (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10);

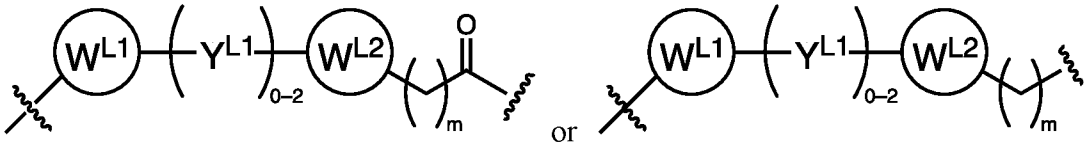
each A^L is independently selected from the group consisting of a bond, $CR^{L1}R^{L2}$, O, S, SO, SO^2, NR^{L3}, SO_2NR^{L3}, $SONR^{L3}$, $CONR^{L3}$, $NR^{L3}CONR^{L4}$, $NR^{L3}SO_2NR^{L4}$, CO, $CR^{L1}=CR^{L2}$, C≡C, $SiR^{L1}R^{L2}$, $P(O)R^{L1}$, $P(O)OR^{L1}$, $NR^{L3}C(=NCN)NR^{L4}$, $NR^{L3}C(=NCN)$, $NR^{L3}C(=CNO_2)NR^{L4}$, C_{3-11}cycloalkyl optionally substituted with 0-6 R^{L1} and/or R^{L2} groups, C_{5-13} spirocycloalkyl optionally substituted with 0-9 R^{L1} and/or R^{L2} groups, C_{3-11}heterocyclyl optionally substituted with 0-6 R^{L1} and/or R^{L2} groups, C_{5-13} spiroheterocycloalkyl optionally substituted with 0-8 R^{L1} and/or R^{L2} groups, aryl optionally substituted with 0-6 R^{L1} and/or R^{L2} groups, heteroaryl optionally substituted with 0-6 R^{L1} and/or R^{L2} groups, where R^{L1} or R^{L2}, each independently are optionally linked to other groups to form cycloalkyl and/or heterocyclyl moiety, optionally substituted with 0-4 R^{L5} groups; and

R^{L1}, R^{L2}, R^{L3}, R^{L4} and R^{L5} are, each independently, H, halo, C_{1-8}alkyl, OC_{1-8}alkyl, SC_{1-8}alkyl, NHC_{1-8}alkyl, $N(C_{1-8}alkyl)_2$, C_{3-11}cycloalkyl, aryl, heteroaryl, C_{3-11}heterocyclyl, OC_{1-8}cycloalkyl, SC_{1-8}cycloalkyl, NHC_{1-8}cycloalkyl, $N(C_{1-8}cycloalkyl)_2$, $N(C_{1-8}cycloalkyl)(C_{1-}$

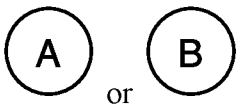
8alkyl), OH, NH_2, SH, SO_2C_{1-8}alkyl, P(O)(OC_{1-8}alkyl)(C_{1-8}alkyl), P(O)(OC_{1-8}alkyl)$_2$, CC-C_{1-8}alkyl, CCH, CH=CH(C_{1-8}alkyl), C(C_{1-8}alkyl)=CH(C_{1-8}alkyl), C(C_{1-8}alkyl)=C(C_{1-8}alkyl)$_2$, $Si(OH)_3$, Si(C_{1-8}alkyl)$_3$, Si(OH)(C_{1-8}alkyl)$_2$, COC_{1-8}alkyl, CO_2H, halogen, CN, CF_3, CHF_2, CH_2F, NO_2, SF_5, SO_2NHC_{1-8}alkyl, $SO_2N(C_{1-8}$alkyl)$_2$, $SONHC_{1-8}$alkyl, $SON(C_{1-8}$alkyl)$_2$, $CONHC_{1-8}$alkyl, $CON(C_{1-8}$alkyl)$_2$, N(C_{1-8}alkyl)CONH(C_{1-8}alkyl), N(C_{1-8}alkyl)CON(C_{1-8}alkyl)$_2$, NHCONH(C_{1-8}alkyl), NHCON(C_{1-8}alkyl)$_2$, $NHCONH_2$, N(C_{1-8}alkyl)$SO_2NH(C_{1-8}$alkyl), N(C_{1-8}alkyl) $SO_2N(C_{1-8}$alkyl)$_2$, $NHSO_2NH(C_{1-8}$alkyl), $NHSO_2N(C_{1-8}$alkyl)$_2$, or $NHSO_2NH_2$.

[102] In some embodiments, q is 1 to 2. In some embodiments, q is 1 to 5. In some embodiments, q is 1 to 10. In some embodiments, q is 1 to 20. In some embodiments, q is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In some embodiments, q is an integer from 1 to 100, 1 to 90, 1 to 80, 1 to 70, 1 to 60, 1 to 50, 1 to 40, or 1 to 30.

[103] In certain embodiments, L is a group of the formula:



wherein:

the symbol " ~~~ " indicates a point of attachment to 

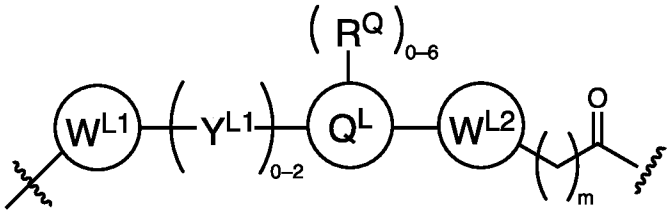
;

W^{L1} and W^{L2} are each independently a 4-8 membered ring with 0-4 heteroatoms, optionally substituted with R^Q; wherein each R^Q is independently a H, halo, OH, CN, CF_3, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 alkoxy, or 2 R^Q groups are taken together with the atom they are attached to, form a 4-8 membered ring system containing 0-4 heteroatoms;

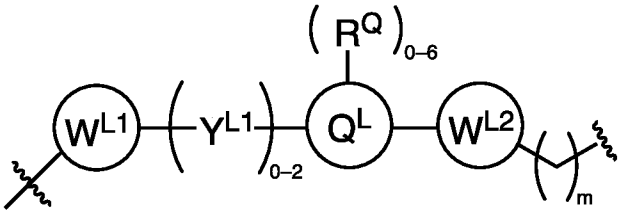
Y^{L1} is each independently a bond, optionally substituted C_1-C_6 alkyl, or optionally substituted 2-8 membered heteroalkyl (*e.g.*, C_1-C_6 alkoxy; and

m is 0-10.

[104] In certain embodiments, L is a group of the formula:



or



wherein:

the symbol " ~~~ " indicates a point of attachment to  or  ,

W^{L1} and W^{L2} are each independently aryl, heteroaryl, cyclic, heterocyclic, C_{1-6} alkyl, bicyclic, biaryl, biheteroaryl,or biheterocyclic, each optionally substituted with R^Q; wherein each R^Q is independently a H, halo, OH, CN, CF_3, hydroxyl, nitro, C≡CH, C_{2-6} alkenyl, C_{2-6} alkynyl, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 alkoxy, optionally substituted O-C_{1-3}alkyl (*e.g.*, C_{1-3} haloalkoxyl), OH, NH_2, $NR^{Y1}R^{Y2}$, CN, or 2 R^Q groups are taken together with the atom they are attached to, form a 4-8 membered ring system containing 0-4 heteroatoms;

Y^{L1} is each independently a bond, NR^{YL1}, O, S, NR^{YL2}, $CR^{YLl}R^{YL2}$, C=O, C=S, SO, SO_2, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 alkoxy;

Q^L is a 3-6 membered alicyclic or aromatic ring with 0-4 heteroatoms, optionally bridged, optionally substituted with 0-6 R^Q; wherein each R^Q is independently H, optionally substituted C_{1-6} alkyl, optionally substituted C_{1-6} alkoxyl, or 2 R^Q groups are taken together with the atom they are attached to, form a 3-8 membered ring system containing 0-2 heteroatoms);

RY^{L1}, RY^{L2} are each independently H, OH, optionally substituted C_{1-6} alkyl, or R^1, R^2 together with the atom they are attached to, form a 3-8 membered ring system containing 0-2 heteroatoms); and

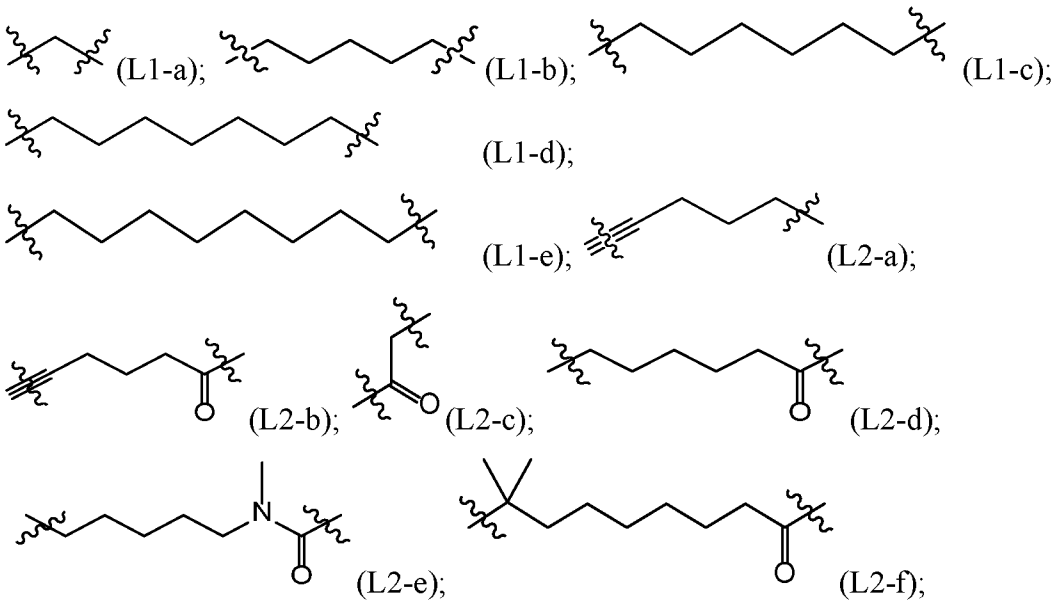
m is 0-10.

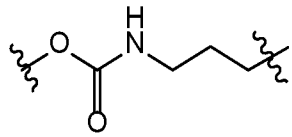
[105] In some embodiments, L is selected from the group consisting of -$NR(CH_2)_m$-(lower alkyl)-, -$NR(CH_2)_m$-(lower alkoxyl)-, -$NR(CH_2)_m$-(lower alkoxyl)-OCH_2-, -$NR(CH_2)_m$-(lower alkoxyl)-(lower alkyl)-OCH_2-, -$NR(CH_2)_m$-(cycloalkyl)-(lower alkyl)-OCH_2-, -$NR(CH_2)_m$-

(hetero cycloalkyl)-, -NR(CH_2CH_2O)$_m$-(lower alkyl)-O-CH_2-, -NR(CH_2CH_2O)$_m$-(hetero cycloalkyl)-O-CH_2-, -NR(CH_2CH_2O)$_m$-Aryl-O-CH_2-, - NR(CH_2CH_2O)$_m$-(hetero aryl)-O-CH_2-, -NR(CH_2CH_2O)$_m$-(cyclo alkyl)-O-(hetero aryl)-O-CH_2-, -NR(CH_2CH_2O)$_m$-(cyclo alkyl)-O-Aryl-O-CH_2-, -NR(CH_2CH_2O)$_m$-(lower alkyl)-NH-Aryl-O-CH_2-, -NR(CH_2CH_2O)$_m$-(lower alkyl)-O-Aryl-CH_2, -NR(CH_2CH_2O)$_m$-cycloalkyl-O-Aryl-, -NR(CH_2CH_2O)$_m$-cycloalky1-O-(heteroaryl), -NR(CH_2CH_2)$_m$ -(cycloalkyl)-O-(heterocycle)-CH_2, -NR(CH_2CH_2)$_m$-(heterocycle)-(heterocycle)-CH_2, - N(R^1R^2)-(heterocycle)-CH_2; wherein m of L can be 0 to 10; R of L can be H, lower alkyl; and R^1 and R^2 of L can form a ring with the connecting N.

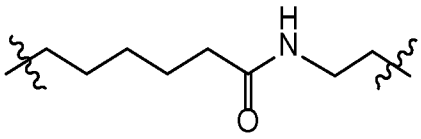
[106] The description above describes multiple embodiments relating to compounds of Formula I or II. The present disclosure specifically contemplates all combinations of the embodiments.

[107] In some embodiments, the linker is represented by any one of structures:

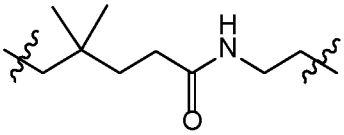




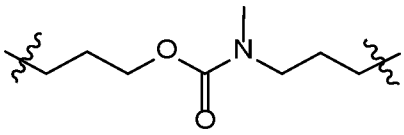
(L2-g);



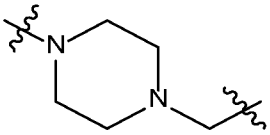
(L3-a);



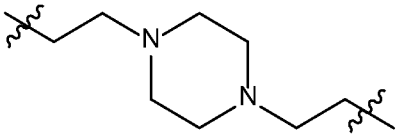
(L3-b);



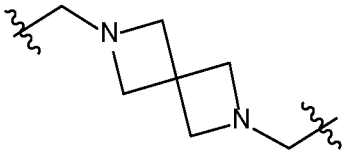
(L3-d);



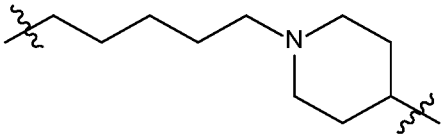
(L4-a);



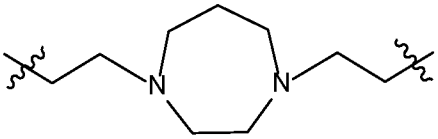
(L4-b);



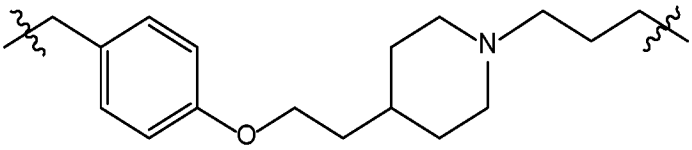
(L4-c);



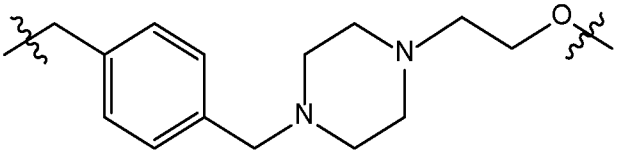
(L4-d);



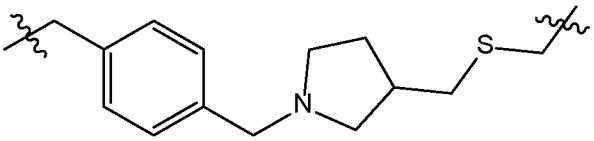
(L4-e);



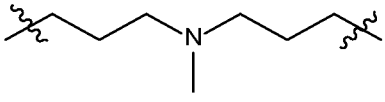
(L6-a);



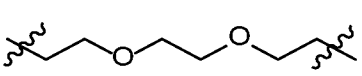
(L6-b);



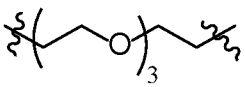
(L6-c);



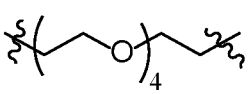
(L7-a);



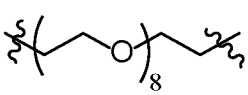
(L8-a);



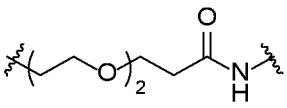
(L8-b);



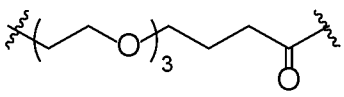
(L8-c);



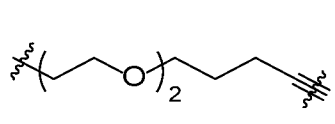
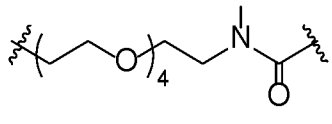
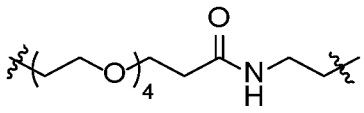
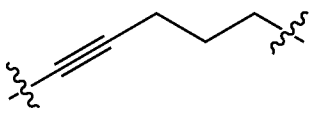
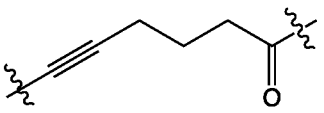
(L8-d);



(L9-a);



(L9-b);


(L9-c);

(L9-d);

(L9-e);

(L9-f); and

(L9-g).

[108] Compounds disclosed herein may be in the form of a free acid or free base, or a pharmaceutically acceptable salt. As used herein, the term "pharmaceutically acceptable" in the context of a salt refers to a salt of the compound that does not abrogate the biological activity or properties of the compound, and is relatively non-toxic, *i.e.*, the compound in salt form may be administered to a subject without causing undesirable biological effects (such as dizziness or gastric upset) or interacting in a deleterious manner with any of the other components of the composition in which it is contained. The term "pharmaceutically acceptable salt" refers to a product obtained by reaction of the compound of the present disclosure with a suitable acid or a base. Examples of pharmaceutically acceptable salts of the compounds of this disclosure include those derived from suitable inorganic bases such as Li, Na, K, Ca, Mg, Fe, Cu, Al, Zn and Mn salts. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate, bisulfate, phosphate, isonicotinate, acetate, lactate, salicylate, citrate, tartrate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, 4-methylbenzenesulfonate or p-toluenesulfonate salts and the like. Certain compounds of the disclosure can form pharmaceutically acceptable salts with various organic bases such as lysine, arginine, guanidine, diethanolamine or metformin.

[109] Compounds as disclosed herein may have at least one chiral center. Therefore, they may be in the form of a stereoisomer. As used herein, the term "stereoisomer" embraces all isomers of individual compounds that differ only in the orientation of their atoms in space. The term stereoisomer includes mirror image isomers (enantiomers which include the (R-) or (S-) configurations of the compounds), mixtures of mirror image isomers (physical mixtures of the enantiomers, and racemates or racemic mixtures) of compounds, geometric (cis/trans or E/Z, R/S) isomers of compounds and isomers of compounds with more than one chiral center that

are not mirror images of one another (diastereoisomers). The chiral centers of the compounds may undergo epimerization *in vivo*; thus, for these compounds, administration of the compound in its (R-) form is considered equivalent to administration of the compound in its (S-) form. Accordingly, the compounds of the present disclosure may be made and used in the form of individual isomers and substantially free of other isomers, or in the form of a mixture of various isomers, *e.g.*, racemic mixtures of stereoisomers.

[110] In some embodiments, a compound disclosed herein is an isotopic derivative in that it has at least one desired isotopic substitution of an atom, at an amount above the natural abundance of the isotope, *i.e.*, enriched. In one embodiment, the compound includes deuterium or multiple deuterium atoms. Substitution with heavier isotopes such as deuterium, *i.e.* ^{2}H, may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased *in vivo* half-life or reduced dosage requirements, and thus may be advantageous in some circumstances.

[111] In addition, compounds of the present disclosure embrace N-oxides, crystalline forms (also known as polymorphs), active metabolites of the compounds having the same type of activity, tautomers, and unsolvated as well as solvated and hydrated forms with pharmaceutically acceptable solvents such as water, ethanol, and the like, of the compounds. The solvated forms of the conjugates presented herein are also considered to be disclosed herein.

Methods of Synthesis

[112] In some embodiments, the present disclosure is directed to methods for making a compound as disclosed herein or a pharmaceutically acceptable salts or stereoisomers thereof. Broadly, the inventive compounds or pharmaceutically-acceptable salts or stereoisomers thereof, may be prepared by any process known to be applicable to the preparation of chemically related compounds. The compounds disclosed herein will be better understood in connection with the synthetic schemes described in various working examples that illustrate non-limiting methods by which the compounds of the disclosure may be prepared.

Methods of Generating Masked-Warhead and Warhead-Bearing DEL Compounds

[113] In some aspects, the present disclosure is directed to methods of creating an electrophilic warhead-bearing DNA-Encoded Library (eDEL) comprising:

coupling the compound of formula I or II with a DNA-Encoded Library to generate a stable masked-warhead DEL (mwDEL) intermediate;

purifying the mwDEL intermediate;

unmasking the mwDEL intermediate to generate an activated eDEL; and

purifying the activated eDEL.

[114] In some embodiments, the coupling of the compound of formula I with a DEL is achieved via an amide coupling reaction (Shi *et al.*, *RSC Adv. 11*(4):2359–2376 (2021), Gartner *et al.*, Angew. Chem. Int. Ed., *41*:1796-1800 (2002)).

[115] In some embodiments, the coupling of the compound of formula I with a DEL is proceeded by base pairing interactions between the DEL barcode and an oligonucleotide tag as described in Gartner *et al.*, Angew. Chem Int. Ed. *42*(12):1370-1375 (2003), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), and Usanov *et al.*, Nat. Chem. *10*(7):704–714 (2018). Base pairing interactions between a DEL barcode and an oligonucleotide tag are also described in International Patent Publication No WO 2019/168654, U.S. Patent Application Publication Nos 2006/223086, 2005/0170376, and 2009/003582, and U.S. Patent No 7,479,472, each of which is incorporated herein by reference.

Methods of Generating Masked-Warhead and Warhead-Bearing DEL Compounds

[116] The present disclosure is directed to a method in which the masked warhead-bearing DEL intermediate comprises an arylsulfone.

[117] In some embodiments, the present disclosure is directed to a method in which the masked warhead-bearing DEL intermediate is a Julia-Kocienski reaction intermediate.

