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(54) Title: MACROCYCLIC KINASE INHIBITORS AND USES THEREOF

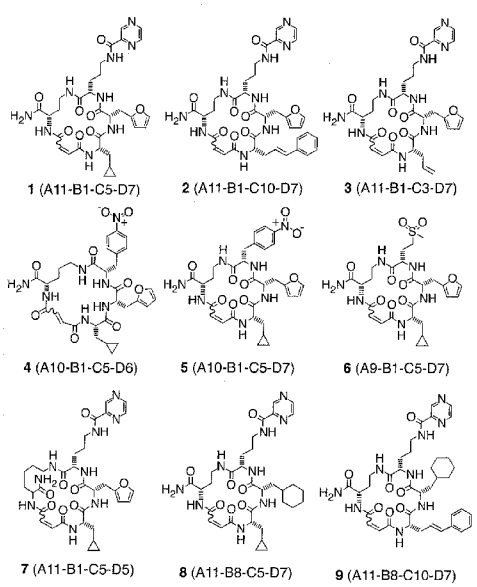

Fig. 5

(57) Abstract: The present invention provides macrocyclic compounds of Formula (I): pharmaceutically acceptable salts thereof; and pharmaceutical compositions thereof, wherein R_1, R_2, R_3, R_4, R^E, R^F, R^G, R^H, R^I, f, g, h, n, and m are as defined herein. The present invention further provides methods of synthesizing these macrocyclic compounds, and methods of their use and treatment. Certain aspects of the present invention relate to modulation of kinase activity, and in the treatment of kinase-associated diseases or disorders.

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— *with sequence listing part of description (Rule 5.2(a))*

MACROCYCLIC KINASE INHIBITORS AND USES THEREOF

RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. provisional patent application, U.S.S.N. 61/368,986, filed July 29, 2010, the entire contents of which are hereby incorporated by reference.

GOVERNMENT SUPPORT

[0002] This invention was made with U.S. Government support under grant R01 GM065865 awarded by the National Institutes of Health (NIH). The U.S. Government has certain rights in the invention.

BACKGROUND

[0003] The discovery of small molecules capable of selectively modulating the activity of biological targets remains a central challenge of chemistry and chemical biology. Such small molecules are commonly discovered through combinatorial[1,2] or diversity-oriented[3] synthesis and high-throughput screening[4] (HTS). In contrast, functional molecules emerge in nature through iterated cycles of translation, selection, and amplification with mutation.[5-8] While scientists have applied components of biological evolution to generate DNA, RNA, and protein molecules with tailor-made catalytic or binding properties, this approach has traditionally been restricted to molecules whose structures are compatible with biosynthetic machinery.[9-16] DNA-templated organic synthesis was recently developed as a method for translating DNA sequences into synthetic small molecules[17-25] (see also published PCT application, WO 2004/016767) and synthetic polymers[26-28] that can be subjected to *in vitro* selection for desired properties.[17,20,23,28,29] Several related approaches to generate and evaluate DNA-encoded small-molecule libraries have also been used successfully in academic[30-37] and industrial settings.[38,39]

[0004] Macrocycles are particularly attractive candidates for the discovery of biologically active small molecules because their rigid scaffolds can decrease the entropic cost of target binding and limit access to non-binding conformations, resulting in higher affinity and greater binding specificity than their corresponding linear counterparts.[40] In addition, macrocyclic peptide-like structures can offer advantages for applications in cell culture and *in vivo* over their linear analogs since they can possess higher bioavailability,

membrane permeability, and resistance to *in vivo* degradation.[40] While synthesizing macrocyclic structures, especially in a library format, can be challenging,[41,42] features of DNA-templated synthesis including compatibility with aqueous solvents, extremely low (nM) reactant concentrations, and the ability of base pairing to hold together relevant reactants at high effective molarities can promote efficient macrocylization.

SUMMARY OF THE INVENTION

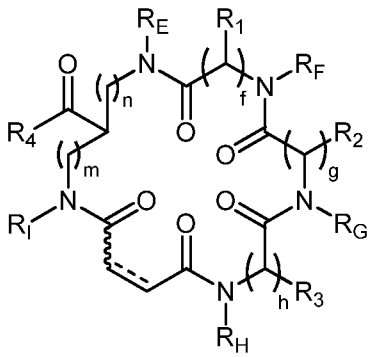
[0005] DNA-templated organic synthesis enables the translation of DNA sequences into synthetic small- molecule libraries suitable for *in vitro* selection. Previously, model *in vitro* selection of a pilot library of 65 macrocycles was described[20]. Subsequent advances in DNA template design and DNA-templated synthesis methods enabled the preparation and characterization of a larger 13,824-membered DNA-templated macrocycle library.[24] Here the discovery of small molecules that modulate the activity of kinase enzymes through the *in vitro* selection of this DNA-templated small-molecule macrocycle library against 36 biomedically relevant protein targets is reported. Some of these protein targets are kinases - enzymes that phosphorylate their substrate, for example, their specific target protein in the case of protein kinases. The human genome contains about 500 protein kinase genes and dysregulation of kinase activity has been implicated in numerous diseases. Accordingly, compounds modulating the activity of specific kinases, for example, the kinases described herein, are useful for clinical as well as basic research applications.

[0006] DNA encoding selection survivors was amplified by PCR and identified by DNA sequencing. Macrocycles corresponding to DNA sequences enriched upon selection against several protein kinases were synthesized on a multi-milligram scale. *In vitro* assays revealed that these macrocycles inhibit (or activate) the kinases against which they were selected with IC_{50} values as low as approximately 680 nM. A family of macrocycles enriched upon selection against Src kinase was characterized in depth, and inhibition was shown to be highly dependent on the identity of macrocycle building blocks as well as on backbone conformation (see, *e.g.*, Figures 5 and 8-11). Two macrocycles in this family exhibited unusually strong Src inhibition selectivity even among kinases closely related to Src. One macrocycle was found to activate, rather than inhibit, its target kinase, VEGFR2. These results establish the use of DNA-templated synthesis and *in vitro* selection to discover novel small molecules that modulate enzyme activities, and also reveal a new molecular scaffold for selective ATP-competitive kinase inhibitors.

[0007] The Src kinase inhibitors discovered through this approach are believed to represent the first examples of synthetic peptidic macrocycles that inhibit protein kinase activity in an ATP-competitive manner. Some of the Src-inhibiting macrocycles exhibited unusual selectivity for Src when screened against a representative panel of human protein kinases. Further, some of the Src-inhibiting macrocylces described herein inhibit mutant forms of kinases, for example, mutant forms of Src kinase (e.g., Src kinase having a gatekeeper residue mutation, e.g., a Thr338Ile mutation). Macrocycles that activate VEGFR2 kinase and that inhibit Akt3, MAPKAPK2, p38α, and Pim1 kinases were also discovered. These results reveal two novel and synthetically versatile scaffolds for the selective inhibition of Src-family protein kinases.

[0008] Some aspects of this invention relate to compounds that bind and modulate the activity of kinases. In some embodiments, macrocyclic compounds are provided that modulate the activity of a kinase. For example, in some embodiments, kinase-inhibitory compounds are provided and in other embodiments, kinase-activating compounds are provided. In some embodiments, macrocyclic compounds that inhibit Src, Akt3, MAPKAPK2, p38α, and/or Pim1 kinases are provided. In some embodiments, compounds that activate VEGFR2 kinase are provided.

[0009] In certain embodiments, a compound that binds and modulates the activity of a kinase as provided herein is of Formula (I):



(I)

or a pharmaceutically acceptable salt thereof;

wherein:

n is 0 or an integer between 1-4, inclusive;

m is 0 an integer between 1-4, inclusive;

f is an integer between 1-3, inclusive;

g is an integer between 1-3, inclusive;

h is an integer between 1-3, inclusive;

------ is a single or double C-C bond, wherein when ------ is a double C-C bond, then ~~~~ indicates that the adjacent C-C double bond is in a *cis* or *trans* configuration;

each instance of R_1 is independently hydrogen; halogen; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; acyl; $-OR_A$; $-N(R_A)_2$; $-SR_A$; =O; -CN; $-NO_2$; -SCN; $-SOR_A$; or $-SO_2R_A$; wherein each occurrence of R_A is independently hydrogen; a protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; acyl; substituted or unsubstituted aryl; or substituted or unsubstituted heteroaryl;

each instance of R_2 is independently hydrogen; halogen; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; acyl; $-OR_B$; $-N(R_B)_2$; $-SR_B$; =O; -CN; $-NO_2$; -SCN; $-SOR_B$; or $-SO_2R_B$; wherein each occurrence of R_B independently hydrogen; a protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; acyl; substituted or unsubstituted aryl; or substituted or unsubstituted heteroaryl;

each instance of R_3 is independently hydrogen; halogen; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; acyl; $-OR_C$; $-N(R_C)_2$; $-SR_C$; =O; -CN; $-NO_2$; -SCN; $-SOR_C$; or $-SO_2R_C$; wherein each occurrence of R_C is independently hydrogen; a protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; acyl; substituted or unsubstituted aryl; or substituted or unsubstituted heteroaryl;

R_4 is substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; $-N(R_D)_2$; $-OR_D$; or $-SR_D$; wherein each occurrence of R_D is independently independently hydrogen; a protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; acyl; substituted or unsubstituted aryl; or substituted or unsubstituted heteroaryl; or two R_D groups are joined to form a substituted or unsubstituted heterocyclic group; optionally wherein R^4 further comprises a label, resin, or therapeutic agent attached thereto; and

each instance of R_E, R_F, R_G, R_H, and R_I is independently hydrogen; acyl; a nitrogen protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; substitute or unsubstituted hydroxyl; substituted or unsubstituted thiol; substituted or unsubstituted amino; or halogen; optionally wherein an R_1 group and R^F are joined to form a substituted or unsubstituted heterocyclic ring; an R_2 group and R^G are joined to form a

substituted or unsubstituted heterocyclic ring; and/or an R_3 group and R^H are joined to form a substituted or unsubstituted heterocyclic ring.

[0010] Some aspects of this invention relate to compositions comprising any of the kinase-modulating compounds provided herein. In some embodiments, compositions of the kinase-modulating macrocyclic compounds are provided. In some embodiments, the provided compositions are pharmaceutical compositions, for example, compositions for clinical use in humans.

[0011] Other aspects of this invention relate to methods of synthesizing a kinase-modulating compound as provided herein. In some embodiments, methods of synthesizing kinase-modulatory macrocyclic compounds via DNA-encoded addition synthesis are provided. In other embodiments, synthesis methods that do not rely on DNA encoded synthesis are provided. In some embodiments, synthetic methods including multiple Fmoc synthesis cycles to generate a peptidic macrocyclization precursor from individual building blocks are provided. In some embodiments, the building blocks are amino acids or amino-acid like compounds. In some embodiments, the synthetic methods include cyclization of a macrocyclization precursor, for example, by Wittig cyclization. In certain embodiments, synthetic methods provided herein are solid-phase synthetic methods.

[0012] Some aspects of this invention relate to methods of using a compound provided herein to modulate the activity of a kinase. In some embodiments, methods that include contacting the kinase with a compound as provided herein are provided to modulate the activity of a specific kinase. In some embodiments, the kinase is a clinically relevant kinase, for example, a Src, Akt3, MAPKAPK2, p38α, Pim1, or VEGFR2 kinase. In some embodiments, the kinase is inhibited or activated by administering to a subject exhibiting an aberrant (*e.g.,* an increased or constitutive) activity of the kinase an effective amount of a compound as provided herein. In some embodiments, the subject is diagnosed with a disease or disorder associated with aberrant kinase activity, for example, with a proliferative disorder, including, but not limited to benign neoplasm, malignant neoplasm, or cancer associated with increased Src, Akt3, MAPKAP2, p38, or Pim1 activity, or with metabolic syndrome, diabetes, insulin resistance, a neurodegenerative disorder, osteoporosis, inflammatory disease, or an autoimmune disorder.