[118] In some embodiments, the present disclosure is directed to a method in which the masked warhead-bearing DEL intermediate is a Julia-Kocienski reaction intermediate, wherein the hydroxyl group is available for elimination. In some embodiments, the hydroxyl group is eliminated through a Smiles rearrangement (Levy *et al.*, J. Chem. Soc., 1931, 3264-3269). In some embodiments, the hydroxyl group is eliminated through a beta elimination reaction (Clayden J; Greeves N; Warren S Organic chemistry. 2nd Edition ed.; Oxford University Press: New York, 201).

[119] In some embodiments, the present disclosure is directed to a method in which the masked warhead-bearing DEL intermediate is a Julia-Kocienski reaction intermediate, wherein the hydroxyl group is attached to a Protecting Group (PG).

[120] In some embodiments, the present disclosure is directed to a method in which the stable masked-warhead DEL intermediate is purified via gel electrophoresis purification.

[121] In some embodiments, the present disclosure is directed to a purification method that

involves polyacrylamide gel electrophoresis.

[122] In some embodiments, the present disclosure is directed to a purification method that involves agarose gel electrophoresis.

[123] In some embodiments, the present disclosure is directed to a purification method that involves capillary gel electrophoresis.

[124] In some embodiments, the present disclosure is directed to a method in which the stable masked-warhead mwDEL intermediate is purified via chromatographic separation.

[125] In some embodiments, the present disclosure is directed to a purification method that involves liquid chromatography (LC).

[126] In some embodiments, the present disclosure is directed to a purification method that involves size-exclusion chromatography (SEC).

[127] In some embodiments, the present disclosure is directed to a purification method that involves ion exchange chromatography (*e.g.*, cation- or anion-exchange chromatography).

[128] In some embodiments, the present disclosure is directed to a method in which the stable masked-warhead DEL intermediate is purified via precipitation (*e.g.*, ethanol precipitation).

[129] In some embodiments, the present disclosure is directed to a method in which the stable masked-warhead DEL intermediate is purified via affinity-based methods.

[130] In some embodiments, the present disclosure is directed to a purification method that involves an avidin protein.

[131] In some embodiments, the present disclosure is directed to a purification method that involves oligonucleotide base-pairing.

[132] In some embodiments, the present disclosure is directed to a purification method that involves antibodies (*e.g.* anti-HA, anti-Flag, anti-Myc, *etc.*).

[133] In some embodiments, two or more purification methods are applied in series.

[134] Other purification methods that may be used are described in Usanov *et al.*, Nat. Chem. *10*(7):704–714 (2018) and in International Patent Publication WO 2019/168654, which is incorporated herein by reference.

[135] In some embodiments, the purified mwDEL intermediate is storable.

[136] In some embodiments, the purified mwDEL intermediate is storable at a temperature between -150 °C and 25 °C.

[137] In some embodiments, the purified mwDEL intermediate is storable at a temperature between 25 °C and 40 °C (room temperature).

[138] In some embodiments, the purified mwDEL intermediate is storable at a temperature between 4 °C and room temperature.

[139] In some embodiments, the purified mwDEL intermediate is storable at a temperature between 0 °C and 4 °C.

[140] In some embodiments, the purified mwDEL intermediate is storable at a temperature between -20 °C and room 0 °C.

[141] In some embodiments, the purified mwDEL intermediate is storable at a temperature between -40 °C and room -20 °C.

[142] In some embodiments, the purified mwDEL intermediate is storable at a temperature between -78 °C and room -40 °C.

[143] In some embodiments, the purified mwDEL intermediate is storable at a temperature between -82 °C and room -78 °C.

[144] In some embodiments, the purified mwDEL intermediate is storable at a temperature between -150 °C and room -80 °C.

[145] In some embodiments, the purified mwDEL intermediate is storable in the presence of oxygen.

[146] In some embodiments, the purified mwDEL intermediate is storable in the presence of water.

[147] In some embodiments, the purified mwDEL intermediate is storable in the presence of organic solvents.

[148] In some embodiments, the purified mwDEL intermediate is storable in the presence of dimethyl sulfoxide.

[149] In some embodiments, the purified mwDEL intermediate is storable for one day to up to two or more years.

[150] In some embodiments, the purified mwDEL intermediate is storable for one day.

[151] In some embodiments, the purified mwDEL intermediate is storable for two or more days.

[152] In some embodiments, the purified mwDEL intermediate is storable for one week.

[153] In some embodiments, the purified mwDEL intermediate is storable for two or more weeks.

[154] In some embodiments, the purified mwDEL intermediate is storable for one month.

[155] In some embodiments, the purified mwDEL intermediate is storable for two or more

months.

[156] In some embodiments, the purified mwDEL intermediate is storable for one year.

[157] In some embodiments, the purified mwDEL intermediate is storable for two or more years.

[158] In some embodiments, an amide coupling reagent activates the carboxylic acid group of formula (I) or II for amide bond formation with a DEL.

[159] In some embodiments, the amide coupling reagent that activates the carboxylic acid group of formula I or II is 1-ethyl-3-(3′-dimethyl-aminopropyl)carbodiimide (EDC); *N,N′*-dicyclohexylcarbodiimide (DCC); *N,N′*-diisopropylcarbodiimide (DIC); 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU); 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU); benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP); bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP); (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU); *N*-methyl-2-chloropyridinium tetrafluoroborate; ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma); 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride (DMTMM); *N*-hydroxysuccinimide (NHS); *N*-hydroxysulfosuccinimide (sulfo-NHS); 1-hydroxybenzotriazole (HOBt); 1-hydroxy-7-azabenzotriazole (HOAt); or 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (DhbtOH).

[160] In some embodiments, the mwDEL unmasking involves the use of an acidic aqueous solution.

[161] In some embodiments, the mwDEL unmasking involves the use of a reducing aqueous solution.

[162] In some embodiments, the mwDEL unmasking involves the use of an oxidizing aqueous solution.

[163] In some embodiments, the mwDEL unmasking involves the use of an alkaline aqueous solution.

[164] In some embodiments, the alkaline solution comprises Tris buffer, phosphate buffer, acetate buffer, ammonium hydroxide buffer, dimethylamine buffer, methylamine buffer, citrate buffer, *N*-(2-hydroxyethyl)piperazine-*N*'-(2-ethanesulfonic acid) (HEPES), 2-(*N*-Morpholino) ethanesulfonic acid (MES), 3-(*N*-Morpholino) propanesulfonic acid (MOPS), or *N*-tris hydroxymethyl methyl-3-aminopropanesulfonic acid (TAPS), 4-morpholinebutanesulfonic

acid (MOBS), 3-morpholino-2-hydroxypropanesulfonic acid (MOPSO), (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES), 3-[4-(2-hydroxyethyl)-1-piperazinyl propanesulfonic acid (EPPS or HEPPS), piperazine-*N*,*N*'-bis(2-ethanesulfonic acid) (PIPES), *N*-(2-hydroxyethyl)piperazine-*N*'-(2-hydroxypropanesulfonic acid) (HEPPSO); *N*-(2-hydroxyethyl)piperazine-*N*'-(4-butanesulfonic acid) (HEPBS), *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES), N-cyclohexyl-3-aminopropanesulfonic acid (CAPS), 4-(cyclohexylamino)-1-butanesulfonic acid (CABS), 3-(cyclohexylamino)-2-hydroxyl-1-propanesulfonic acid (CAPSO), *N*,*N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), bis-(2-hydroxyethyl)amino-tris(hydroxymethyl)methane (BisTris), bis(2-hydroxyethyl) aminotris(hydroxymethyl) methane (Bicine), piperazine-*N*,*N*'-Bis[2-hydroxypropanesulfonic acid] (Tricine), or *N*,*N*-bis(2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid (DIPSO).

[165] In some embodiments, the mwDEL unmasking involves the use of an inorganic hydroxide (OH^-) base, such as alkali hydroxides, (*e.g.*, sodium hydroxide, potassium hydroxide), alkaline earth metal hydroxides, (*e.g.*, barium hydroxide), or alkali metal or alkaline earth metal alkoxides, (*e.g.*, sodium methoxide, potassium methoxide, sodium ethoxide, potassium ethoxide, potassium tert-butoxide, *etc.*).

[166] In some embodiments, the mwDEL unmasking involves the use of an inorganic carbonate base, such as alkali metal carbonates (*e.g.*, sodium carbonate, potassium carbonate or caesium carbonate) or alkaline earth metal carbonates (*e.g.*, calcium carbonate, *etc.*).

[167] In some embodiments, the mwDEL unmasking involves the use of an organic base or amino base (trialkyl(C_1-C_6)amines), such as triethylamine, (dialkyl(C_1-C_6)amines), such as dimethylamnine, (alkyl(C_1-C_6)amines) such as methylamine, ammonia, hydrazines, phosphazenes, or heterocycles, such as 1,4-diazabicyclo[2.2.2]octane (DABCO), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 1,5-diazabicyclo[4.3.0]non-5-ene (DBN), pyridine, diaminopyridine, 4-dimethylaminopyridine (DMAP), methylpiperidine, or morpholine.

[168] In some embodiments, the mwDEL unmasking involves the use of a hydroxide (OH^-) salt solution.

[169] In some embodiments, the mwDEL unmasking involves the use of a base with pKa >14.

[170] In some embodiments, the mwDEL unmasking reaction is promoted by a solution of pH less than 7.

[171] In some embodiments, the mwDEL unmasking reaction is promoted by a solution of

pH 7 to 8.

[172] In some embodiments, the mwDEL unmasking reaction is promoted by a solution of pH 8 to 9.

[173] In some embodiments, the mwDEL unmasking reaction is promoted by a solution of pH 9 to 10.

[174] In some embodiments, the mwDEL unmasking reaction is promoted by a solution of pH 10 to 11.

[175] In some embodiments, the mwDEL unmasking reaction is promoted by a solution of pH 11 to 12.

[176] In some embodiments, the mwDEL unmasking reaction is promoted by a solution of pH more than 12.

[177] In some embodiments, the activated eDEL comprises acrylamide moieties from the unmasking reaction.

Methods of Use

[178] In some aspects, the present disclosure is directed to an eDEL generated from the methods described above and methods of using the same.

[179] In some embodiments, the eDEL is used in an in vitro selection assay followed by DNA sequencing. In some embodiments, the in vitro selection assay comprises screening protein ligands.

[180] In some embodiments, the in vitro selection assay comprises screening ligands that covalently modify a residue of a protein.

[181] In some embodiments, the in vitro selection assay comprises screening ligands that covalently modify the thiol group of a Cysteine residue of a protein.

[182] In some embodiments, the in vitro selection assay comprises screening ligands that covalently modify the imidazole ring of a Histidine residue of a protein.

[183] In some embodiments, the in vitro selection assay comprises screening ligands that covalently modify the amino group of a Lysine residue of a protein.

[184] In some embodiments, the in vitro selection assay comprises screening ligands that covalently modify the hydroxyl group of a Serine residue of a protein.

[185] In some embodiments, the in vitro selection assay comprises screening ligands that covalently modify the hydroxyl group of a Threonine residue of a protein.

[186] In some embodiments, the in vitro selection assay comprises screening ligands that

covalently modify the phenolic hydroxyl of a Tyrosine residue of a protein.

[187] In some embodiments, the in vitro selection assay comprises screening ligands that covalently modify a carboxylate group of a protein.

[188] In some embodiments, the in vitro selection assay comprises screening ligands that covalently modify an amide group of a protein.

[189] In some embodiments, the in vitro selection assay comprises screening ligands that covalently modify an amino group of a protein.

[190] In some embodiments, the in vitro selection assay comprises screening ligands that covalently modify a residue in the active site of a protein.

[191] In some embodiments, the in vitro selection assay comprises screening ligands that covalently modify a residue in a non-orthosteric site of a protein.

[192] In some embodiments, the in vitro selection assay comprises screening ligands that covalently modify a residue in an allosteric site of a protein.

[193] In some embodiments, the in vitro selection assay comprises screening ligands that covalently modify a residue in a non-catalytic domain of a protein.

[194] The present disclosure also provides uses of the eDELs in the in vitro selection assays described herein.

[195] In some embodiments, the active site (*e.g.*, the orthosteric site, or the catalytic site) is a pocket of a protein comprising amino acids that participate in the catalysis of a chemical reaction (*e.g.*, by H-bonding, acid/base catalysis, transition-state stabilization, proximity, water activation, *etc.*), the binding interactions with an enzymatic cofactor (*e.g.*, ATP, GTP, NADH, heme, vitamins, Zn, Mg, Ca, redox-active metals, *etc.*), or the binding interactions with a substrate (*e.g.*, DNA, RNA, peptide, protein, metabolite, cofactor, vitamins, *etc.*) (Jacobson *et al.*, Trends Biochem Sci *39*(8):363-371 (2014); Izidoro *et al.*, Bioinformatics *31*(6):864–870 (2015); Feehan *et al.*, Nat Commun. *12*(1):3712 (2021)). In some embodiments, the active site comprises the binding pocket defined by nearby amino acids that are within at least about 2, 3, 5, 10, 15, or 20 Å from those amino acids that participate in a chemical reaction, cofactor, or substrate binding.

[196] In some embodiments, the non-orthosteric site is a region of a protein distinct from the active site, which has defined or undefined functions that are accessory or complementary to the active site (*e.g.*, protein-protein interactions, homodimerization, heterodimerization, homo-oligomerization, hetero-oligomerization, protein-DNA interactions, subcellular localization,

compartmentalization, protein stability, protein destabilization, acceptor for ubiquitination, acceptor for phosphorylation, acceptor for acetylation, modulation of ternary structure, domain-domain contacts, substrate recruitment, conformational changes, *etc.*). In some embodiments, the non-orthosteric site comprises a binding pocket defined by amino acids that are at least about 5, 10, 15, or 20 Å away from the amino acids that participate in a chemical reaction, cofactor, or substrate binding. Examples of non-orthosteric sites include pockets in domains such as SH2, PTB, SH3, WW, WD40, PDZ, PH, RING, HECT, UBA, Tudor, CHROMO, BROMO, BIR, TRAF, CARD, pseudokinases, kinase C-lobe, kinase N-lobe pockets, among others (Berdasco *et al.*, Nat Rev Genet, *20*(2):109–127 (2019); Scott *et al.*, Nat Rev Drug Discov. *15*(8):533–550 (2016); Jin *et al.*, Sci Signal., *2*(98):ra76 (2009); Stanton *et al.*, Science *359*(6380):eaao5902 (2018)).

[197] In some embodiments, the allosteric site is a type of non-orthosteric pocket that has defined or undefined functions that are accessory or complementary to the active site by imparting conformational changes to the protein upon the binding of a ligand, substrate, ion, protein partner, or cofactor (*e.g.*, changes in secondary structure, changes in ternary structure, conformational activation, conformational inhibition, transition-state destabilization, change in substrate affinity, *etc.*) (Changeux *et al.*, Nat Rev Mol Cell Biol, *14*(12):819-829 (2013); Ubersax *et al.*, Nat Rev Mol Cell Biol. *8*(7):530-541 (2007)). In some aspects, the allosteric site imparts conformational changes within at least about 2, 3, 5, 10, 15, or 20 Å from those amino acids that participate in a chemical reaction, cofactor, or substrate binding.

[198] In some embodiments, the non-catalytic domain of a protein is distinct from the region comprising the active site (alternatively, the protein has no active site) and this domain has functions that do not immediately produce changes to the chemical structure or composition of a substrate (*e.g.*, proline amide bond isomerization, phospholipid bilayer organization, membrane binding, membrane fusion, membrane translocation, force, movement, switch behavior, sensors, channels, transporters, antiporters, translocation, storage, proximity, recruitment, protein-protein interactions, protein-DNA interactions, homodimerization, heterodimerization, homo-oligomerization, hetero-oligomerization, subcellular localization, compartmentalization, scaffolding, protein stability, protein destabilization, acceptor for ubiquitination, acceptor for phosphorylation, acceptor for acetylation, modulation of ternary structure, domain-domain contacts, substrate recruitment to a multi-protein complex, *etc.*). Examples of non-catalytic domains and non-catalytic proteins include proline isomerases,

pseudokinases, chaperones, ion channels, voltage-gated ion channels, scaffold proteins, SNAREs, motor proteins, amyloid fibrils, phospholipid flippases, GTPase exchange factors (GEFs), extracellular receptors, transcription factors, ATPases, epigenetic mark readers, and non-orthosteric domains exemplified above, among others (Berdasco *et al.*, Nat Rev Genet, *20*(2):109–127 (2019); Scott *et al.*, Nat Rev Drug Discov. *15*(8):533–550 (2016); Jin *et al.*, Sci Signal., *2*(98):ra76 (2009)).

[199] Methods of screening for covalent ligands using DELs are known in the art, for example, Chan, *et al.*, Curr. Opin. Chem. Biol. *26*:55-61 (2015), and Zhu *et al.*, SLAS Discov. Adv. Sci. Drug Discov. *24*(2):169–174 (2019)). The methods of screening for covalent ligands involve the incubation of a warhead-bearing DEL with a target protein, followed by: solid-supported separation of the protein and protein-bound DEL library members for Polymerase chain reaction (PCR) amplification and DNA sequencing analysis; or alternatively, interaction-dependent PCR and DNA sequencing analysis (Chan *et al.*, J. Am. Chem. Soc. *139*(30):10192–10195 (2017); Gorin *et al.*, J. Am. Chem. Soc. *131*:9189-9191 (2009)).

[200] Additional methods of screening for covalent ligands using DELs are described in Guilinger *et al.*, Bioorg. Med. Chem. *42:*116223 (2021), Zimmermann *et al.*, Chem. Eur. J. *23*(34):8152–8155 (2017), Zambaldo, *et al.*, *MedChemComm 7*(7):1340–1351 (2016), and Cochrane, *et al.*, ACS Comb. Sci. *21*(5):425–435 (2019).

EXAMPLES

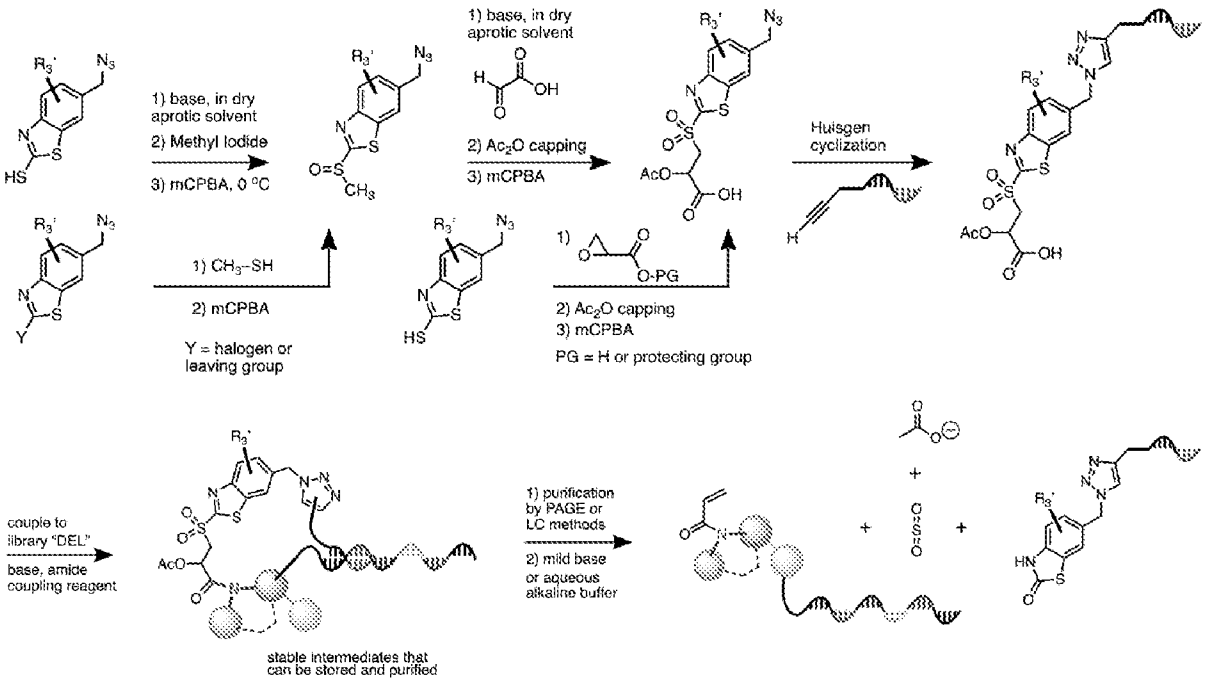
[201] These and other aspects of the present disclosure will be further appreciated upon consideration of the following Examples, which are intended to illustrate certain particular embodiments of the disclosure but are not intended to limit its scope, as defined by the claims.

Abbreviation	**Description**
Ac	acetyl
aq	aqueous
Bt	benzothiazole
DAST-F or DAST	diethylaminosulfur trifluoride
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DEL	DNA-encoded library of small molecules

DIPEA	*N*,*N*-diisopropylethylamine
DMF	dimethylformamide
DNA	deoxyribonucleic acid
DPPA	diphenylphosphoryl azide
EDC	1-ethyl-3-(3′-dimethyl-aminopropyl)carbodiimide
eDEL	electrophilic DEL / electrophilic warhead-bearing DEL
EGFR	epidermal growth factor receptor
Et	ethyl
HA	hemagglutinin antigen
HATU	1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate
HMDS	hexamethyldisilazane
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
i-Pr	isopropyl
LAH	lithium aluminum hydride
LC	liquid chromatography
LG	leaving group
LiHDMS	lithium hexamethyldisilazane
mCPBA	meta-chloroperoxybenzoic acid
Me	methyl
mwDEL	masked-warhead DEL
n-BuLi	n-butyllithium
oligo	oligonucleotide
Oxyma	ethyl cyanohydroxyiminoacetate
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PG	protecting group
Ph	phenyl
RT	room temperature
SEC	size-exclusion chromatography

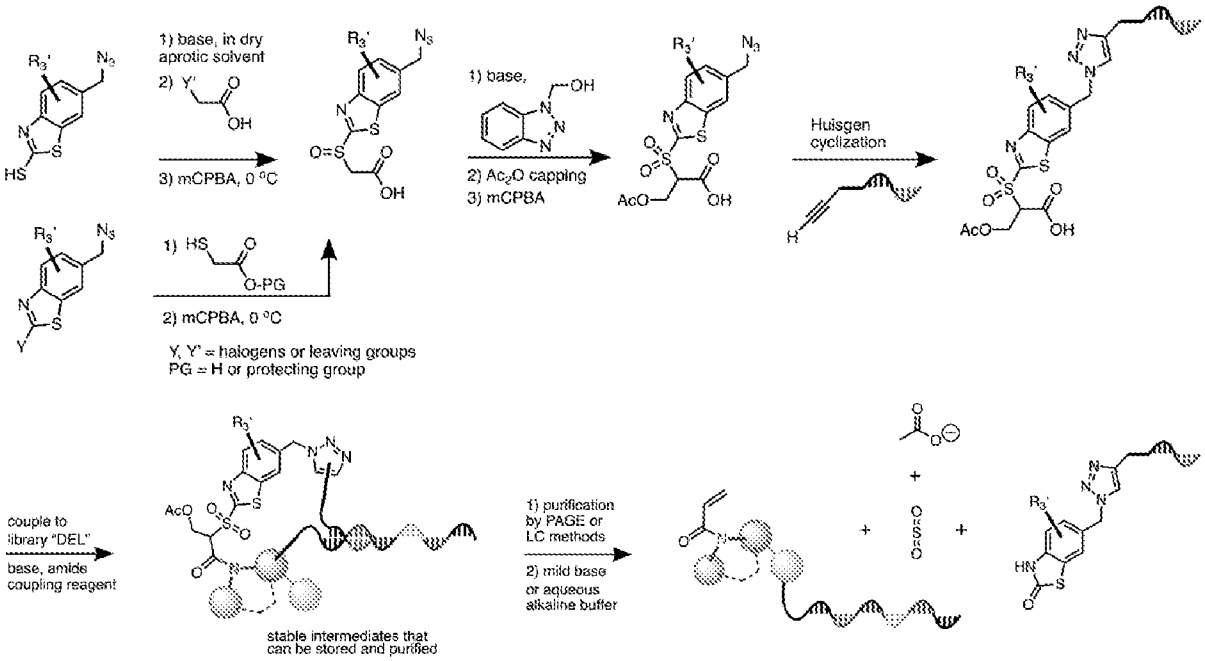
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilane
Ts	toluenesulfonyl

[202] Example 1. Synthesis of benzothiazole sulfone DNA-tagged masked-warhead reagents.



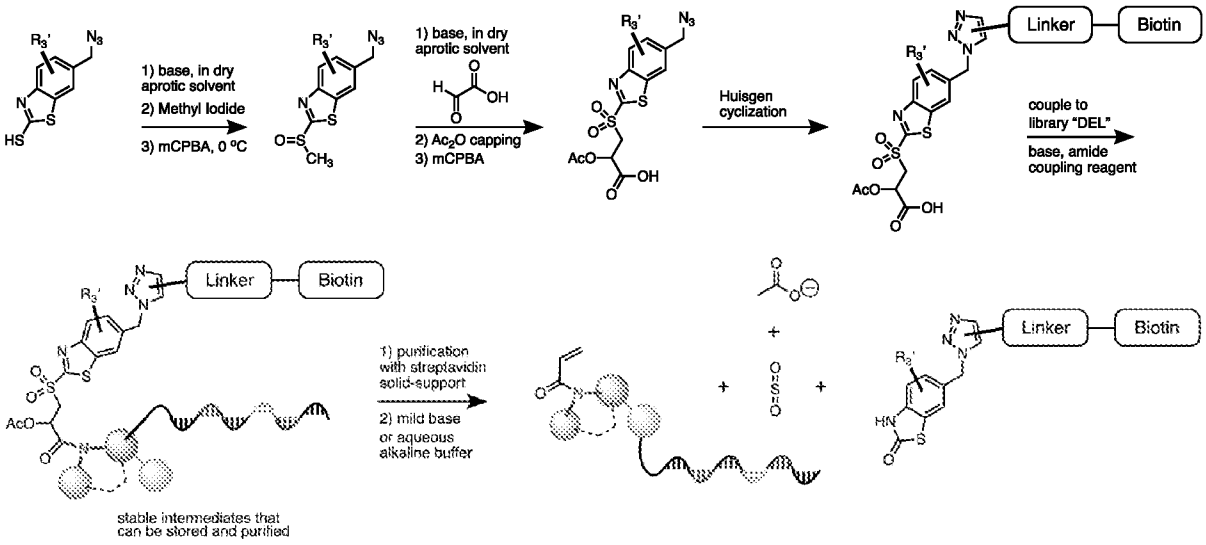
[203] Benzothiazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121*(12):7122–7154 (2021), Gartner *et al.,* Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10*(7):704–714 (2018), and International Patent Publication No. WO 2019/168654.

[204] Example 2. Synthesis of benzothiazole sulfone DNA-tagged masked-warhead reagents.



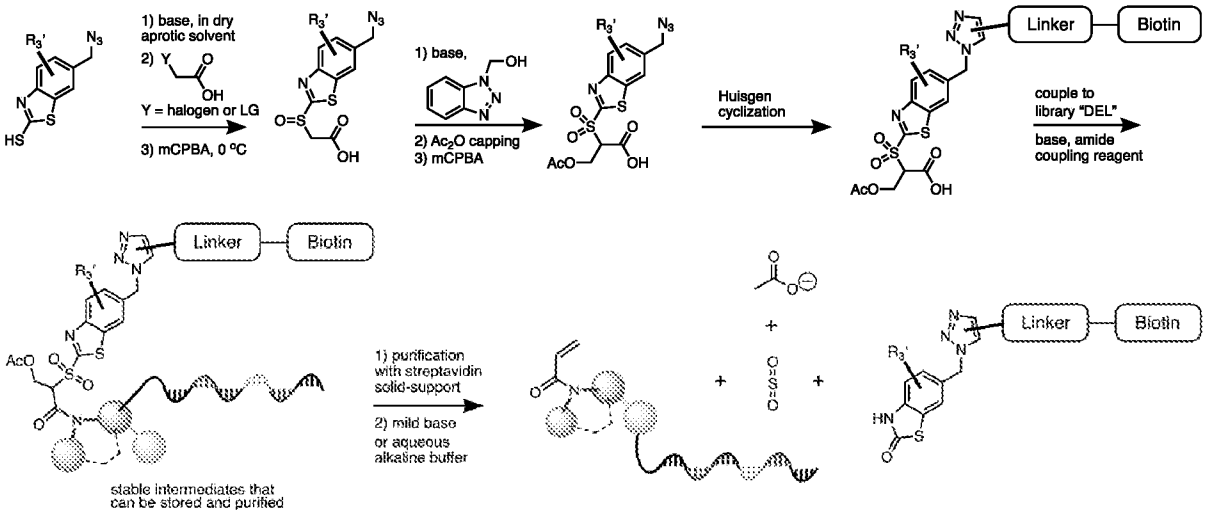
[205] Benzothiazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121*(12):7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10*(7):704–714 (2018), and International Patent Publication No. WO 2019/168654.

[206] Example 3. Synthesis of benzothiazole sulfone biotin-tagged masked-warhead reagents.



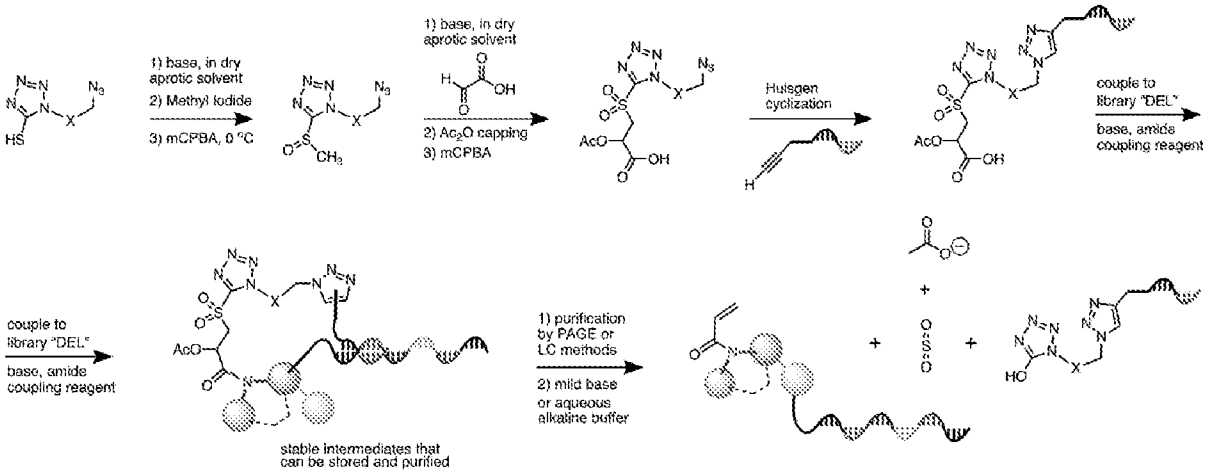
[207] Benzothiazole sulfone biotin-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121*(12):7122–7154 (2021), Gartner *et al.,* Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10*(7):704–714 (2018), and International Patent Publication No. WO 2019/168654.

[208] Example 4. Synthesis of benzothiazole sulfone biotin-tagged masked-warhead reagents.



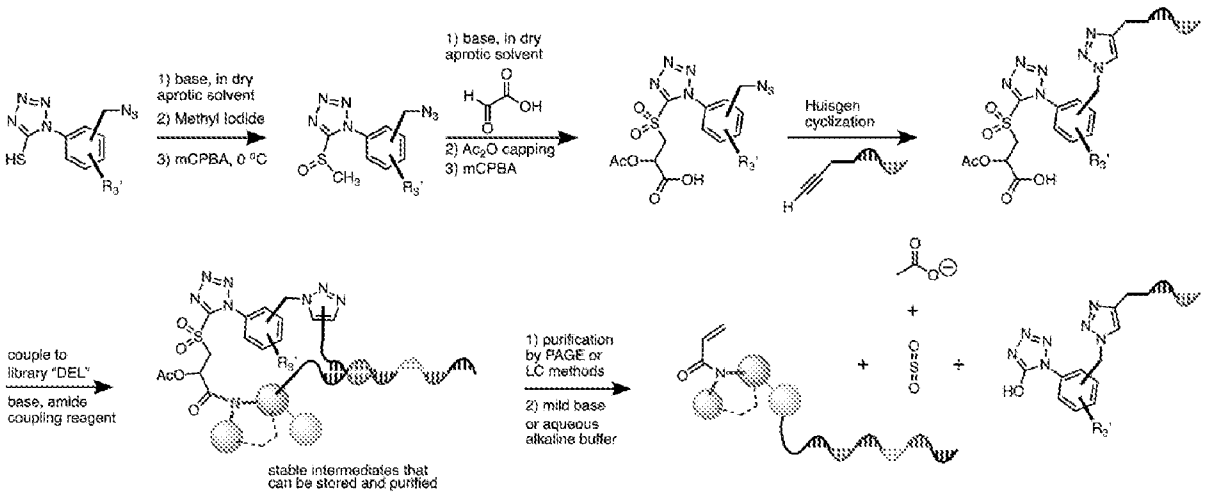
[209] Benzothiazole sulfone biotin-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, In Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121*(12):7122–7154 (2021), Gartner *et al.,* Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10*(7):704–714 (2018), and International Patent Publication No. WO 2019/168654.

[210] Example 5. Synthesis of 1-alkyl-1*H*-tetrazole-5-yl sulfone DNA-tagged masked-warhead reagents.



[211] Alkytetrazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121*(12):7122–7154 (2021), Gartner *et al.,* Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10*(7):704–714 (2018), and International Patent Publication No. WO 2019/168654.

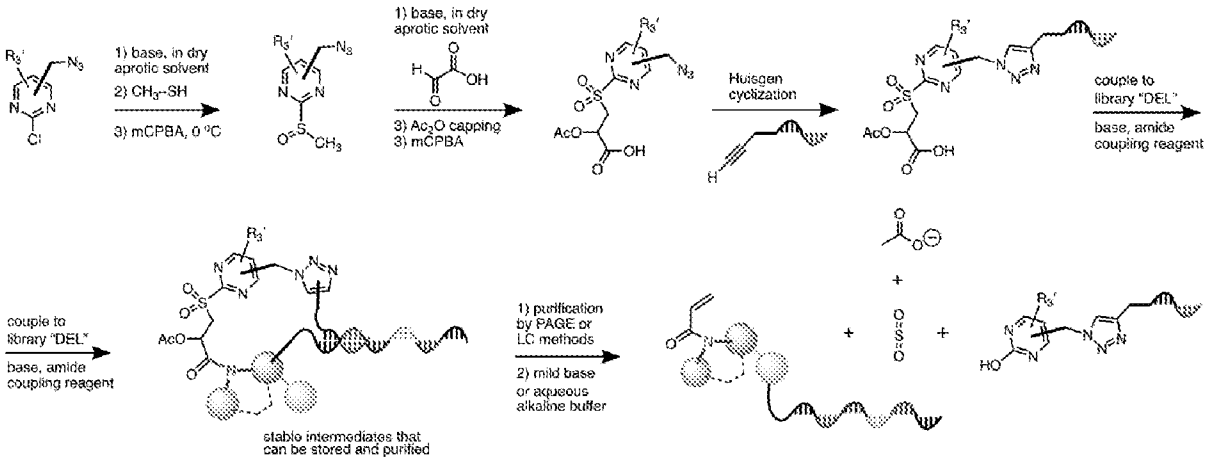
[212] Example 6. Synthesis of 1-phenyl-1*H*-tetrazole-5-yl sulfone DNA-tagged masked-warhead reagents.



[213] Phenyltetrazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251

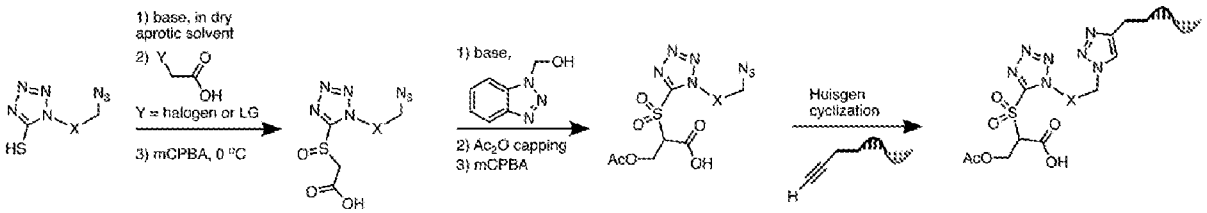
(2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

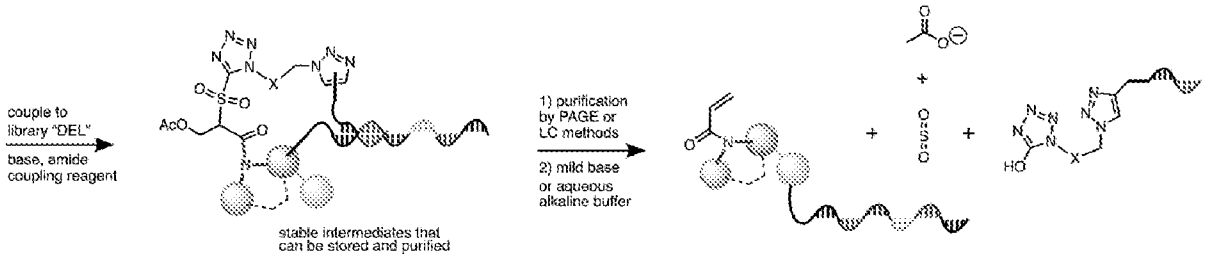
[214] Example 7. Synthesis of pyrimidine-2-yl sulfone DNA-tagged masked-warhead reagents.



[215] Pyrimidyl sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

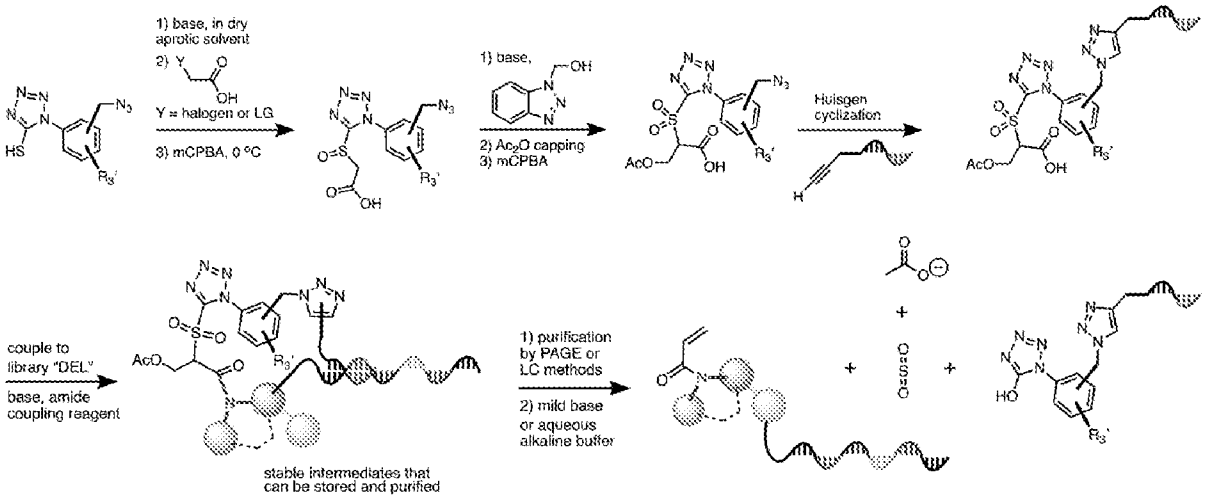
[216] Example 8. Synthesis of 1-alkyl-1*H*-tetrazole-5-yl sulfone DNA-tagged masked-warhead reagents.





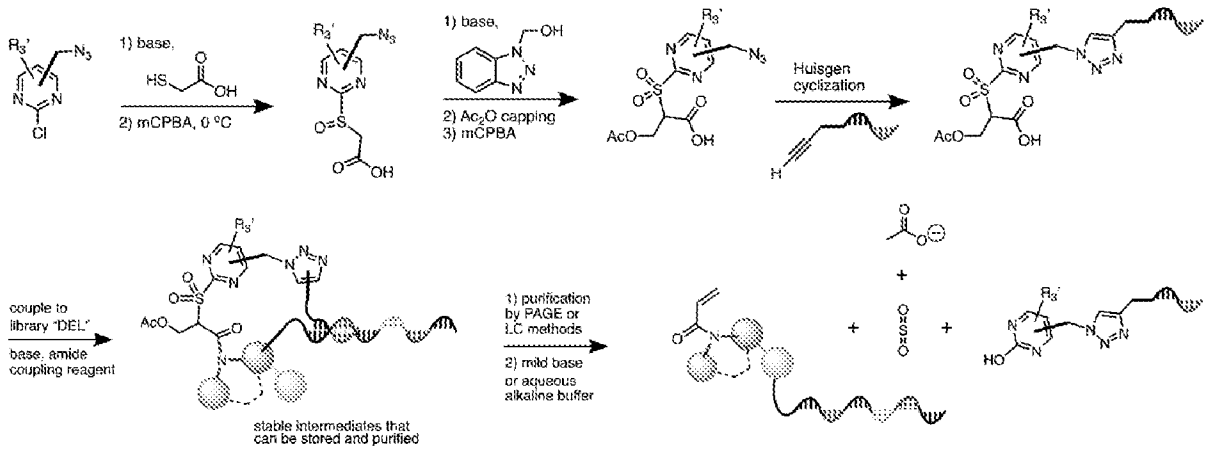
[217] Alkytetrazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

[218] Example 9. Synthesis of 1-phenyl-1*H*-tetrazole-5-yl sulfone DNA-tagged masked-warhead reagents.



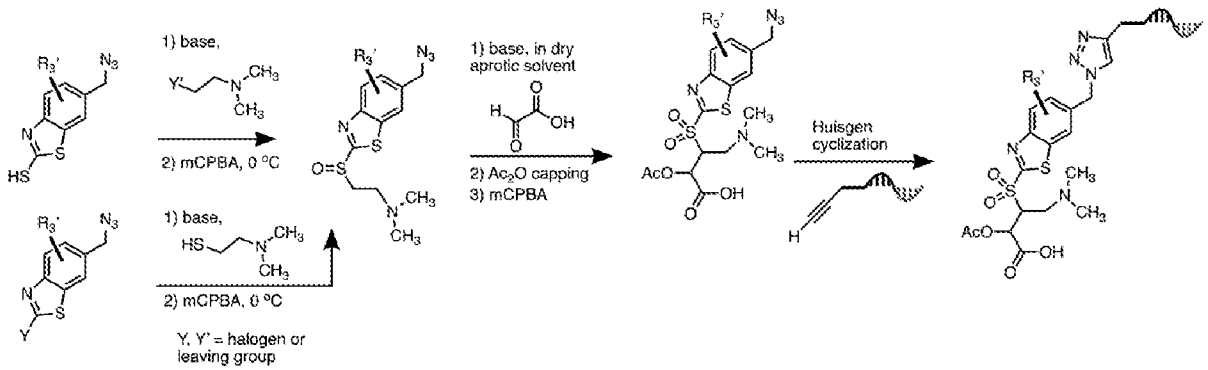
[219] Phenyltetrazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

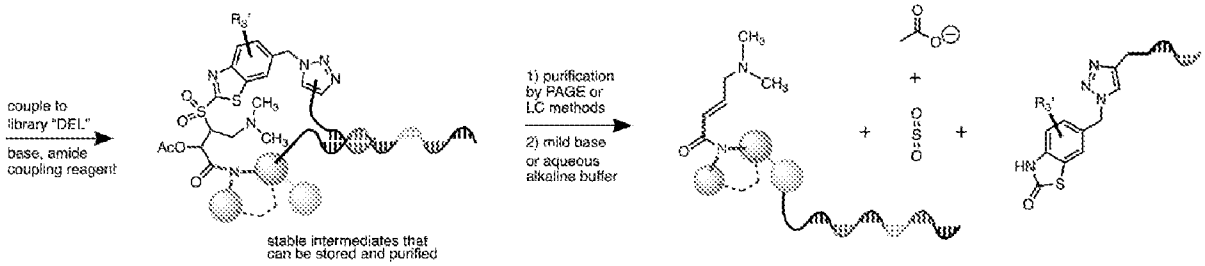
[220] Example 10. Synthesis of pyrimidine-2-yl sulfone DNA-tagged masked-warhead reagents.