[0013] Other advantages, features, and uses of the invention will become apparent from the following detailed description of non-limiting embodiments of the invention when considered in conjunction with the accompanying drawings. All publications, patents, patent applications, database entries, and other referenes cited herein are incorporated in their

entirety by reference into this application.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] **Figure 1.** Restriction endonuclease digestion to reveal enrichment of BAK peptide-linked DNA after selection for binding to immobilized GST-BcL-xL, followed by PCR amplification of eluted molecules.

[0015] **Figure 2.** 13,824-membered DNA-templated small-molecule macrocycle library. a) Scheme for the multistep DNA-templated synthesis of the macrocycle library.[24] b) Amino-acid building blocks used in the library synthesis. Step one building blocks: **A1**-**A12**; step two building blocks: **B1**-**B12;** step three building blocks: **C1**-**C12;** and the macrocycle scaffolds: (**D1**-**D8**) are shown.

[0016] **Figure 3.** *In vitro* selection of the DNA-templated macrocycle library against 36 protein targets. Following affinity selection of the library against an immobilized GST-fused target protein, library members possessing target affinity were eluted and their attached DNA templates were amplified by PCR using DNA primers containing a barcode sequence that uniquely identifies the protein target. Barcoded PCR amplicons from 36 target protein-binding selections, one control selection for binding immobilized GST, and eight aliquots of unselected library were pooled and submitted as one sample for ultra high-throughput DNA sequencing.

[0017] **Figure 4.** Deconvolution and analysis of high-throughput sequencing results. (a) Paired-end reads were assembled and binned computationally by barcode. The enrichment factor for each library member in each selection (~497,000 total possible combinations of library member and target) were calculated as (post-selection abundance)÷(pre-selection abundance) using a PERL script. (b) Plot of enrichment factor vs. sequence abundance for library members after selection for binding to Src kinase. Each blue or red dot represents the DNA sequence corresponding to a single library member. The structures of macrocycles **1**-**9** are shown in **Figure 5**. Enrichment factors for low-abundance library members vary widely due to statistical undersampling. Only enrichment factors that were substantially higher than background values were considered potential positives (shown in red).

[0018] **Figure 5.** Chemical structures of macrocycles exhibiting significant enrichment above background and common structural motifs after selection against Src kinase. Macrocycle numbering corresponds to that used in **Figure 4b**.

[0019] **Figure 6.** Plots of enrichment factor vs. sequence abundance for library

members after selection for binding to Akt3, MAPKAPK2, Pim1, and VEGFR2. Macrocycles **10-19** exhibit significant enrichment above background.

[0020] **Figure 7.** Milligram-scale synthesis of macrocycle A11-B1-C5-D7. The route was adapted from Gartner *et al.*[20] Methyl hydrogen 2,3-*O*-isopropylidene-(L)-tartrate was synthesized as reported by Musich and Rapoport[69].

[0021] **Figure 8.** A representative set of Src kinase inhibition assay data.

[0022] **Figure 9.** Inhibition of kinase activity using macrocycles *cis*-A10-B1-C11-D5 and *cis*-A10-B8-C11-D5.

[0023] **Figure 10.** VEGFR2 activity increases upon treatment with macrocycle *cis*-A10-B1-C11-D5. Kinase activity was measured as in **Table 6**. Activation was calculated by dividing the amount of substrate phosphorylation observed in the presence of the macrocycle by the amount of substrate phosphorylation observed in the absence of the macrocycle.

[0024] **Figure 11.** Kinase inhibition activities of macrocycles of the *cis*-A12-B8-C10-DX family.

[0025] **Figure 12.** Macrocycle *cis*-A11-B1-C5-D7 is an ATP-competitive inhibitor of Src kinase. a) The apparent K_M of Src for ATP was measured in the presence of increasing concentrations of macrocycle *cis*-A11-B1-C5-D7. Kinase activity was measured as in **Table 6**. b) The resulting relationship is linear in accord with the following equation for a classical competitive inhibitor: apparent $K_M = K_M*(1+[\text{inhibitor}]/K_i)$.

[0026] **Figure 13.** Macrocycle *trans*-A10-B1-C5-D6 is an ATP-competitive Src inhibitor. Src kinase activity was measured as a function of ATP concentration at 3 μM and 9 μM concentrations of macrocycle *trans*-A10-B1- C5-D6 and compared against Src activity in the presence of DMSO control. Macrocycle *trans*-A10-B1-C5-D6 induces a shift in the observed $K_{M,\,ATP}$ indicating ATP-competitive inhibition. Kinase activity was measured as described in **Table 6**.

[0027] **Figure 14.** Selectivity of macrocycles *cis*-A11-B1-C5-D7 and *trans*-A10-B1-C5-D6 among Src-related kinases, including all nine Src-family kinases. Both macrocycles were assayed at 5 μM concentration against the indicated kinases. Assays of *cis*-A11-B1-C5-D7 against Abl and Hck were performed at 50 μM macrocycle concentration instead of 5 μM. Assay data for Src kinase is from **Figure 8** and **Table 6**. All non-Src assay points were measured by the Invitrogen Select Screen Profiling Service

[0028] **Figure 15.** Structure-activity relationship (SAR) analysis of Src-inhibiting macrocycles. a) Single-alanine mutants of *cis*-A11-B1-C5- D7 were assayed against Src-

family kinases Src and Fgr at 5 μM concentration as described previously. b) Linear diacetylated peptides corresponding to macrocycles A11-B1-C5-D7 and A10- B1-C5-D6 were synthesized by Fmoc solid-phase peptide synthesis and assayed against Src kinase. c) Macrocycle *trans*-Phe-B1-C5-D6 (replacing nitrophenylalanine with phenylalanine) was assayed against Src as described previously.

[0029] **Figure 16.** MM2 energy-minimized (Chem3D, CambridgeSoft) models of macrocycles *cis*-A11-B1- C5-D7 and *trans*-A10-B1-C5-D6. The models are oriented such that the α carbon of each scaffold amino acid is in the same spatial orientation.