[221] Pyrimidyl sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

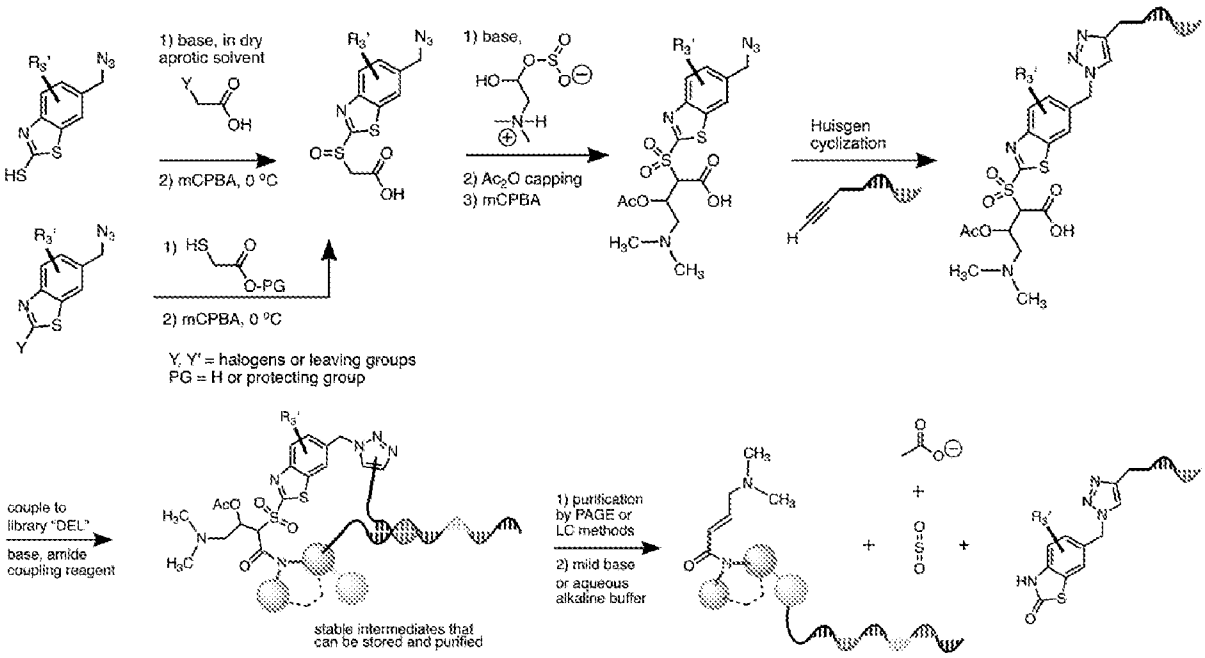
[222] Example 11. Synthesis of benzothiazole sulfone DNA-tagged masked-warhead reagents.





[223] Benzothiazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

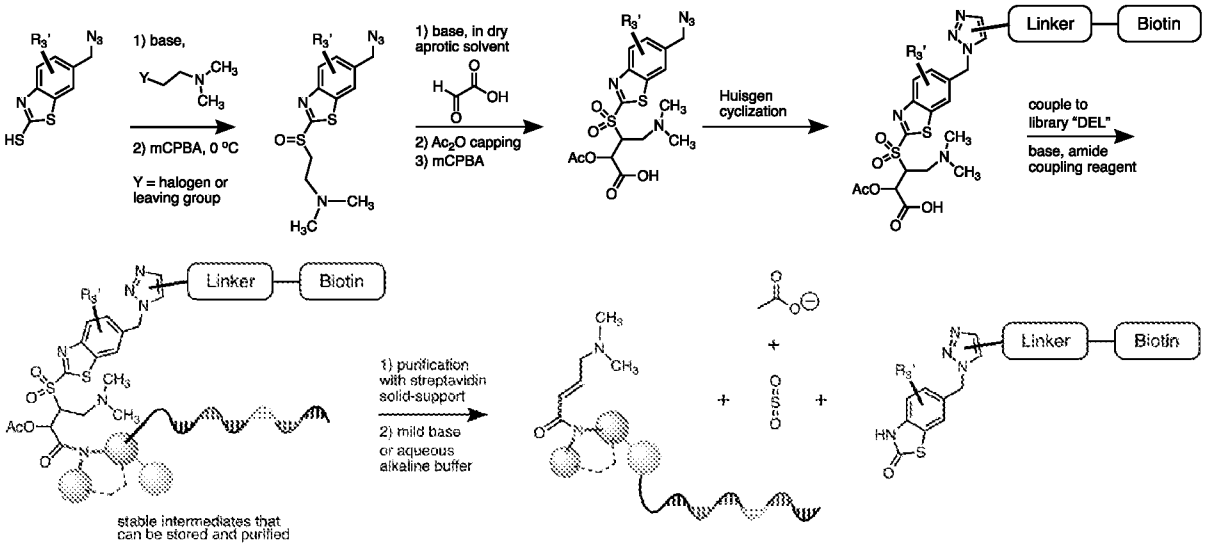
[224] Example 12. Synthesis of benzothiazole sulfone DNA-tagged masked-warhead reagents.



[225] Benzothiazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev.

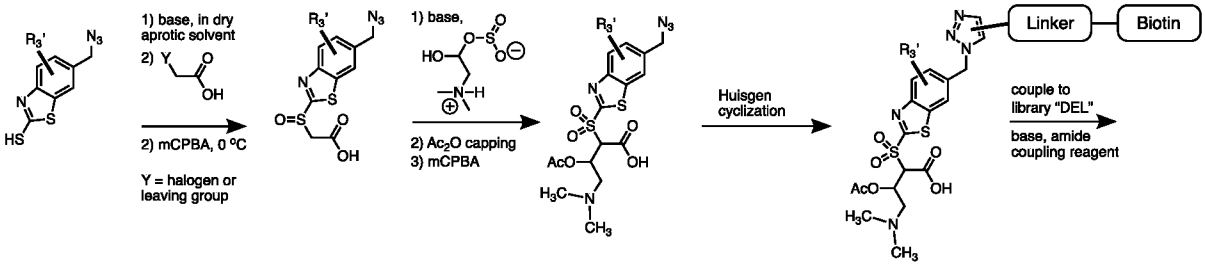
121(12):7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

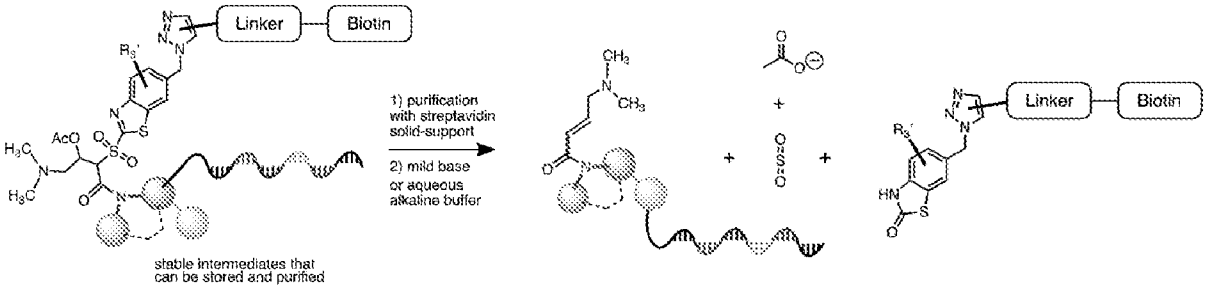
[226] Example 13. Synthesis of benzothiazole sulfone biotin-tagged masked-warhead reagents.



[227] Benzothiazole sulfone biotin-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

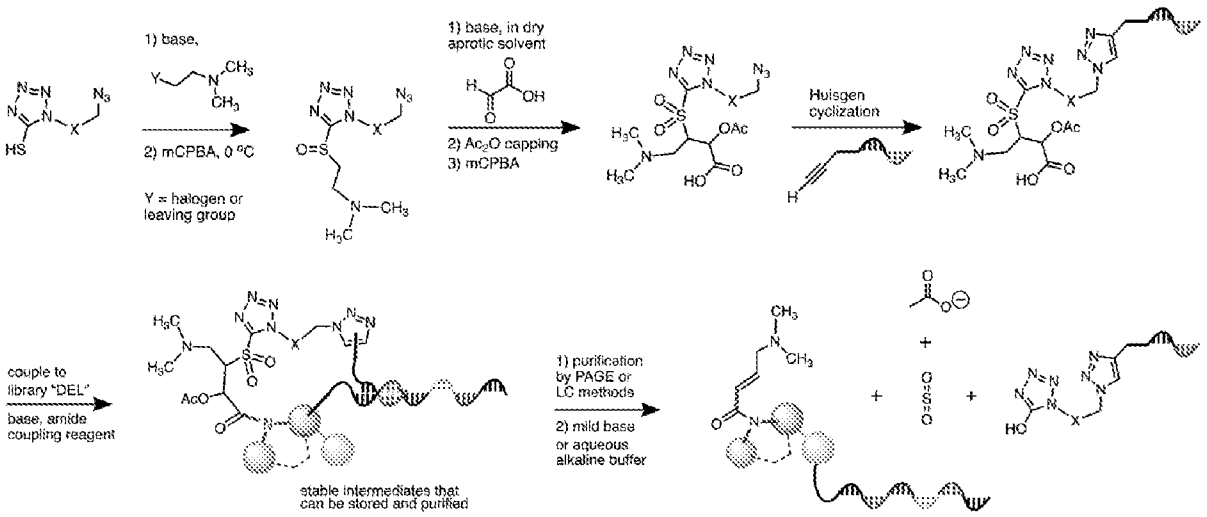
[228] Example 14. Synthesis of benzothiazole sulfone biotin-tagged masked-warhead reagents.





[229] Benzothiazole sulfone biotin-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

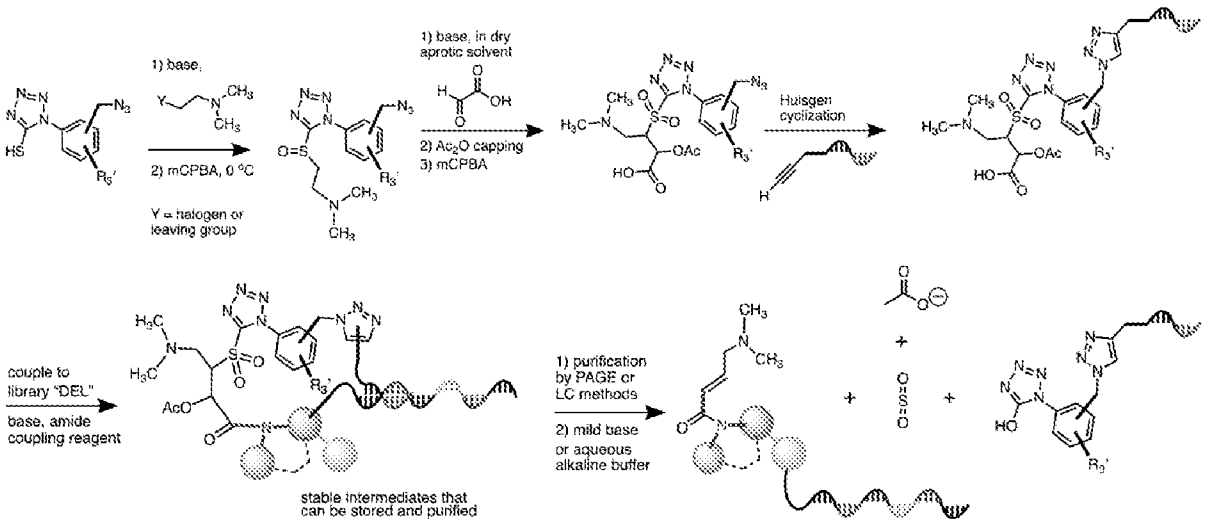
[230] Example 15. Synthesis of 1-alkyl-1*H*-tetrazole-5-yl sulfone DNA-tagged masked-warhead reagents.



[231] Alkytetrazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and

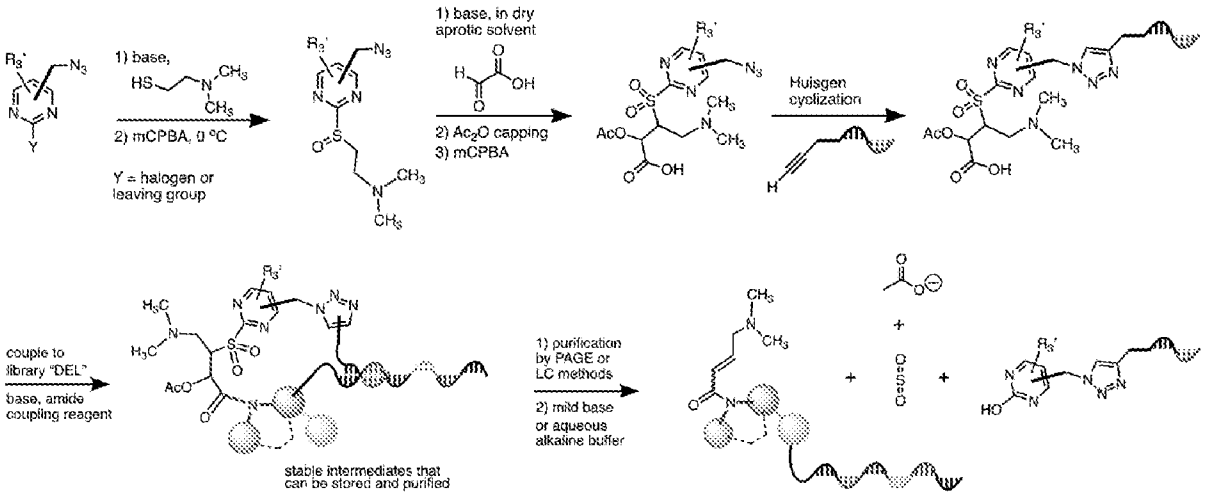
International Patent Publication No. WO 2019/168654.

[232] Example 16. Synthesis of 1-phenyl-1*H*-tetrazole-5-yl sulfone DNA-tagged masked-warhead reagents.



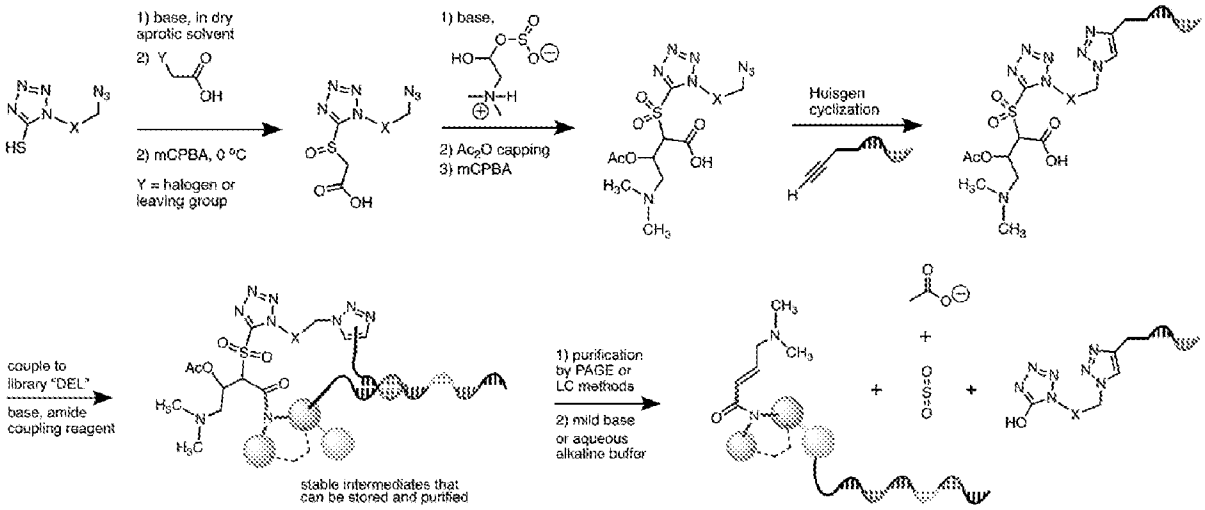
[233] Phenyltetrazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

[234] Example 17. Synthesis of pyrimidine-2-yl sulfone DNA-tagged masked-warhead reagents.



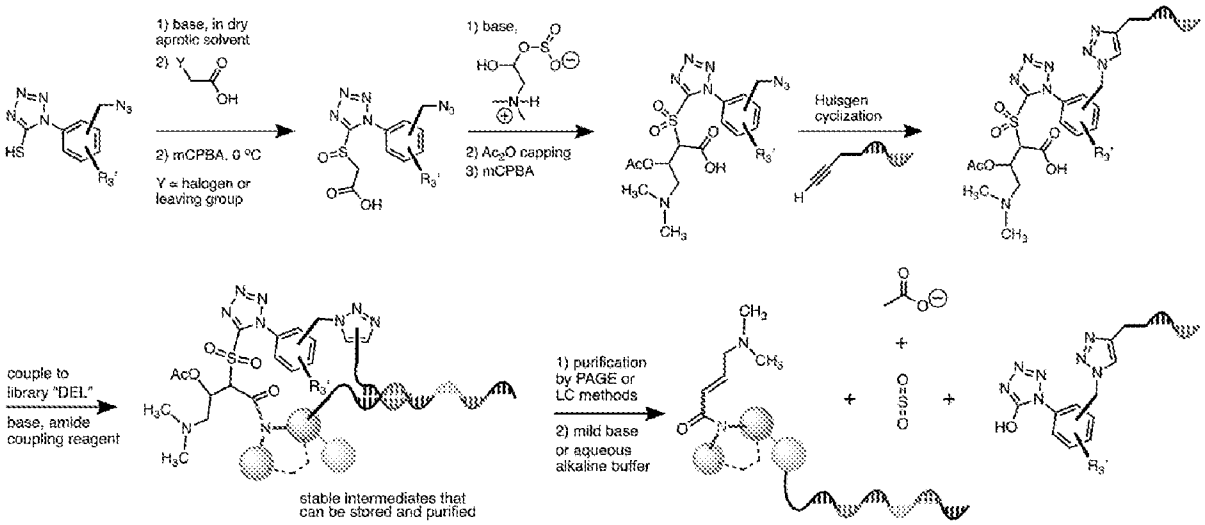
[235] Pyrimidyl sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

[236] Example 18. Synthesis of 1-alkyl-1*H*-tetrazole-5-yl sulfone DNA-tagged masked-warhead reagents.



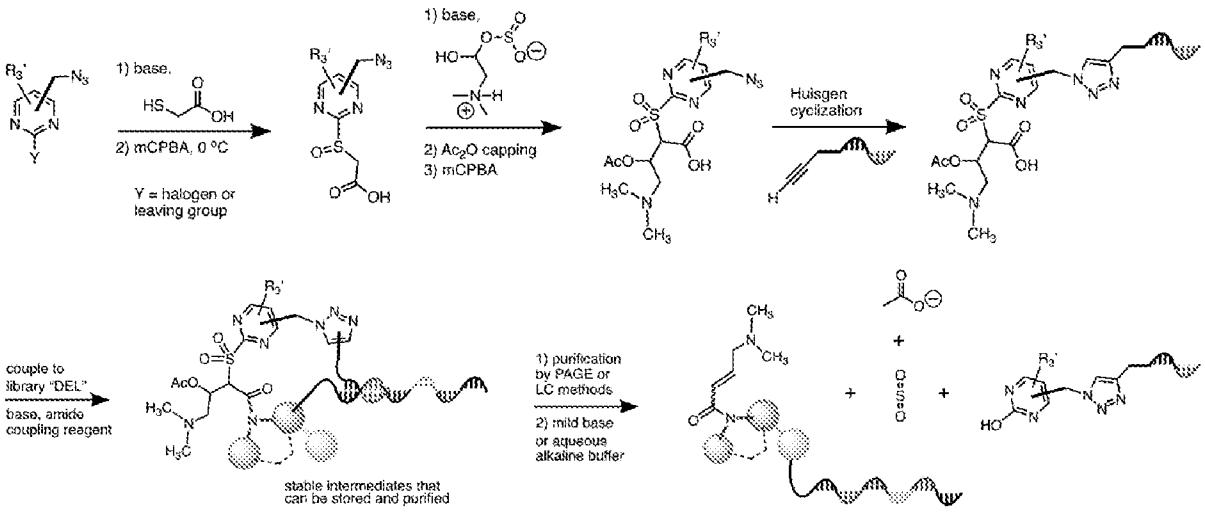
[237] Alkytetrazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

[238] Example 19. Synthesis of 1-phenyl-1*H*-tetrazole-5-yl sulfone DNA-tagged masked-warhead reagents.



[239] Phenyltetrazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

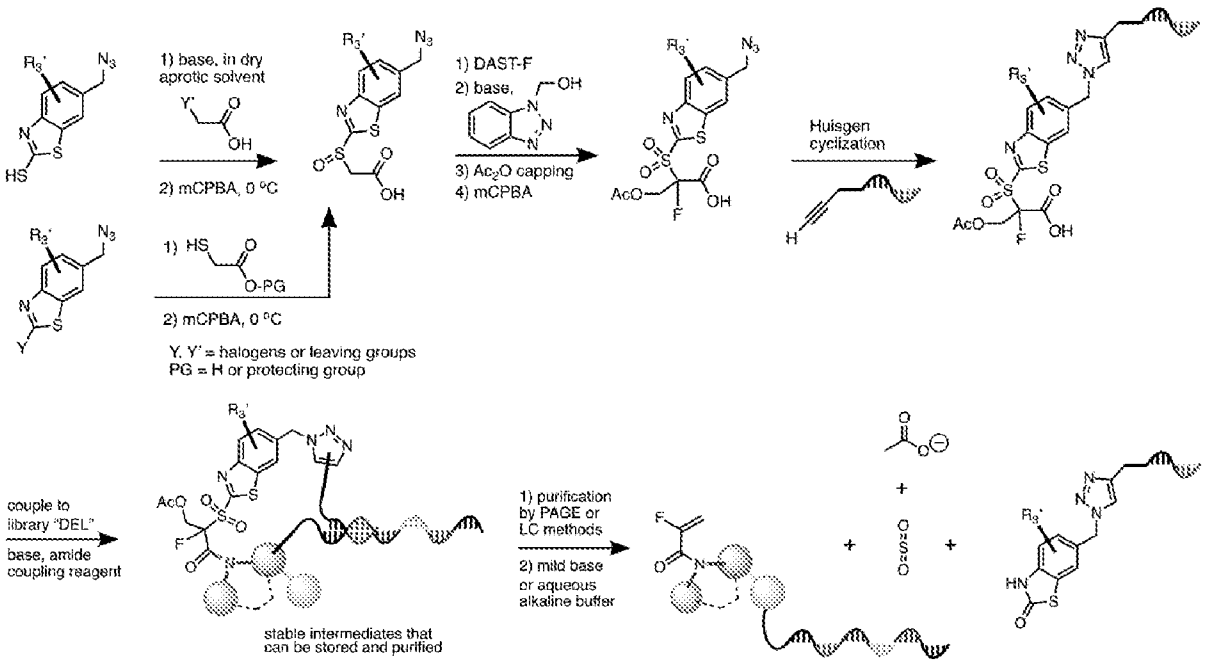
[240] Example 20. Synthesis of pyrimidine-2-yl sulfone DNA-tagged masked-warhead reagents.



[241] Pyrimidyl sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018,

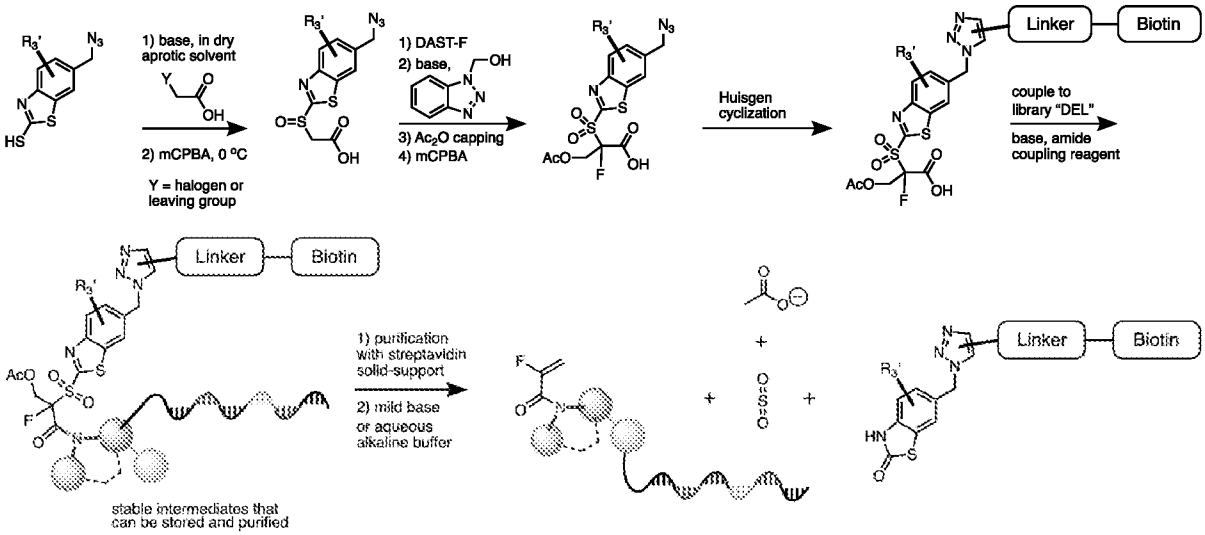
pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

[242] Example 21. Synthesis of benzothiazole sulfone DNA-tagged masked-warhead reagents.



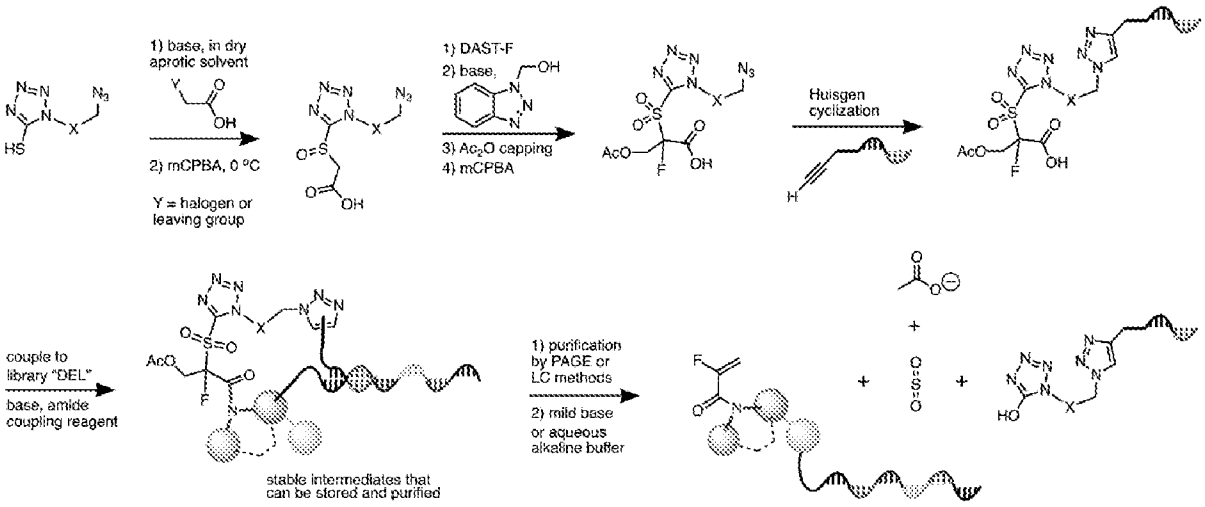
[243] Benzothiazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

[244] Example 22. Synthesis of benzothiazole sulfone biotin-tagged masked-warhead reagents.



[245] Benzothiazole sulfone biotin-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

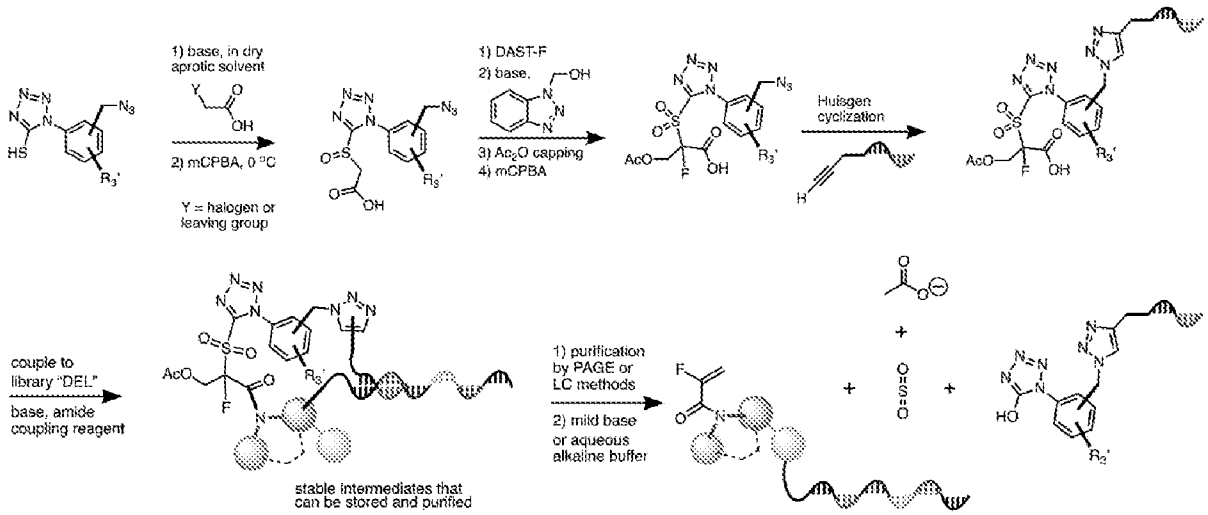
[246] Example 23. Synthesis of 1-alkyl-1*H*-tetrazole-5-yl sulfone DNA-tagged masked-warhead reagents.



[247] Alkytetrazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ,

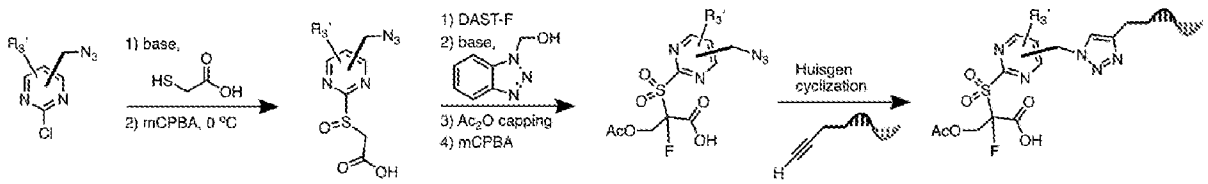
USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

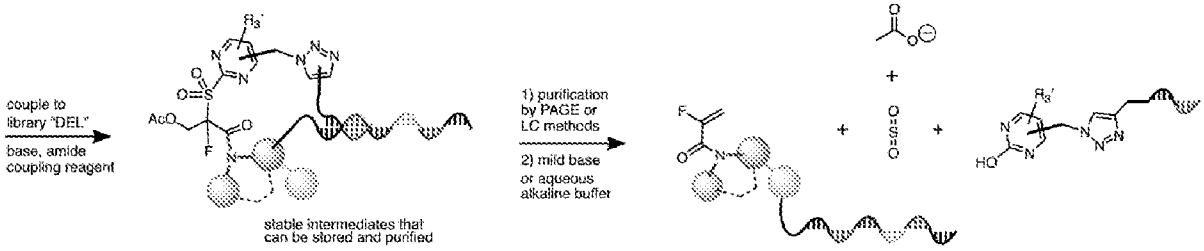
[248] Example 24. Synthesis of 1-phenyl-1*H*-tetrazole-5-yl sulfone DNA-tagged masked-warhead reagents.



[249] Phenyltetrazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

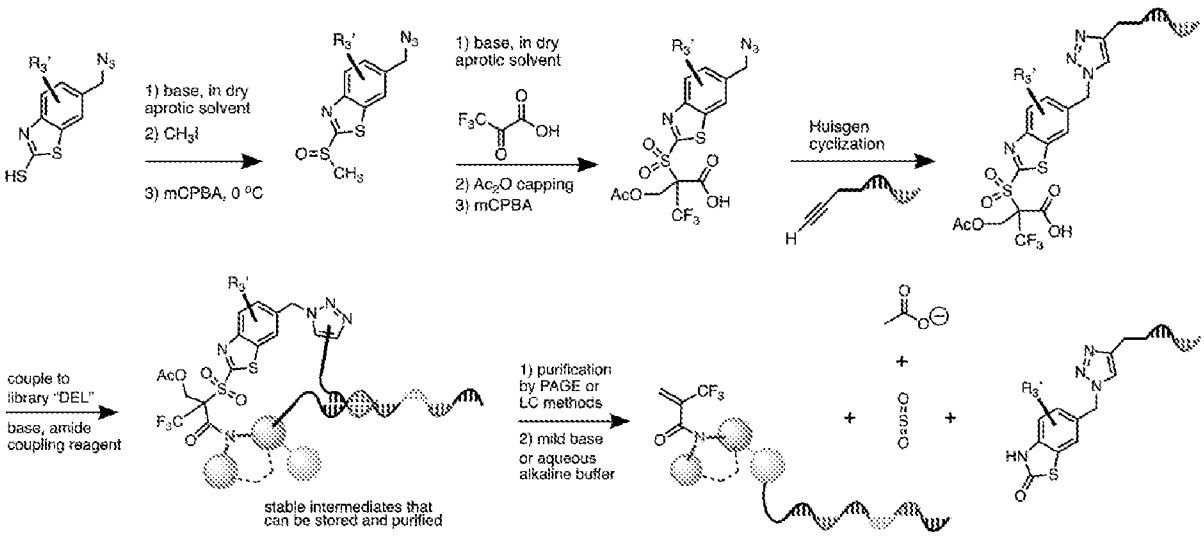
[250] Example 25. Synthesis of pyrimidine-2-yl sulfone DNA-tagged masked-warhead reagents.





[251] Pyrimidyl sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

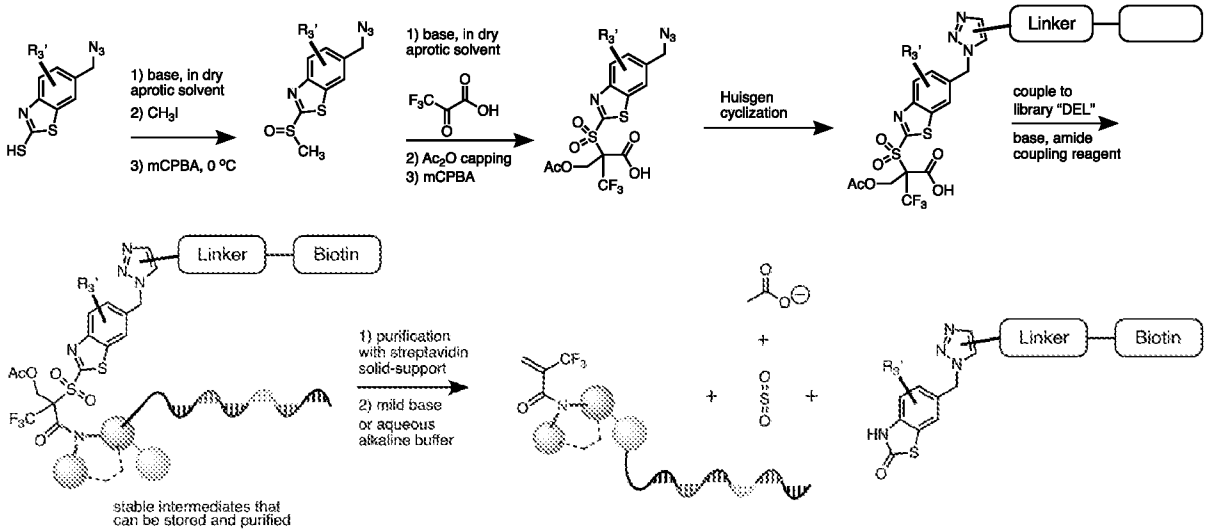
[252] Example 26. Synthesis of benzothiazole sulfone DNA-tagged masked-warhead reagents.



[253] Benzothiazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and

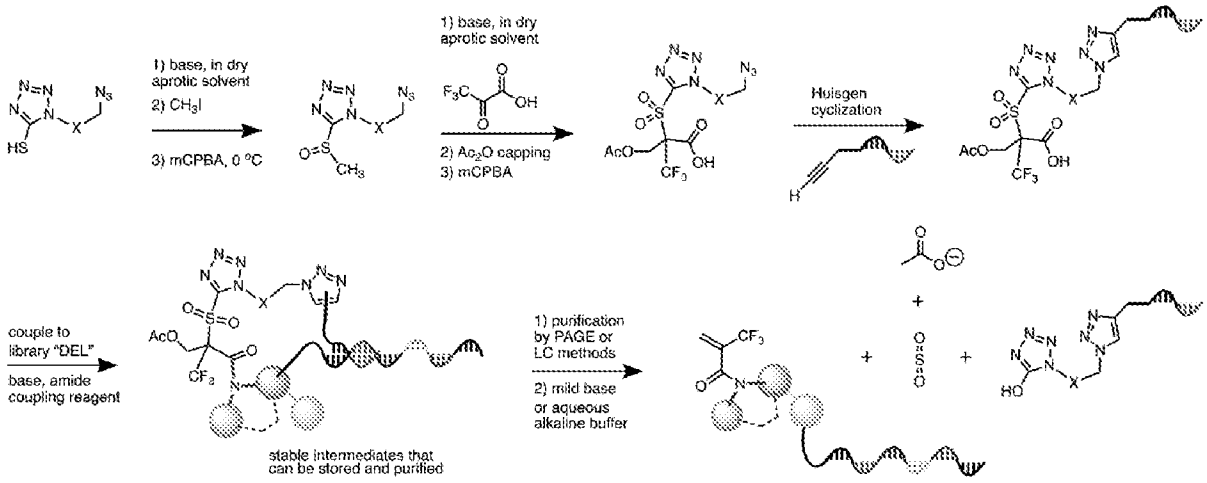
International Patent Publication No. WO 2019/168654.

[254] Example 27. Synthesis of benzothiazole sulfone biotin-tagged masked-warhead reagents.



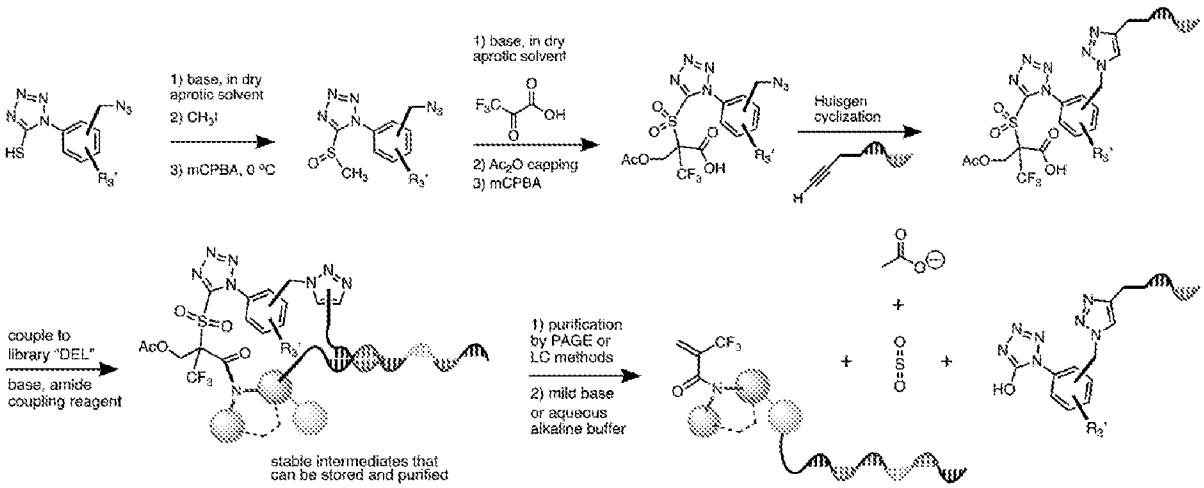
[255] Benzothiazole sulfone biotin-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

[256] Example 28. Synthesis of 1-alkyl-1*H*-tetrazole-5-yl sulfone DNA-tagged masked-warhead reagents.



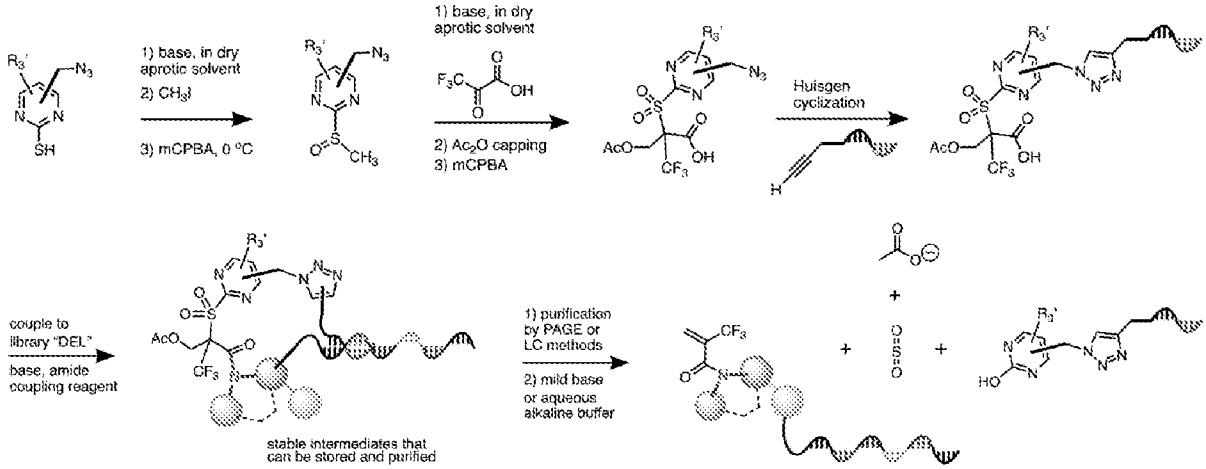
[257] Alkytetrazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

[258] Example 29. Synthesis of 1-phenyl-1*H*-tetrazole-5-yl sulfone DNA-tagged masked-warhead reagents.