[0030] **Figure 17.** Enzyme titration test for promiscuous aggregator behavior. The specified macrocycles were assayed in the presence of varying concentrations of enzyme (shown in the legend). Significant dependence of apparent IC_{50} on enzyme concentration was observed for macrocycle *cis*-A11-B8-C10- D5 when assayed against Akt3, consistent with the behavior of a promiscuous aggregator, but not for the other macrocycles assayed.

[0031] **Figure 18.** NMR spectra and LC/MS data for certain macrocycles.

[0032] **Figure 19.** Macrocycle kinase inhibitors with improved inhibition potency. Macrocycle compounds **MC1** (trans A11-B1-C5-D7), **MC1.2** (trans A11-Phe-Cha-D7), **MC4** (cis A10-B1-C5-D6), **MC4.1** (trans A10-Phe-Cha-D6), **MC4.3** (trans A10-[4-F-Phe]-Cha-D6),-and **MC9** (trans A11-B8-C10-D7). Macrocycles **MC1**, **MC1.2**, and **MC9** are based off a diaminobutyric acid scaffold, and contain a pyrazine group in the A position (shown in red). Macrocycles **MC4**, **MC4.1**, **MC4.2**, and **MC4.3** are based off an ornithine scaffold, and contains a nitrophenylalanine in position A. Positions B and C varied for each compound, resulting in different affinities for Src kinase. Adapted from Kleiner, et al. JACS (2010).

[0033] **Figure 20.** The macrocycle compounds specificity for Src kinase over Hck, Lck, and Abl kinases. **(A)** IC_{50} values of macrocycles **MC1**, **MC1.2**, **MC4**, **MC4.1**, and **MC9**. IC_{50} values of the macrocycle compounds were determined in the presence of 5 μM ATP and 100 μM Src optimal substrate peptide. Concentrations of kinase used were 0.25 μM for Src, 0.042 μM for Hck, 0.5 μM for Lck, and 0.5 μM for Abl. **(B)** Src83-533 is more potently inhibited by the Macrocycle compounds than the kinase domain of Src. IC_{50} values of the macrocycle compounds were determined in the presence of 5 μM ATP and 100 μM Src optimal substrate peptide and 0.66 μM of Src83-533. *value is an upper estimate of the IC_{50} because it is 2-fold less than the concentration of kinase used. **(C)** Macrocycle compounds are mixed inhibitors. IC_{50} values of the Macrocycle compounds for Src kinase domain in response to a 50-fold decrease in ATP (5 μM to 250 μM), and a 3-fold change in Src optimal

peptide (100 μM to 300 μM). IC_{50} values decrease as ATP and substrate peptide decrease, showing that the macrocycle compounds compete with both ATP and substrate peptide. *values are an upper estimate of the IC_{50} because it is 2-fold less than the concentration of kinase used.

[0034] **Figure 21. (A)** Macrocylces A11-B1-C5-D7 and A10-B1-C5-D6 are ATP- and substrate-competitive Src kinase inhibitors. Inhibition of Src decreases for both compounds with increasing ATP concentration. K_D of fluorescein-tagged macrocycles increases with increasing concentrations of src substrate peptide. **(B)** K_D of the fluorescein labeled Macrocycles **MC1**, **MC4**, and **MC9** for Src kinase domain in the presence of 250 μM AMP-PNP and 300 μM Src optimal peptide. In the presence of AMP-PNP, or Src optimal peptide, the KD increases, showing that the macrocycles compete with both ATP and peptide for binding.

[0035] **Figure 22**. Inhibition of the Src gatekeeper mutant T338I by cis-A11-B1-C5-D7 and trans-A10-B1-C5-D6. The T338I mutation ("gatekeeper" residue) confers resistance to most ATP-competitive inhibitors and promotes oncogenic activation of Src.

[0036] **Figure 23.** Src kinase inhibition in mammalian cells.

DEFINITIONS

Chemical definitions

[0037] Definitions of specific functional groups and chemical terms are described in more detail below. For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, *Handbook of Chemistry and Physics*, 75th Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in *Organic Chemistry*, Thomas Sorrell, University Science Books, Sausalito, 1999; Smith and March *March's Advanced Organic Chemistry*, 5th Edition, John Wiley & Sons, Inc., New York, 2001; Larock, *Comprehensive Organic Transformations*, VCH Publishers, Inc., New York, 1989; Carruthers, *Some Modern Methods of Organic Synthesis*, 3rd Edition, Cambridge University Press, Cambridge, 1987.

[0038] Compounds described herein can comprise one or more asymmetric centers, and thus can exist in various isomeric forms, *e.g.*, enantiomers and/or diastereomers. For example, the compounds described herein can be in the form of an individual enantiomer, diastereomer or geometric isomer, or can be in the form of a mixture of stereoisomers, including racemic mixtures and mixtures enriched in one or more stereoisomer. Isomers can

be isolated from mixtures by methods known to those skilled in the art, including chiral high pressure liquid chromatography (HPLC) and the formation and crystallization of chiral salts; or preferred isomers can be prepared by asymmetric syntheses. See, for example, Jacques *et al.*, *Enantiomers, Racemates and Resolutions* (Wiley Interscience, New York, 1981); Wilen *et al.*, *Tetrahedron* 33:2725 (1977); Eliel, *Stereochemistry of Carbon Compounds* (McGraw–Hill, NY, 1962); and Wilen, *Tables of Resolving Agents and Optical Resolutions* p. 268 (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN 1972). The invention additionally encompasses compounds described herein as individual isomers substantially free of other isomers, and alternatively, as mixtures of various isomers.