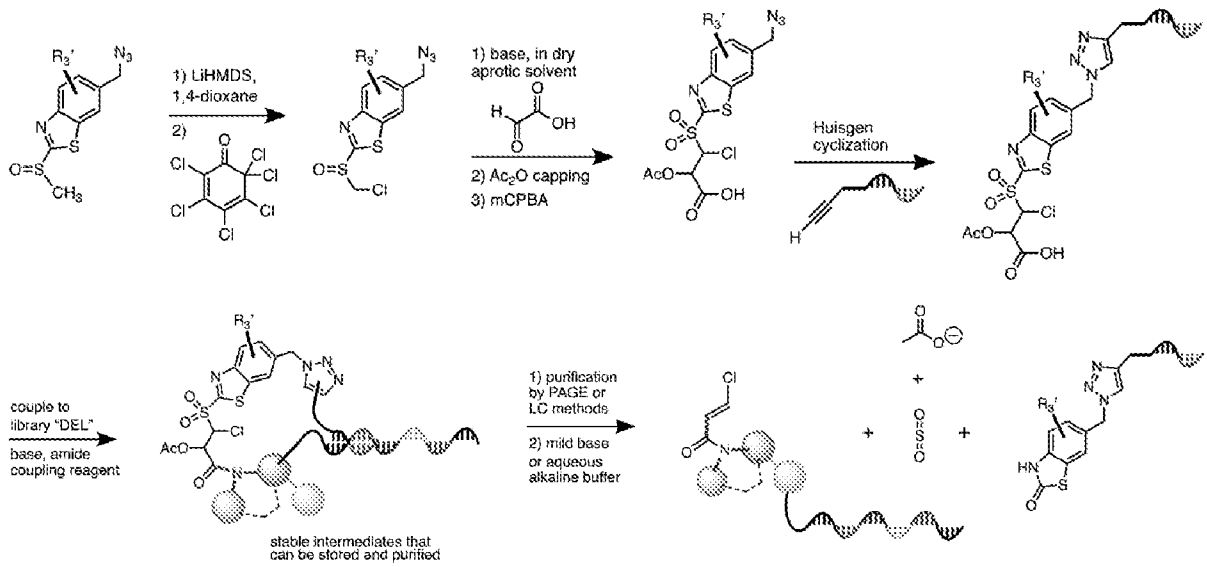
[259] Phenyltetrazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

[260] Example 30. Synthesis of pyrimidine-2-yl sulfone DNA-tagged masked-warhead reagents.



[261] Pyrimidyl sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

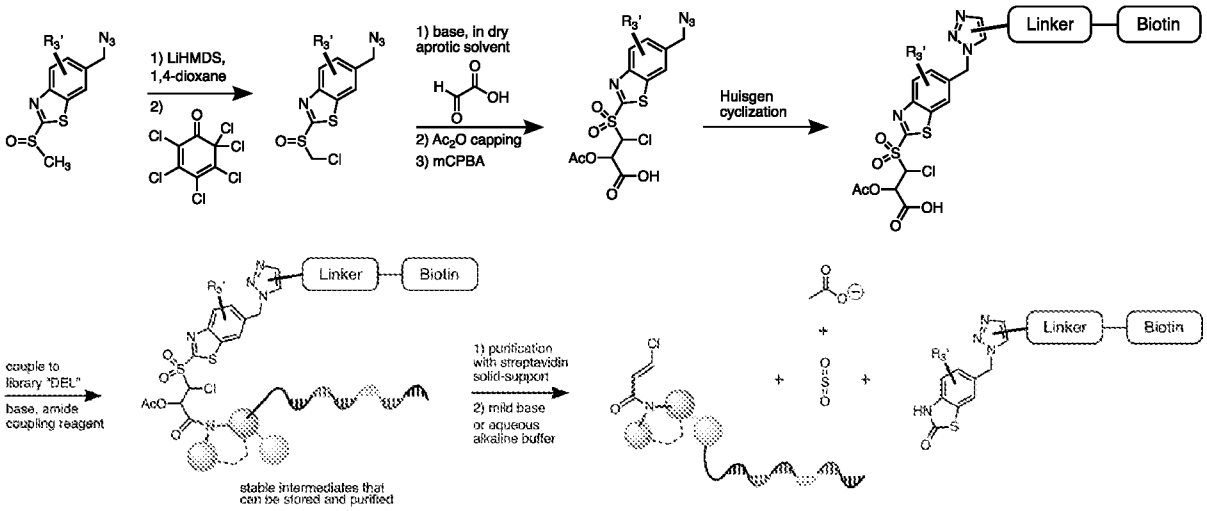
[262] Example 31. Synthesis of benzothiazole sulfone DNA-tagged masked-warhead reagents.



[263] Benzothiazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ,

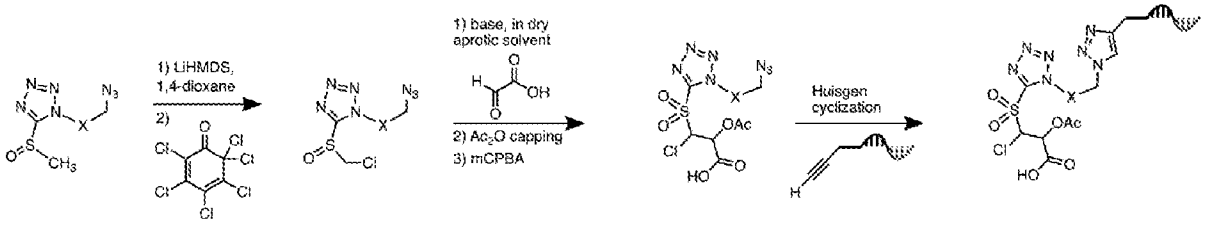
USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

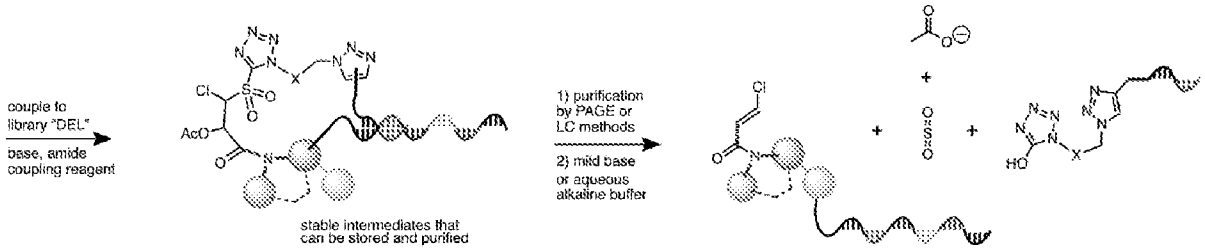
[264] Example 32. Synthesis of benzothiazole sulfone biotin-tagged masked-warhead reagents.



[265] Benzothiazole sulfone biotin-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

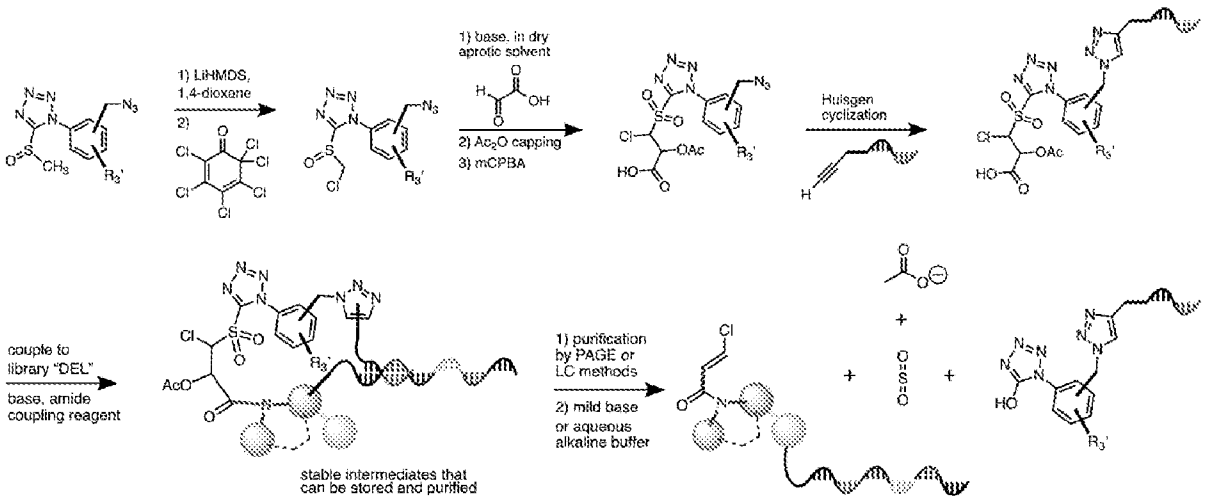
[266] Example 33. Synthesis of 1-alkyl-1*H*-tetrazole-5-yl sulfone DNA-tagged masked-warhead reagents.





[267] Alkytetrazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

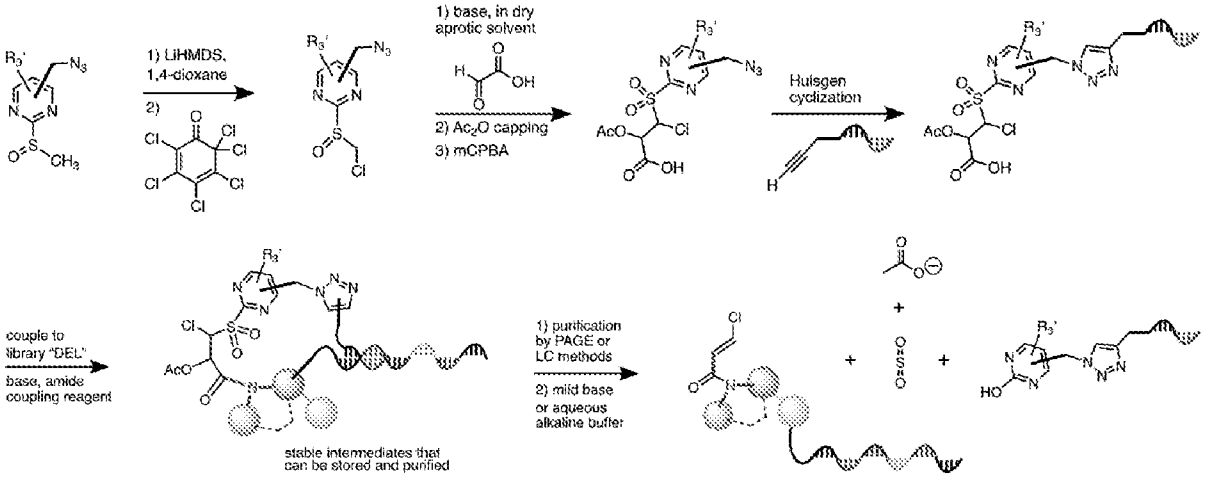
[268] Example 34. Synthesis of 1-phenyl-1*H*-tetrazole-5-yl sulfone DNA-tagged masked-warhead reagents.



[269] Phenyltetrazole sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and

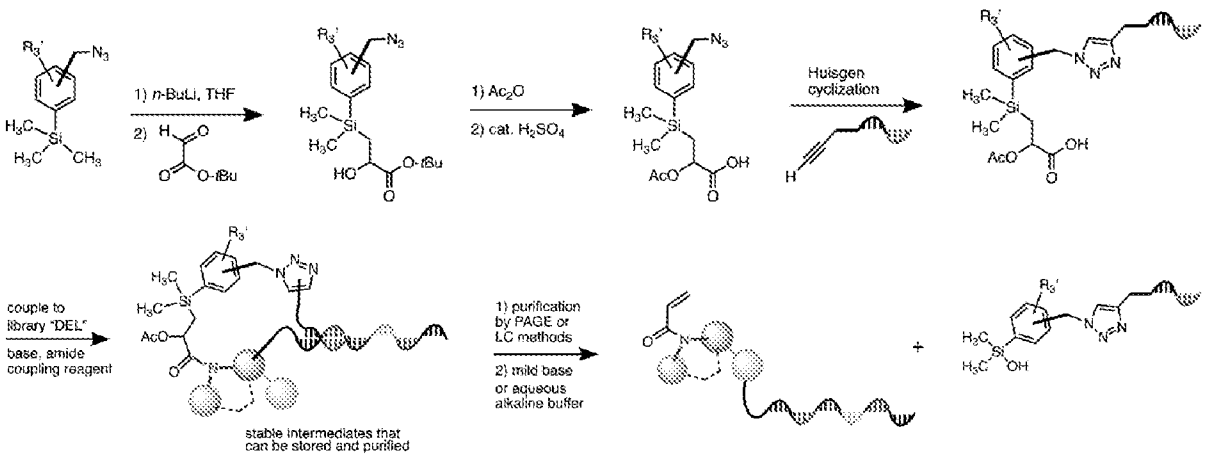
International Patent Publication No. WO 2019/168654.

[270] Example 35. Synthesis of pyrimidine-2-yl sulfone DNA-tagged masked-warhead reagents.



[271] Pyrimidyl sulfone DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

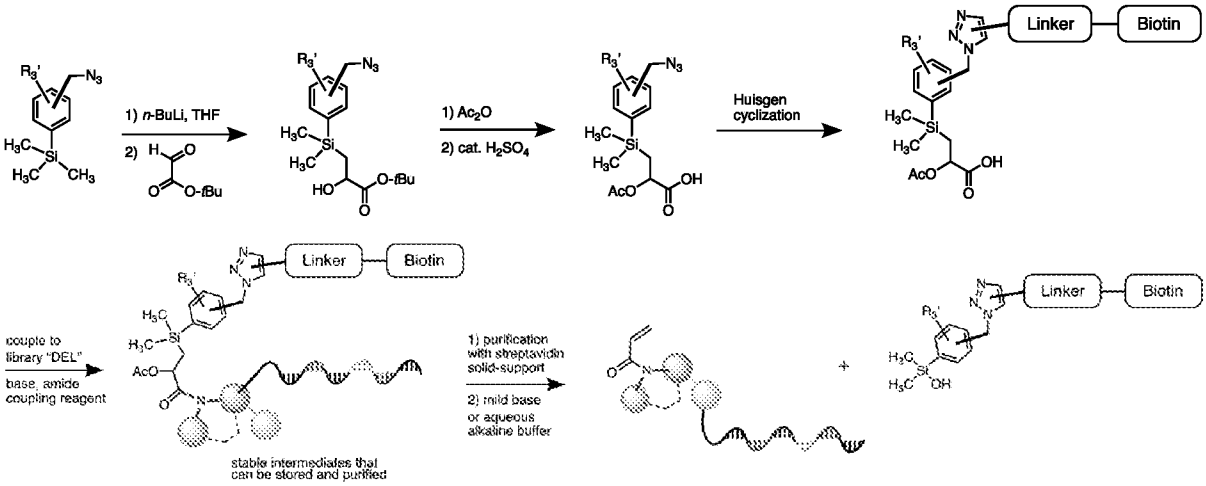
[272] Example 36. Synthesis of arylsilyl DNA-tagged masked-warhead reagents.



[273] Arylsilyl DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Bishop *et al.*, J. Org. Chem.

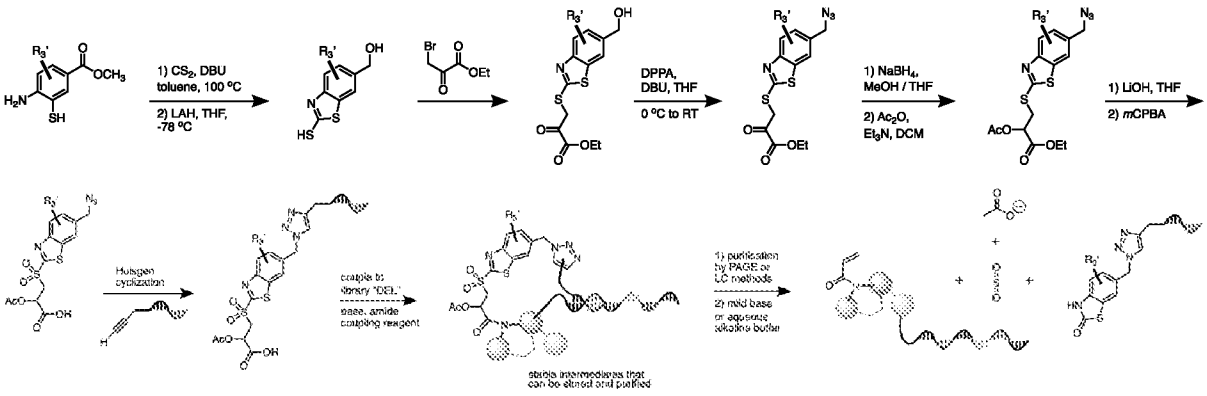
56(17):5079-5091 (1991), Ager, D.J. Synthesis; *1984*(5): 384-398 (1984), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704-714 (2018), and International Patent Publication No. WO 2019/168654.

[274] Example 37. Synthesis of arylsilyl biotin-tagged masked-warhead reagents.



[275] Arylsilyl DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Bishop *et al.*, J. Org. Chem. *56*(17):5079-5091 (1991), Ager, D.J. Synthesis; *1984*(5):384-398 (1984), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

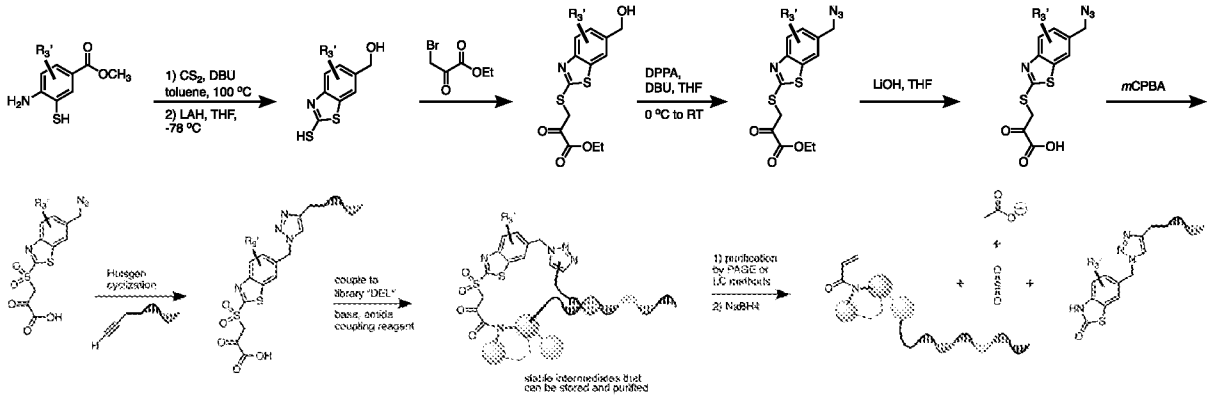
[276] Example 38. Synthesis of benzothiazole sulfone DNA-tagged masked-warhead reagents using a 3-bromo-pyruvate ester and an arylthiol synthetic intermediates.



[277] Arylsilyl DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018,

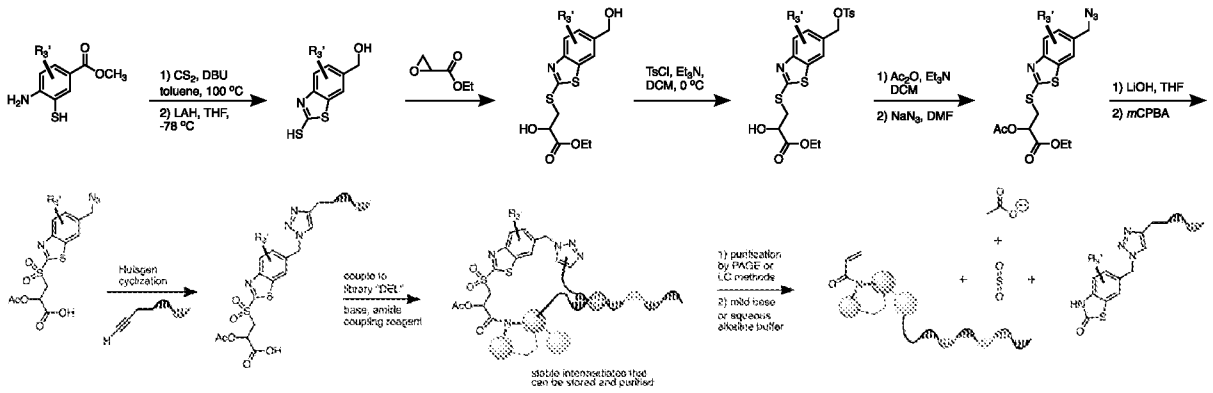
pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

[278] Example 39. Synthesis of benzothiazole sulfone DNA-tagged masked-warhead reagents, wherein a ketone reduction step mediates the release of the acrylamide eDEL.