[0039] Where an isomer/enantiomer is preferred, it may, in some embodiments, be provided substantially free of the corresponding enantiomer, and may also be referred to as "optically enriched." "Optically enriched," as used herein, means that the compound is made up of a significantly greater proportion of one enantiomer. In certain embodiments the compound of the present invention is made up of at least about 90% by weight of a preferred enantiomer. In other embodiments the compound is made up of at least about 95%, 98%, or 99% by weight of a preferred enantiomer. Preferred enantiomers may be isolated from racemic mixtures by any method known to those skilled in the art, including chiral high pressure liquid chromatography (HPLC) and the formation and crystallization of chiral salts or prepared by asymmetric syntheses. See, for example, Jacques *et al.*, *Enantiomers, Racemates and Resolutions* (Wiley Interscience, New York, 1981); Wilen *et al.*, *Tetrahedron* 33:2725 (1977); Eliel, *Stereochemistry of Carbon Compounds* (McGraw–Hill, NY, 1962); Wilen, *Tables of Resolving Agents and Optical Resolutions* p. 268 (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN 1972).

[0040] When a range of values is listed, it is intended to encompass each value and sub–range within the range. For example "C_{1-6} alkyl" is intended to encompass, C_1, C_2, C_3, C_4, C_5, C_6, C_{1-6}, C_{1-5}, C_{1-4}, C_{1-3}, C_{1-2}, C_{2-6}, C_{2-5}, C_{2-4}, C_{2-3}, C_{3-6}, C_{3-5}, C_{3-4}, C_{4-6}, C_{4-5}, and C_{5-6} alkyl.

[0041] The term "aliphatic," as used herein, includes both saturated and unsaturated, straight chain (*i.e.*, unbranched), branched, acyclic, and cyclic (*i.e.*, carbocyclic) hydrocarbons, which are optionally substituted with one or more functional groups. It is understood from the above description that the term "aliphatic," whether preceded by the terms substituted or unsubstituted, and unless otherwise specified, encompasses "cyclic or acyclic" and "branched or unbranched" groups. As will be appreciated by one of ordinary skill in the art, "aliphatic" is intended herein to include, but is not limited to, alkyl, alkenyl,

alkynyl, and carbocyclyl (cycloalkyl, cycloalkenyl, and cycloalkynyl) moieties. In certain embodiments, as used herein, "aliphatic" is used to indicate those aliphatic groups (cyclic, acyclic, substituted, unsubstituted, branched or unbranched) having 1–20 carbon atoms. Unless otherwise specified, each instance of an aliphatic group is independently unsubstituted or substituted with one or more substituents, as valency permits, and which results in a stable compound. Exemplary substituents are further described herein.

[0042] The term "alkyl" refers to a radical of a straight–chain or branched saturated hydrocarbon group having from 1 to 20 carbon atoms ("C_{1-20} alkyl"). In some embodiments, an alkyl group has 1 to 10 carbon atoms ("C_{1-10} alkyl"). In some embodiments, an alkyl group has 1 to 9 carbon atoms ("C_{1-9} alkyl"). In some embodiments, an alkyl group has 1 to 8 carbon atoms ("C_{1-8} alkyl"). In some embodiments, an alkyl group has 1 to 7 carbon atoms ("C_{1-7} alkyl"). In some embodiments, an alkyl group has 1 to 6 carbon atoms ("C_{1-6} alkyl"). In some embodiments, an alkyl group has 1 to 5 carbon atoms ("C_{1-5} alkyl"). In some embodiments, an alkyl group has 1 to 4 carbon atoms ("C_{1-4} alkyl"). In some embodiments, an alkyl group has 1 to 3 carbon atoms ("C_{1-3} alkyl"). In some embodiments, an alkyl group has 1 to 2 carbon atoms ("C_{1-2} alkyl"). In some embodiments, an alkyl group has 1 carbon atom ("C_1 alkyl"). In some embodiments, an alkyl group has 2 to 6 carbon atoms ("C_{2-6} alkyl"). Examples of C_{1-6} alkyl groups include methyl (C_1), ethyl (C_2), n–propyl (C_3), isopropyl (C_3), n–butyl (C_4), tert–butyl (C_4), sec–butyl (C_4), iso–butyl (C_4), n–pentyl (C_5), 3–pentanyl (C_5), amyl (C_5), neopentyl (C_5), 3–methyl–2–butanyl (C_5), tertiary amyl (C_5), n–hexyl (C_6), and the like, which may bear one or more substituents. Additional examples of alkyl groups include n–heptyl (C_7), n–octyl (C_8) and the like, which may bear one or more sustitutents. Unless otherwise specified, each instance of an alkyl group is independently unsubstituted or substituted with one or more substituents, as valency permits, and which results in a stable compound. Exemplary substituents are further described herein.

[0043] The term "perhaloalkyl" is a substituted alkyl group as defined herein wherein all of the hydrogen atoms are independently replaced by a halogen, *e.g.,* fluoro, bromo, chloro, or iodo. In some embodiments, the alkyl moiety has 1 to 8 carbon atoms ("C_{1-8} perhaloalkyl"). In some embodiments, the alkyl moiety has 1 to 6 carbon atoms ("C_{1-6} perhaloalkyl"). In some embodiments, the alkyl moiety has 1 to 4 carbon atoms ("C_{1-4} perhaloalkyl"). In some embodiments, the alkyl moiety has 1 to 3 carbon atoms ("C_{1-3} perhaloalkyl"). In some embodiments, the alkyl moiety has 1 to 2 carbon atoms ("C_{1-2} perhaloalkyl"). In some embodiments, all of the hydrogen atoms are replaced with fluoro. In some embodiments, all of the hydrogen atoms are replaced with chloro. Examples of

perhaloalkyl groups include $-CF_3$, $-CF_2CF_3$, $-CF_2CF_2CF_3$, $-CCl_3$, $-CFCl_2$, $-CF_2Cl$, and the like.