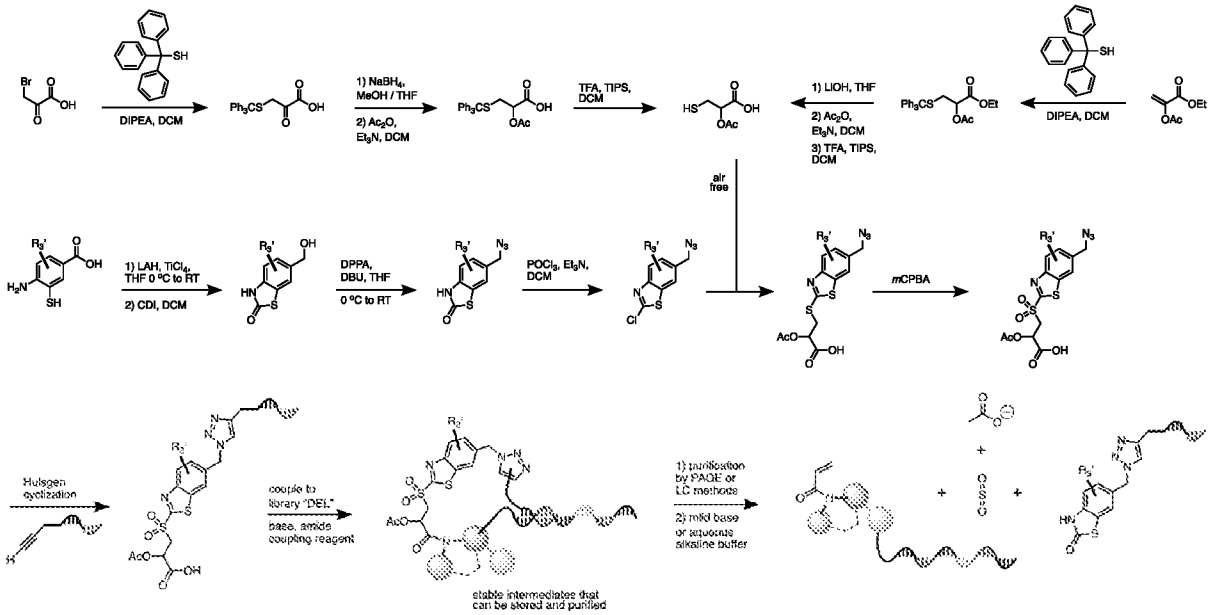
[279] Arylsilyl DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

[280] Example 40. Synthesis of benzothiazole sulfone DNA-tagged masked-warhead reagents using an epoxide and arylthiol synthetic intermediates.



[281] Arylsilyl DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

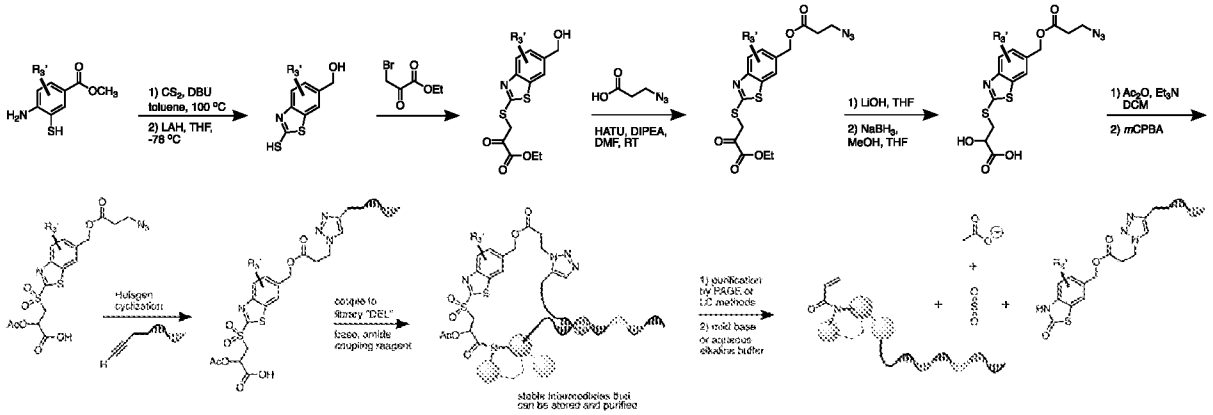
[282] Example 41. Synthesis of benzothiazole sulfone DNA-tagged masked-warhead reagents using a 2-chlorobenzothiazole intermediate and 2-acetoxy-3-mercaptopropanoic acid.



[283] Arylsilyl DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Galardon *et al.*, ChemBioChem, 19(16) 1702-1705 (2018), Reddy *et al.*, Org. Lett. 2019, 21, 24, 9965–9969, Batt-Coutrot *et al.*, Macromol. Chem. Phys., 206, 1709-1717 (2005), Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

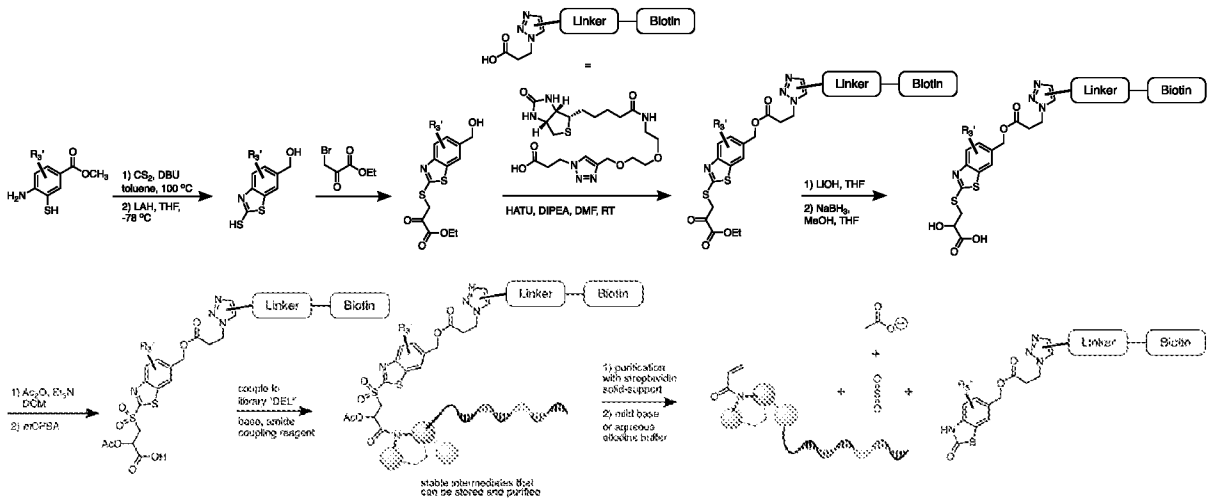
[284] Example 42. Synthesis of benzothiazole sulfone DNA-tagged masked-warhead

reagents by acylation of a Click Chemistry handle onto a mercaptobenzothiazole intermediate.



[285] Arylsilyl DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et al.*, Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

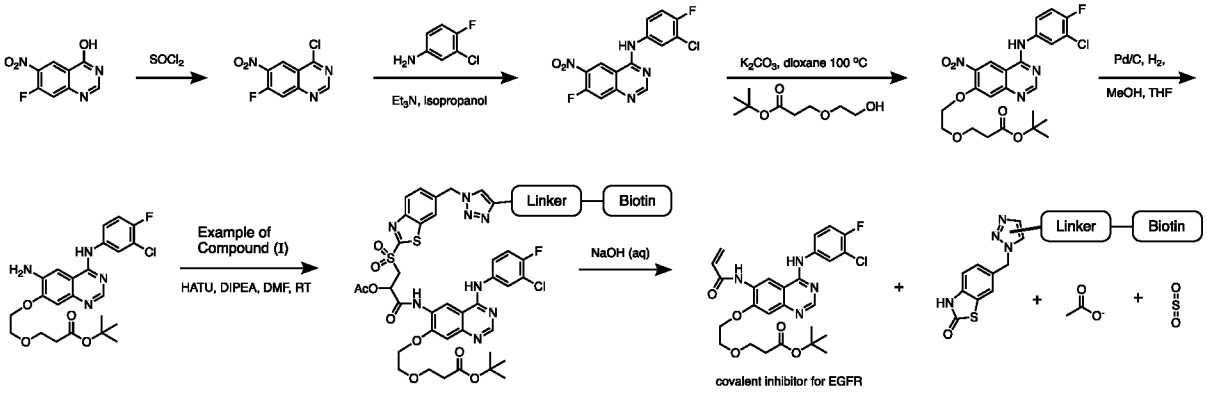
[286] Example 43. Synthesis of benzothiazole sulfone biotin-tagged masked-warhead reagents by acylation of a biotinylated linker onto a mercaptobenzothiazole intermediate.



[287] Arylsilyl DNA-tagged masked-warhead reagents are synthesized as illustrated in the above scheme. The reactions are performed as described in Blakemore *et al.*, The Julia-Kocienski Olefination, Organic Reactions, John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2018, pp 1–261, Keri *et al.*, European Journal of Medicinal Chemistry, *89*:207–251 (2015), Kolb *et*

al., Angew. Chem. Int. Ed., *40*:2004-2021 (2001), Fantoni *et al.*, Chem. Rev. *121(12)*:7122–7154 (2021), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018), and International Patent Publication No. WO 2019/168654.

[288] Example 44. Synthesis of an analog of the covalent EGFR inhibitor "afatinib" using a biotin-tagged masked warhead reagent to install the acrylamide group.



[289] An advanced intermediate of "afatinib" is synthesized as illustrated in the above scheme. The reactions are performed as described in International Patent Publication No. WO 2013/131424.

[290] The Examples comprise illustrative single- and multi-step functional group transformations that are well known in the art, as described in, for example, "Comprehensive Organic Synthesis" (B.M. Trost & I. Fleming, eds., 1991-1992) and that may be replaced or superseded by other known transformations and functional groups that nonetheless serve the broader purpose of producing the claimed structures. The modular synthetic routes illustrated in Scheme 1 to Scheme 44 can also be readily modified by one of skill in the art to provide additional substituted masked warheads, acrylamides, and related compounds by conducting functional group transformations on the intermediates and final compounds.

[291] Certain examples in Scheme 1 to Scheme 44 comprise a leaving group (LG) which is a covalently attached group that can be eliminated from the molecule during a chemical reaction (*e.g.*, tosylate, mesylate, acetate, hydroxide, halogens, *etc.*) (B.M. Trost & I. Fleming, eds., 1991-1992).

[292] The present invention is not limited to any one combinatorial chemistry route or library size chosen to generate DELs, including drug-like small molecule chemical space (Reymond *et al*, Acc. Chem. Res. *48(3)*:722–730 (2015)), with the exception that attachment sites should

be present on the DEL "scaffolds" or "appendages" to enable acylation by the masked-warhead reagents. Previously, other chemists in the DEL field have also recognized the potential of DELs featuring warheads, and specifically their promise to discover new covalent ligands in unbiased screens for biomedical target proteins (Zimmermann *et al.*, Chem.- Eur. J. *23(34)*: 8152–8155 (2017); Zambaldo *et al.*, MedChemComm. *7(7)*:1340–1351 (2016); Kuai *et al.*, SLAS Discov. Adv. Sci. Drug Discov. *23(5)*:405–416 (2018); Zhu *et al.*, SLAS Discov. Adv. Sci. Drug Discov. *24(2)*:169–174 (2019); Guilinger *et al.*, Bioorg. Med. Chem. *42*:116223 (2021)). Over the past decade DELs have been impactful and useful for reversible ligand discovery (Goodnow *et al.*, Nat. Rev. Drug Discov. *16(2)*:131–147 (2017)); however, DELs featuring covalent warheads have not been realized for discovery of human therapeutics to-date (Vita, E.D., Future Med. Chem. *13(2)*, 193–210 (2021); Sutanto *et al*, RSC Med. Chem. *11(8)*, 876–884 (2020); Gehringer *et al*, J. Med. Chem. *62(12)*:5673–5724 (2020)). Therefore, other chemists in the field have previously attempted brute-forcing warhead acylation reactions on DELs (Guilinger *et al.*, Bioorg. Med. Chem. *42:*116223 (2021); Zambaldo *et al*, MedChemComm. *7(7)*, 1340–1351 (2016)). Despite selecting DELs known to be particularly favorable this reaction, the outcomes of brute-forcing acylation approaches have been unpurifiable library mixtures, comprising the non-acylated library members as well as synthetic intermediates, capped, truncated, and other byproducts that are nonetheless attached to identical DNA barcodes as the desired warhead products (Guilinger *et al.*, Bioorg. Med. Chem. *42:*116223 (2021); Zambaldo *et al*, MedChemComm. *7(7)*, 1340–1351 (2016)).

[293] The masked-warhead reagents described herein solve the challenges associated with library-format small-molecule DEL purification by connecting the temporary masking group to a DNA-tag and/or an affinity-tag that are useful to separate the desired warhead-connected eDEL away from non-acylated library members and byproducts, using either gel electrophoresis and/or affinity-based purification methods (Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008); Usanov *et al.*, Nat. Chem. *10(7)*:704–714 (2018)). Additionally, the warhead-masking reagents are useful due to their connection to a DNA-tag that enables DNA base-pairing to enforce the acylation reaction under high effective molarity (Gartner *et al.*, *Angew.* Chem Int. Ed. *42(12)*:1370-1375 (2003), Gartner *et al.*, Science *305*:1601-1605 (2004), Tse *et al.*, J. Am. Chem. Soc. *130*:15611-15626 (2008), and Usanov *et al.*, Nat. Chem. *10*(7):704–714 (2018)), which will promote the acylation of warheads on a broader scope of low reactivity attachment sites that are typically encountered on diverse small-molecule

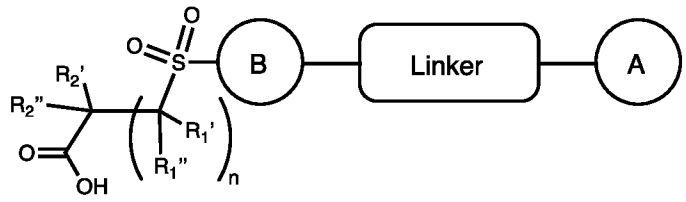
scaffolds and appendage structures (e.g., anilines, sterically hindered amines, hydroxyls, heterocyclic nitrogens, *etc.*) (Shi *et al.*, RSC Adv. *11*(4):2359–2376 (2021)). The reagents and methods described in the present disclosure will find broad utility in the synthesis of structurally diverse and highly pure DNA-encoded libraries of small molecules featuring electrophilic warheads for covalent ligand discovery.

[294] All patent publications and non-patent publications are indicative of the level of skill of those skilled in the art to which this disclosure pertains. All these publications (including any specific portions thereof that are referenced) are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated as being incorporated by reference.

[295] Although the disclosure herein has been described with reference to particular embodiments, it is to be understood that these embodiments are merely illustrative of the principles and applications of the present disclosure. It is therefore to be understood that numerous modifications may be made to the illustrative embodiments and that other arrangements may be devised without departing from the spirit and scope of the invention as defined by the appended claims.

What is claimed is:

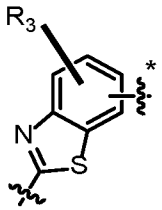
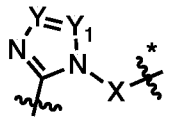
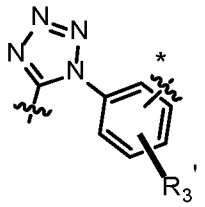
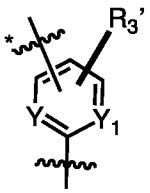
1. A compound represented by formula (I) or a pharmaceutical salt or stereoisomer thereof:



(I), wherein

(A) is an oligonucleotide tag represented by  or an affinity tag;

Linker is a linker that covalently attaches (A) to (B);

(B) is  ,  ,  , or  , wherein

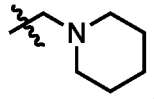
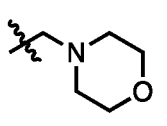
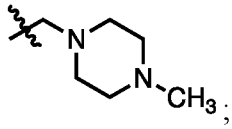
 is the connection to the sulfone group,

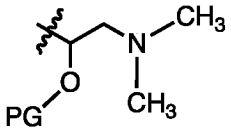
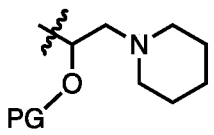
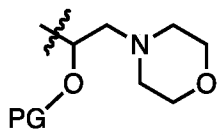
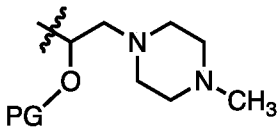
 is the connection to the linker,

R_3' is H, halogen, amino, hydroxyl, (C_1-C_6) alkyl, (C_1-C_6) hydroxyalkyl, (C_1-C_6) aminoalkyl, (C_3-C_6) carbocyclyl, 4- to 6-membered heterocyclyl, (C_1-C_6) alkyl-(C_3-C_6) carbocyclyl, or (C_1-C_6) alkyl-4- to 6-membered heterocyclyl, wherein said alkyl, hydroxyalkyl, aminoalkyl, carbocyclyl, or heterocyclyl is further optionally substituted by one or more, identical or different R_{1a} groups, wherein each R_{1a} is independently (C_1-C_6) alkyl, (C_1-C_6) alkoxy, (C_1-C_6) alkyl-(C_1-C_3) alkoxy, halogen, amino, hydroxyl, (C_1-C_6) haloalkyl, NH-(C_1-C_6) alkyl, N((C_1-C_6)alkyl)$_2$, (C_3-C_6) carbocyclyl, or 4- to 6-membered heterocyclyl,

X is (C_1-C_6) alkyl, wherein said alkyl is further optionally substituted by one or more, identical or different R_{1a} groups, wherein each R_{1a} is independently (C_1-C_6) alkyl, (C_1-C_6) alkoxy, (C_1-C_6) alkyl-(C_1-C_3) alkoxy, halogen, amino, hydroxyl, (C_1-C_6) haloalkyl, NH-(C_1-C_6) alkyl, N((C_1-C_6)alkyl)$_2$, (C_3-C_6) carbocyclyl, or 4- to 6-membered heterocyclyl, and

Y and Y_1 are each independently CH or N;

R_1' and R_1'' are each independently H, CH_3, CF_3, halogen, CH_2NMe_2,  ,  , or  ;

R_2' and R_2'' are each independently H, halogen, CF_3, OH, OAc, CH_2OH, $CH(CH_3)OH$, $C(CH_3)_2OH$, CH_2OAc, CH_2OPG,  ,  ,  , or  wherein PG is a Protecting Group, provided that both R_2' and R_2'' are not H, or R_2' and R_2'' can be joined to form =O; and

n is 0 or 1.

2. A method of creating an electrophilic warhead-bearing DNA-Encoded Library (eDEL) comprising:

coupling the compound of claim 1 with a DNA-Encoded Library to generate a stable masked-warhead DEL (mwDEL) intermediate;

purifying the mwDEL intermediate;

unmasking the mwDEL intermediate to generate an activated eDEL; and

purifying the activated eDEL.

3. An eDEL, which is generated from the method of claim 2.

4. The eDEL of claim 3, which is used in an in vitro selection assay followed by DNA sequencing.

5. The eDEL of claim 3 or 4, wherein the in vitro selection assay comprises screening protein ligands.

6. The eDEL of claim 3 or 4, wherein the in vitro selection assay comprises screening ligands that covalently modify a residue of a protein.

7. The eDEL of claim 6, wherein the in vitro selection assay comprises screening ligands that covalently modify the thiol group of a Cysteine residue, the imidazole ring of a Histidine residue, the amino group of a Lysine residue, the hydroxyl group of a Serine residue, the hydroxyl group of a Threonine residue, the phenolic hydroxyl of a Tyrosine residue, a carboxylate group, or an amide group of the protein.

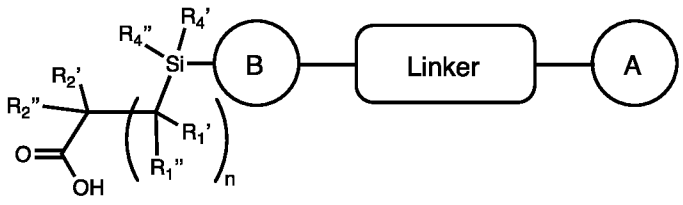
8. The eDEL of claim 6, wherein the in vitro selection assay comprises for screening ligands that covalently modify a residue in the active site of the protein.

9. The eDEL of claim 6, wherein the in vitro selection assay comprises screening ligands that covalently modify a residue in a non-orthosteric site of the protein.

10. The eDEL of claim 6, wherein the in vitro selection assay comprises screening ligands that covalently modify a residue in an allosteric site of the protein.

11. The eDEL of claim 6, wherein the in vitro selection assay comprises screening ligands that covalently modify a residue in a non-catalytic domain of a protein.

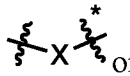
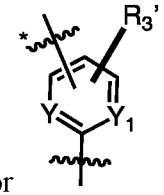
12. A compound represented by formula (II) or a pharmaceutical salt or stereoisomer thereof:



(II), wherein

(A) is an oligonucleotide tag represented by  or an affinity tag;

Linker is a linker that covalently attaches (A) to (B);

Ⓑ is  or  , wherein

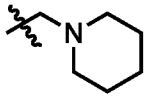
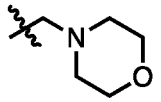
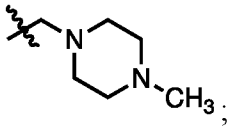
 is the connection to the silyl group,

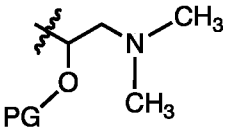
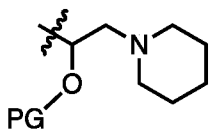
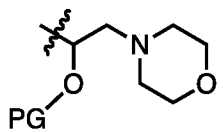
 is the connection to the linker,

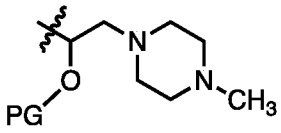
R_3' is H, halogen, amino, hydroxyl, (C_1-C_6) alkyl, (C_1-C_6) hydroxyalkyl, (C_1-C_6) aminoalkyl, (C_3-C_6) carbocyclyl, 4- to 6-membered heterocyclyl, (C_1-C_6) alkyl-(C_3-C_6) carbocyclyl, or (C_1-C_6) alkyl-4- to 6-membered heterocyclyl, wherein said alkyl, hydroxyalkyl, aminoalkyl, carbocyclyl, or heterocyclyl is further optionally substituted by one or more, identical or different R_{1a} groups, wherein each R_{1a} is independently (C_1-C_6) alkyl, (C_1-C_6) alkoxy, (C_1-C_6) alkyl-(C_1-C_3) alkoxy, halogen, amino, hydroxyl, (C_1-C_6) haloalkyl, NH-(C_1-C_6) alkyl, N((C_1-C_6)alkyl)$_2$, (C_3-C_6) carbocyclyl, or 4- to 6-membered heterocyclyl,

X is (C_1-C_6) alkyl, wherein said alkyl is further optionally substituted by one or more, identical or different R_{1a} groups, wherein each R_{1a} is independently (C_1-C_6) alkyl, (C_1-C_6) alkoxy, (C_1-C_6) alkyl-(C_1-C_3) alkoxy, halogen, amino, hydroxyl, (C_1-C_6) haloalkyl, NH-(C_1-C_6) alkyl, N((C_1-C_6)alkyl)$_2$, (C_3-C_6) carbocyclyl, or 4- to 6-membered heterocyclyl, and

Y and Y_1 are each independently CH or N;

R_1' and R_1'' are each independently H, CH_3, CF_3, halogen, CH_2NMe_2,  ,  , or  ;

R_2' and R_2'' are each independently H, halogen, CF_3, OH, OAc, CH_2OH, $CH(CH_3)OH$, $C(CH_3)_2OH$, CH_2OAc, CH_2OPG,  ,  ,  , or



wherein PG is a Protecting Group, provided that both R_2' and R_2" are not H, or R_2' and R_2" can be joined to form =O;

R_4' and R_4'' are each independently alkyl or aryl; and

n is 0 or 1.