[0044] The term "alkenyl" refers to a radical of a straight–chain or branched hydrocarbon group having from 2 to 20 carbon atoms, one or more carbon–carbon double bonds, and no triple bonds ("C_{2-20} alkenyl"). In some embodiments, an alkenyl group has 2 to 10 carbon atoms ("C_{2-10} alkenyl"). In some embodiments, an alkenyl group has 2 to 9 carbon atoms ("C_{2-9} alkenyl"). In some embodiments, an alkenyl group has 2 to 8 carbon atoms ("C_{2-8} alkenyl"). In some embodiments, an alkenyl group has 2 to 7 carbon atoms ("C_{2-7} alkenyl"). In some embodiments, an alkenyl group has 2 to 6 carbon atoms ("C_{2-6} alkenyl"). In some embodiments, an alkenyl group has 2 to 5 carbon atoms ("C_{2-5} alkenyl"). In some embodiments, an alkenyl group has 2 to 4 carbon atoms ("C_{2-4} alkenyl"). In some embodiments, an alkenyl group has 2 to 3 carbon atoms ("C_{2-3} alkenyl"). In some embodiments, an alkenyl group has 2 carbon atoms ("C_2 alkenyl"). The one or more carbon–carbon double bonds can be internal (such as in 2–butenyl) or terminal (such as in 1–butenyl). Examples of C_{2-4} alkenyl groups include ethenyl (C_2), 1–propenyl (C_3), 2–propenyl (C_3), 1–butenyl (C_4), 2–butenyl (C_4), butadienyl (C_4), and the like, which may bear one or more substituents. Examples of C_{2-6} alkenyl groups include the aforementioned C_{2-4} alkenyl groups as well as pentenyl (C_5), pentadienyl (C_5), hexenyl (C_6), and the like, which may bear one or more substituents. Additional examples of alkenyl include heptenyl (C_7), octenyl (C_8), octatrienyl (C_8), and the like, which may bear one or more substituents. Unless otherwise specified, each instance of an alkenyl group is independently unsubstituted or substituted with one or more substituents, as valency permits, and which results in a stable compound. Exemplary substituents are further described herein.

[0045] The term "alkynyl" refers to a radical of a straight–chain or branched hydrocarbon group having from 2 to 20 carbon atoms, one or more carbon–carbon triple bonds, and optionally one or more double bonds ("C_{2-20} alkynyl"). In some embodiments, an alkynyl group has 2 to 10 carbon atoms ("C_{2-10} alkynyl"). In some embodiments, an alkynyl group has 2 to 9 carbon atoms ("C_{2-9} alkynyl"). In some embodiments, an alkynyl group has 2 to 8 carbon atoms ("C_{2-8} alkynyl"). In some embodiments, an alkynyl group has 2 to 7 carbon atoms ("C_{2-7} alkynyl"). In some embodiments, an alkynyl group has 2 to 6 carbon atoms ("C_{2-6} alkynyl"). In some embodiments, an alkynyl group has 2 to 5 carbon atoms ("C_{2-5} alkynyl"). In some embodiments, an alkynyl group has 2 to 4 carbon atoms ("C_{2-4} alkynyl"). In some embodiments, an alkynyl group has 2 to 3 carbon atoms ("C_{2-3} alkynyl"). In some embodiments, an alkynyl group has 2 carbon atoms ("C_2 alkynyl"). The one or more

carbon–carbon triple bonds can be internal (such as in 2–butynyl) or terminal (such as in 1–butynyl). Examples of C_{2-4} alkynyl groups include, without limitation, ethynyl (C_2), 1–propynyl (C_3), 2–propynyl (C_3), 1–butynyl (C_4), 2–butynyl (C_4), and the like, which may bear one or more substituents. Examples of C_{2-6} alkenyl groups include the aforementioned C_{2-4} alkynyl groups as well as pentynyl (C_5), hexynyl (C_6), and the like, which may bear one or more substituents. Additional examples of alkynyl include heptynyl (C_7), octynyl (C_8), and the like, which may bear one or more substituents. Unless otherwise specified, each instance of an alkynyl group is independently unsubstituted or substituted with one or more substituents, as valency permits, and which results in a stable compound. Exemplary substituents are further described herein.

[0046] The term "carbocyclyl" or "carbocyclic" refers to a radical of a non–aromatic cyclic hydrocarbon group having from 3 to 10 ring carbon atoms ("C_{3-10} carbocyclyl") and zero heteroatoms in the non–aromatic ring system. In some embodiments, a carbocyclyl group has 3 to 8 ring carbon atoms ("C_{3-8} carbocyclyl"). In some embodiments, a carbocyclyl group has 3 to 6 ring carbon atoms ("C_{3-6} carbocyclyl"). In some embodiments, a carbocyclyl group has 3 to 6 ring carbon atoms ("C_{3-6} carbocyclyl"). In some embodiments, a carbocyclyl group has 5 to 10 ring carbon atoms ("C_{5-10} carbocyclyl"). Exemplary C_{3-6} carbocyclyl groups include, without limitation, cyclopropyl (C_3), cyclopropenyl (C_3), cyclobutyl (C_4), cyclobutenyl (C_4), cyclopentyl (C_5), cyclopentenyl (C_5), cyclohexyl (C_6), cyclohexenyl (C_6), cyclohexadienyl (C_6), and the like. Exemplary C_{3-8} carbocyclyl groups include, without limitation, the aforementioned C_{3-6} carbocyclyl groups as well as cycloheptyl (C_7), cycloheptenyl (C_7), cycloheptadienyl (C_7), cycloheptatrienyl (C_7), cyclooctyl (C_8), cyclooctenyl (C_8), bicyclo[2.2.1]heptanyl (C_7), bicyclo[2.2.2]octanyl (C_8), and the like. Exemplary C_{3-10} carbocyclyl groups include, without limitation, the aforementioned C_{3-8} carbocyclyl groups as well as cyclononyl (C_9), cyclononenyl (C_9), cyclodecyl (C_{10}), cyclodecenyl (C_{10}), octahydro–1*H*–indenyl (C_9), decahydronaphthalenyl (C_{10}), spiro[4.5]decanyl (C_{10}), and the like. As the foregoing examples illustrate, in certain embodiments, the carbocyclyl group is either monocyclic ("monocyclic carbocyclyl") or contain a fused, bridged or spiro ring system such as a bicyclic system ("bicyclic carbocyclyl") and can be saturated or can be partially unsaturated. "Carbocyclyl" also includes ring systems wherein the carbocyclyl ring, as defined above, is fused with one or more aryl or heteroaryl groups wherein the point of attachment is on the carbocyclyl ring, and in such instances, the number of carbons continue to designate the number of carbons in the carbocyclic ring system. Unless otherwise specified, each instance of a carbocyclyl group is

independently unsubstituted or substituted with one or more substituents, as valency permits, and which results in a stable compound. Exemplary substituents are further described herein.