13. The compound of claim 12, wherein R_4' and R_4'' are each independently CH_3, CH_2CH_3, CF_3, propyl, isopropyl, butyl, isobutyl, alkyl, or phenyl.

14. A method of creating an electrophilic warhead-bearing DNA-Encoded Library (eDEL) comprising:

coupling the compound of claim 12 with a DNA-Encoded Library to generate a stable masked-warhead DEL (mwDEL) intermediate;

purifying the mwDEL intermediate;

unmasking the mwDEL intermediate to generate an activated eDEL; and

purifying the activated eDEL.

15. An eDEL, which is generated from the method of claim 14.

FIG. 1A

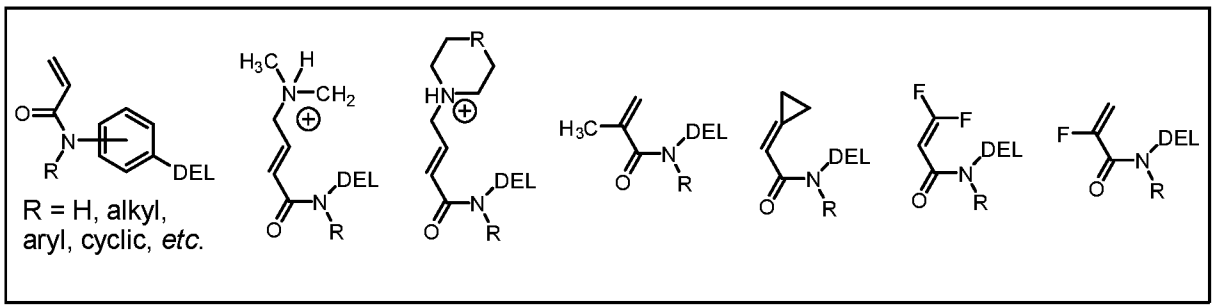

R = H, alkyl,
aryl, cyclic, etc.
DEL

FIG. 1B

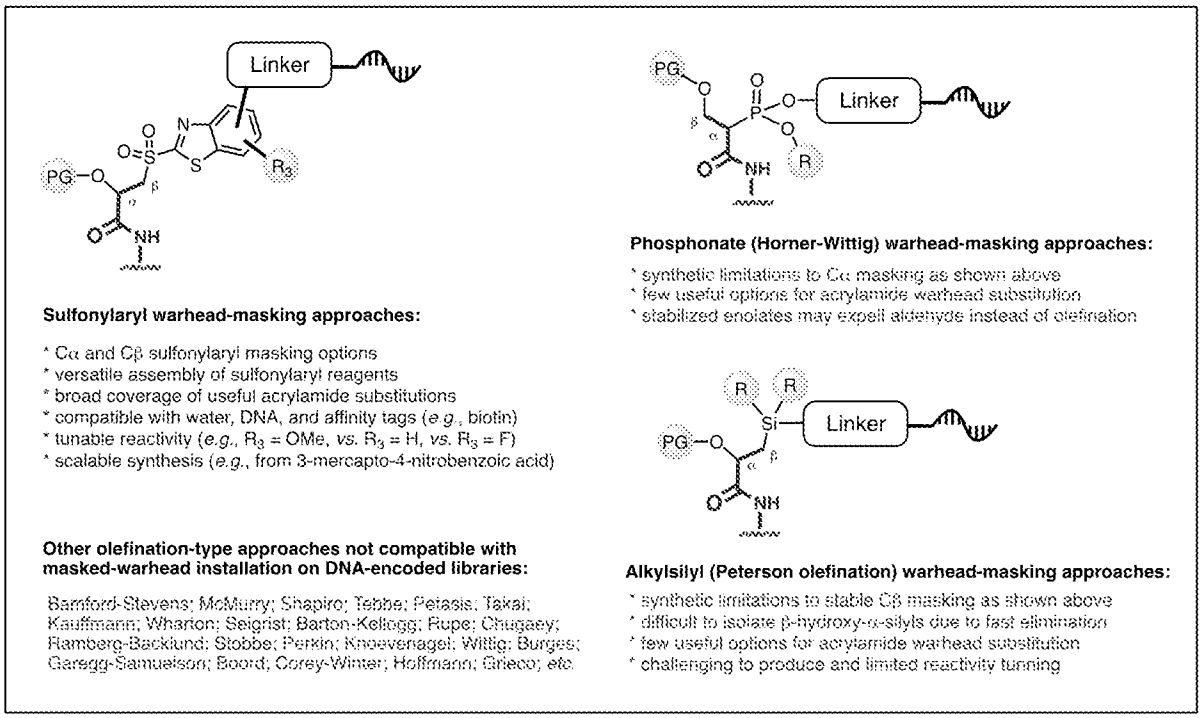

Linker
PG
R3
NH
Sulfonylaryl warhead-masking approaches:
* Cα and Cβ sulfonylaryl masking options
* versatile assembly of sulfonylaryl reagents
* broad coverage of useful acrylamide substitutions
* compatible with water, DNA, and affinity tags (e.g., biotin)
* tunable reactivity (e.g., R3 = OMe, vs. R3 = H, vs. R3 = F)
* scalable synthesis (e.g., from 3-mercapto-4-nitrobenzoic acid)
Other olefination-type approaches not compatible with masked-warhead installation on DNA-encoded libraries:
Bamford-Stevens; McMurry; Shapiro; Tebbe; Peiasis; Takai; Kauffmann; Wharton; Seigrist; Barton-Kellogg; Rupe; Chugaev; Ramberg-Backlund; Stobbe; Perkin; Knoevenagel; Wittig; Burges; Garegg-Samuelson; Boord; Corey-Winter; Hoffmann; Grieco; etc.
Phosphonate (Horner-Wittig) warhead-masking approaches:
* synthetic limitations to Cα masking as shown above
* few useful options for acrylamide warhead substitution
* stabilized enolates may expell aldehyde instead of olefination
Alkylsilyl (Peterson olefination) warhead-masking approaches:
* synthetic limitations to stable Cβ masking as shown above
* difficult to isolate β-hydroxy-α-silyls due to fast elimination
* few useful options for acrylamide warhead substitution
* challenging to produce and limited reactivity tunning

FIG. 1C

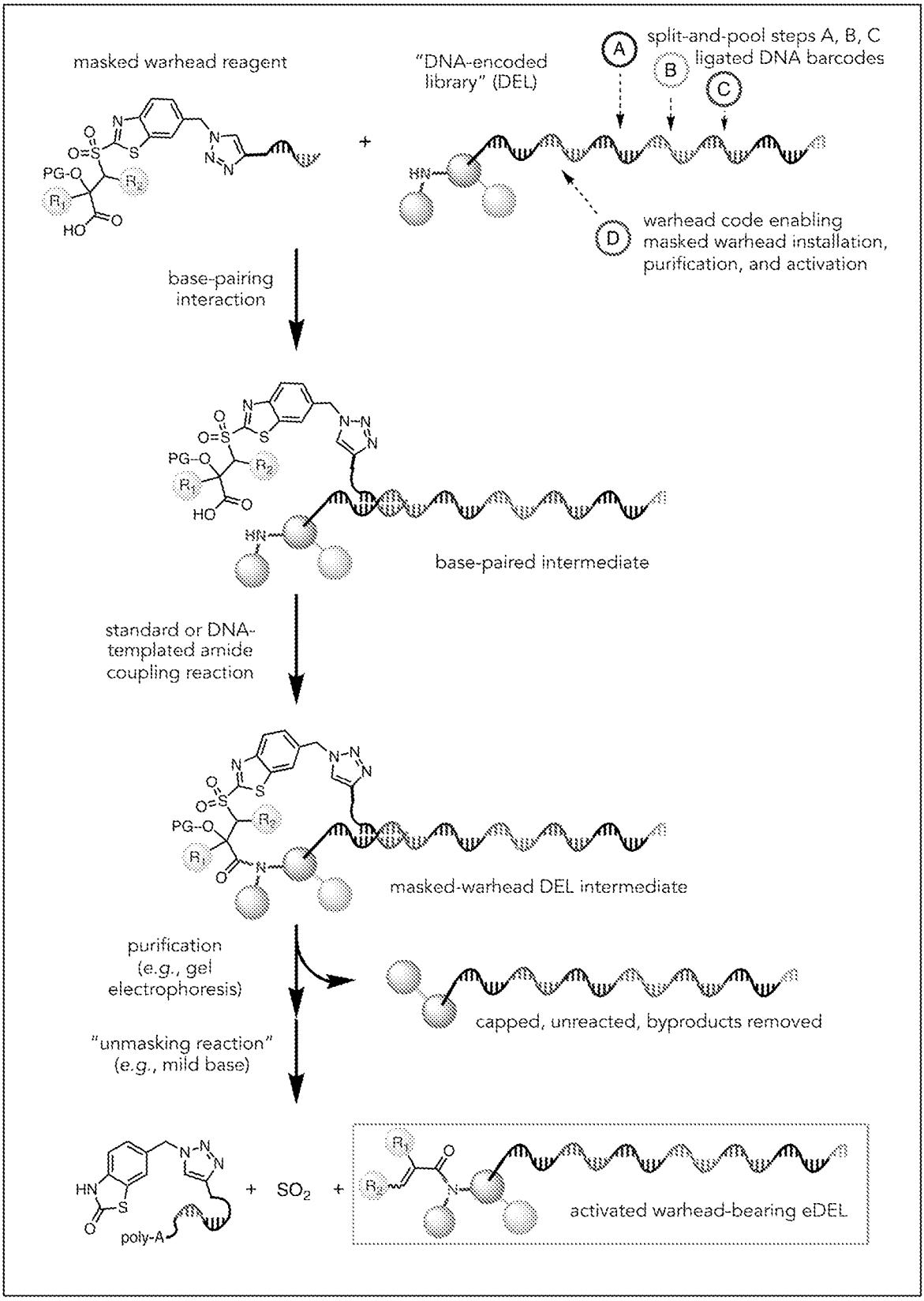

masked warhead reagent
"DNA-encoded library" (DEL)
split-and-pool steps A, B, C ligated DNA barcodes
warhead code enabling masked warhead installation, purification, and activation
base-pairing interaction
base-paired intermediate
standard or DNA-templated amide coupling reaction
masked-warhead DEL intermediate
purification (e.g., gel electrophoresis)
capped, unreacted, byproducts removed
"unmasking reaction" (e.g., mild base)
poly-A
activated warhead-bearing eDEL

FIG. 2A

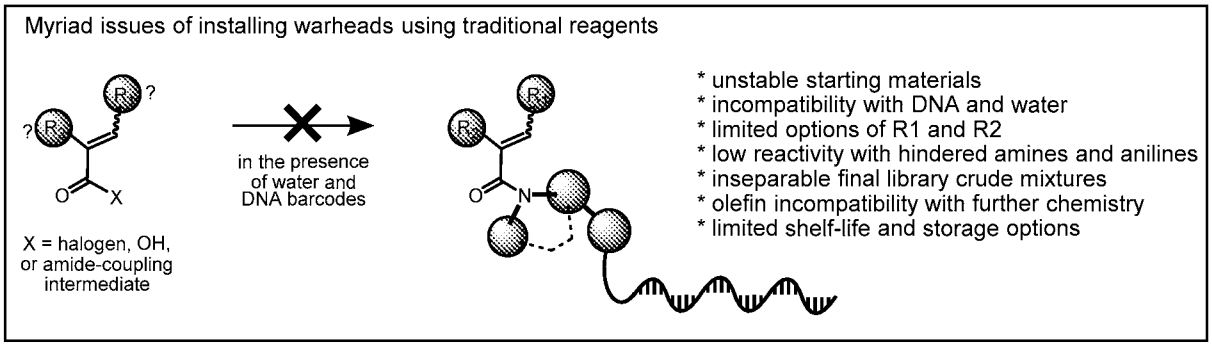

Myriad issues of installing warheads using traditional reagents
in the presence of water and DNA barcodes
X = halogen, OH, or amide-coupling intermediate
* unstable starting materials
* incompatibility with DNA and water
* limited options of R1 and R2
* low reactivity with hindered amines and anilines
* inseparable final library crude mixtures
* olefin incompatibility with further chemistry
* limited shelf-life and storage options

FIG. 2B

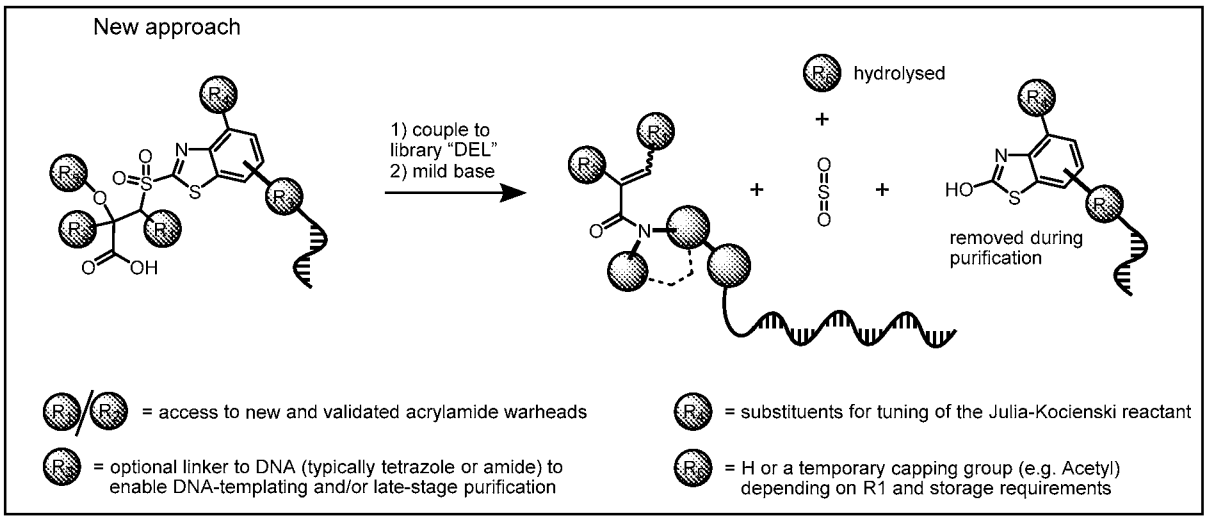
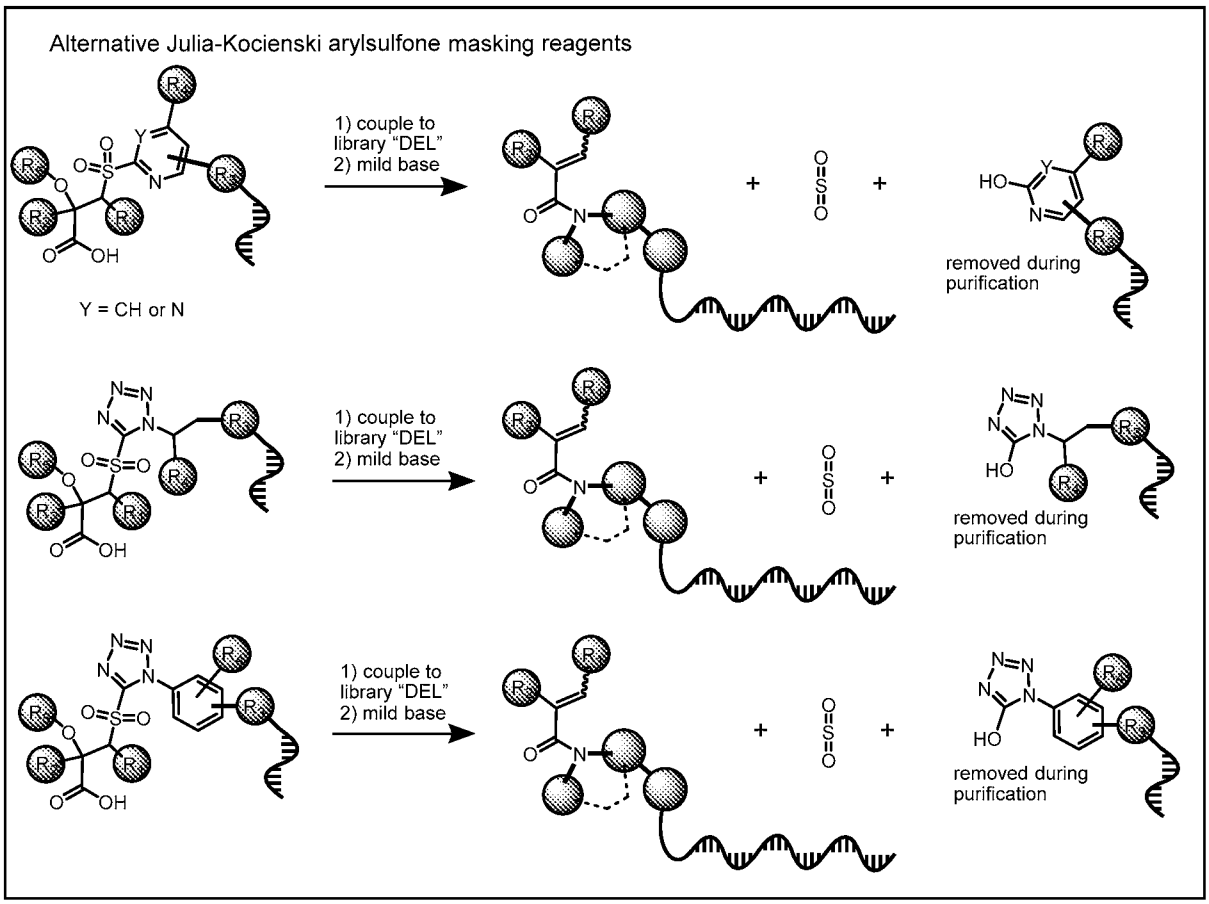

New approach
1) couple to library "DEL"
2) mild base
hydrolysed
removed during purification
= access to new and validated acrylamide warheads
= substituents for tuning of the Julia-Kocienski reactant
= optional linker to DNA (typically tetrazole or amide) to enable DNA-templating and/or late-stage purification
= H or a temporary capping group (e.g. Acetyl) depending on R1 and storage requirements

FIG. 2C



4853-3990-6108.1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US22/78783

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C07H 21/04; C12N 15/10; C12Q 1/68; C40B 40/08; C40B 50/16; G01N 33/68 (2022.01)
ADD.

CPC - INV. C07H 21/04; C12N 15/1065; C12N 15/1068; C12Q 1/68; C40B 50/16; G01N 33/68

ADD. C12N 2310/3517; C12Q 2563/179

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)
See Search History document

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

Electronic database consulted during the international search (name of database and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. "(Benzothiazole-2-sulfonyl)-acetic acid: PUBCHEM CID 693337" Pubchem entry (online). 07 July 2005; Retrieved from the Internet on 28 December 2022: [URL: https://pubchem.ncbi.nlm.nih.gov/compound/693337]; page 2, see 2D structure	1-11
A	NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. "2-Pyrimidin-2-ylsulfonylacetic acid: PUBCHEM CID 54342084" Pubchem entry (online). 04 December 2011; Retrieved from the Internet on 27 December 2022: [URL: https://pubchem.ncbi.nlm.nih.gov/compound/54342084]; page 2, see 2D structure	1-11
A	NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. "(Phenylsulfonyl)acetic acid: PUBCHEM CID 59543" Pubchem entry (online). 26 March 2005; Retrieved from the Internet on 27 December 2022: [URL: https://pubchem.ncbi.nlm.nih.gov/compound/59543]; page 2, see 2D structure	1-11
A	US 2021/0269863 A1 (PURDUE RESEARCH FOUNDATION) 02 September 2021; paragraphs [0055], [0066]-[0067], [0081]	1-15
A	US 2007/0042401 A1 (MORGAN, B) 22 February 2007; paragraphs [0005], [0030]	1-15
A	US 2021/0002630 A1 (X-CHEM, INC.) 07 January 2021; paragraphs [0002], [0046], [0056], [0060], [0094]-[0095], [0134]	1-15
A	NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. "2-(Ethyldimethylsilyl)acetic acid: PUBCHEM CID 55299497" Pubchem entry (online). 24 January 2012; Retrieved from the	12-15

☒ 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
"D" document cited by the applicant in the international application
"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	Date of mailing of the international search report
29 December 2022 (29.12.2022)	FEB 02 2023
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US22/78783

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Internet on 28 December 2022: [URL: https://pubchem.ncbi.nlm.nih.gov/compound/55299497]; page 2, see 2D structure	
A	NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. "3-(Phenyldimethylsilyl)propionic acid: PUBCHEM CID 2755265" Pubchem entry (online). 19 July 2005; Retrieved from the Internet on 28 December 2022: [URL: https://pubchem.ncbi.nlm.nih.gov/compound/2755265]; page 2, see 2D structure	12-15