[0047] In some embodiments, "carbocyclyl" is a monocyclic, saturated carbocyclyl group having from 3 to 10 ring carbon atoms ("C_{3-10} cycloalkyl"). In some embodiments, a cycloalkyl group has 3 to 8 ring carbon atoms ("C_{3-8} cycloalkyl"). In some embodiments, a cycloalkyl group has 3 to 6 ring carbon atoms ("C_{3-6} cycloalkyl"). In some embodiments, a cycloalkyl group has 5 to 6 ring carbon atoms ("C_{5-6} cycloalkyl"). In some embodiments, a cycloalkyl group has 5 to 10 ring carbon atoms ("C_{5-10} cycloalkyl"). Examples of C_{5-6} cycloalkyl groups include cyclopentyl (C_5) and cyclohexyl (C_5). Examples of C_{3-6} cycloalkyl groups include the aforementioned C_{5-6} cycloalkyl groups as well as cyclopropyl (C_3) and cyclobutyl (C_4). Examples of C_{3-8} cycloalkyl groups include the aforementioned C_{3-6} cycloalkyl groups as well as cycloheptyl (C_7) and cyclooctyl (C_8). Unless otherwise specified, each instance of a cycloalkyl group is independently unsubstituted or substituted with one or more substituents, as valency permits, and which results in a stable compound. Exemplary substituents are further described herein.

[0048] The term "heteroaliphatic," as used herein, refers to an aliphatic moiety, as defined herein, which includes both saturated and unsaturated, nonaromatic, straight chain (*i.e.*, unbranched), branched, acyclic or cyclic (*i.e.*, heterocyclic) groups which are optionally substituted with one or more substituents, and which contain one or more oxygen, sulfur, nitrogen, phosphorus, or silicon atoms, *e.g.*, in place of carbon atoms. It is understood from the above description that the term "heteroaliphatic," whether preceded by the terms substituted or unsubstituted, and unless otherwise specified, encompasses "cyclic or acyclic" and "branched or unbranched" groups. It is also understood, similar to aliphatic, that "heteroaliphatic" is intended to encompass heteroalkyl, heteroalkenyl, heteroalkynyl, and heterocyclic (heterocycloalkyl, heterocycloalkenyl, and heterocycloalkynyl) moieties. The terms "heteroalkyl," "heteroalkenyl," and "heteroalkynyl" are defined similarly, *i.e.*, respectively refer to an alkyl, alkenyl, and alkynyl group, as defined herein, which are optionally substituted with one or more substituents, and which contain one or more oxygen, sulfur, nitrogen, phosphorus, or silicon atoms, *e.g.*, in place of carbon atoms. Unless otherwise specified, each instance of a heteroaliphatic group is independently unsubstituted or substituted with one or more substituents, as valency permits, and which results in a stable compound. Exemplary substituents are further described herein.

[0049] The term "heterocyclic," "heterocycles," or "heterocyclyl," as used herein, refers to a cyclic heteroaliphatic group. A heterocyclic group refers to a non–aromatic,

partially unsaturated or fully saturated, 3– to 10–membered ring system, which includes single rings of 3 to 8 atoms in size, and bi– and tri–cyclic ring systems which may include aromatic five– or six–membered aryl or heteroaryl groups fused to a non–aromatic ring. These heterocyclic rings include those having from one to four heteroatoms independently selected from oxygen, sulfur, and nitrogen, in which the nitrogen and sulfur heteroatoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. In certain embodiments, the term heterocylic refers to a non–aromatic 5–, 6–, or 7–membered ring or polycyclic group wherein at least one ring atom is a heteroatom selected from O, S, and N (wherein the nitrogen and sulfur heteroatoms may be optionally oxidized), and the remaining ring atoms are carbon, the radical being joined to the rest of the molecule via any of the ring atoms. Heterocycyl groups include, but are not limited to, a bi– or tri–cyclic group, comprising fused five, six, or seven–membered rings having between one and three heteroatoms independently selected from the oxygen, sulfur, and nitrogen, wherein (i) each 5–membered ring has 0 to 2 double bonds, each 6–membered ring has 0 to 2 double bonds, and each 7–membered ring has 0 to 3 double bonds, (ii) the nitrogen and sulfur heteroatoms may be optionally oxidized, (iii) the nitrogen heteroatom may optionally be quaternized, and (iv) any of the above heterocyclic rings may be fused to an aryl or heteroaryl ring. In the instance of ring fusion, it is understood that "heterocyclyl" refers to a ring system wherein the heterocyclyl ring, as defined above, is fused with one or more carbocyclyl groups wherein the point of attachment is either on the carbocyclyl or heterocyclyl ring, or ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more aryl or heteroaryl groups, wherein the point of attachment is on the heterocyclyl ring, and in such instances, the number of ring members continue to designate the number of ring members in the heterocyclyl ring system. Unless otherwise specified, each instance of a heterocyclyl group is independently unsubstituted or substituted with one or more substituents, as valency permits, and which results in a stable compound. Exemplary substituents are further described herein.

[0050] In some embodiments, a heterocyclyl group is a 5- to 10-membered non–aromatic ring system having ring carbon atoms and 1–4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5- to 10-membered heterocyclyl"). In some embodiments, a heterocyclyl group is a 5- to 8-membered non–aromatic ring system having ring carbon atoms and 1–4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5- to 8-membered heterocyclyl"). In some embodiments, a heterocyclyl group is a 5- to 6-membered non–aromatic ring system having ring carbon atoms and 1–4 ring heteroatoms, wherein each

heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5- to 6-membered heterocyclyl"). In some embodiments, the 5–6 membered heterocyclyl has 1–3 ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, the 5- to 6-membered heterocyclyl has 1–2 ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, the 5–6 membered heterocyclyl has one ring heteroatom selected from nitrogen, oxygen, and sulfur.

[0051] Exemplary 3–membered heterocyclyl groups containing one heteroatom include, without limitation, azirdinyl, oxiranyl, thiorenyl. Exemplary 4–membered heterocyclyl groups containing one heteroatom include, without limitation, azetidinyl, oxetanyl and thietanyl. Exemplary 5–membered heterocyclyl groups containing one heteroatom include, without limitation, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothiophenyl, dihydrothiophenyl, pyrrolidinyl, dihydropyrrolyl and pyrrolyl–2,5–dione. Exemplary 5–membered heterocyclyl groups containing two heteroatoms include, without limitation, dioxolanyl, oxasulfuranyl, disulfuranyl, and oxazolidin-2-one. Exemplary 5–membered heterocyclyl groups containing three heteroatoms include, without limitation, triazolinyl, oxadiazolinyl, and thiadiazolinyl. Exemplary 6–membered heterocyclyl groups containing one heteroatom include, without limitation, piperidinyl, tetrahydropyranyl, dihydropyridinyl, and thianyl. Exemplary 6–membered heterocyclyl groups containing two heteroatoms include, without limitation, piperazinyl, morpholinyl, dithianyl, dioxanyl. Exemplary 6–membered heterocyclyl groups containing two heteroatoms include, without limitation, triazinanyl. Exemplary 7–membered heterocyclyl groups containing one heteroatom include, without limitation, azepanyl, oxepanyl and thiepanyl. Exemplary 8–membered heterocyclyl groups containing one heteroatom include, without limitation, azocanyl, oxecanyl and thiocanyl. Exemplary 5-membered heterocyclyl groups fused to a C_6 aryl ring (also referred to herein as a 5,6-bicyclic heterocyclic ring) include, without limitation, indolinyl, isoindolinyl, dihydrobenzofuranyl, dihydrobenzothienyl, benzoxazolinonyl, and the like. Exemplary 6-membered heterocyclyl groups fused to an aryl ring (also referred to herein as a 6,6-bicyclic heterocyclic ring) include, without limitation, tetrahydroquinolinyl, tetrahydroisoquinolinyl, and the like.

[0052] The term "aryl" refers to a radical of a monocyclic or polycyclic (*e.g.,* bicyclic or tricyclic) 4n+2 aromatic ring system (*e.g.,* having 6, 10, or 14 π electrons shared in a cyclic array) having 6–14 ring carbon atoms and zero heteroatoms provided in the aromatic ring system ("C_{6-14} aryl"). In some embodiments, an aryl group has six ring carbon atoms ("C_6

aryl"; *e.g.,* phenyl). In some embodiments, an aryl group has ten ring carbon atoms ("C_{10} aryl"; *e.g.,* naphthyl such as 1–naphthyl and 2–naphthyl). In some embodiments, an aryl group has fourteen ring carbon atoms ("C_{14} aryl"; *e.g.,* anthracyl). "Aryl" also includes ring systems wherein the aryl ring, as defined above, is fused with one or more carbocyclyl or heterocyclyl groups wherein the radical or point of attachment is on the aryl ring, and in such instances, the number of carbon atoms continue to designate the number of carbon atoms in the aryl ring system. Unless otherwise specified, each instance of an aryl group is independently unsubstituted or substituted with one or more substituents, as valency permits, and which results in a stable compound. Exemplary substituents are further described herein.

[0053] The term "heteroaryl" refers to a radical of a 5–10 membered monocyclic or bicyclic 4n+2 aromatic ring system (*e.g.,* having 6 or 10 π electrons shared in a cyclic array) having ring carbon atoms and 1–4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen and sulfur ("5–10 membered heteroaryl"). In heteroaryl groups that contain one or more nitrogen atoms, the point of attachment can be a carbon or nitrogen atom, as valency permits. Heteroaryl bicyclic ring systems can include one or more heteroatoms in one or both rings. "Heteroaryl" includes ring systems wherein the heteroaryl ring, as defined above, is fused with one or more carbocyclyl or heterocyclyl groups wherein the point of attachment is on the heteroaryl ring, and in such instances, the number of ring members continue to designate the number of ring members in the heteroaryl ring system. "Heteroaryl" also includes ring systems wherein the heteroaryl ring, as defined above, is fused with one or more aryl groups wherein the point of attachment is either on the aryl or heteroaryl ring, and in such instances, the number of ring members designates the number of ring members in the fused (aryl/heteroaryl) ring system. Bicyclic heteroaryl groups wherein one ring does not contain a heteroatom (*e.g.,* indolyl, quinolinyl, carbazolyl, and the like) the point of attachment can be on either ring, *i.e.,* either the ring bearing a heteroatom (*e.g.,* 2–indolyl) or the ring that does not contain a heteroatom (*e.g.,* 5–indolyl). Unless otherwise specified, each instance of a heteroaryl group is independently unsubstituted or substituted with one or more substituents, as valency permits, and which results in a stable compound. Exemplary substituents are further described herein.

[0054] In some embodiments, a heteroaryl group is a 5–10 membered aromatic ring system having ring carbon atoms and 1–4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur

("5–10 membered heteroaryl"). In some embodiments, a heteroaryl group is a 5–8 membered aromatic ring system having ring carbon atoms and 1–4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5–8 membered heteroaryl"). In some embodiments, a heteroaryl group is a 5–6 membered aromatic ring system having ring carbon atoms and 1–4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5–6 membered heteroaryl"). In some embodiments, the 5–6 membered heteroaryl has 1–3 ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, the 5–6 membered heteroaryl has 1–2 ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, the 5–6 membered heteroaryl has 1 ring heteroatom selected from nitrogen, oxygen, and sulfur. Unless otherwise specified, each instance of a heteroaryl group is independently optionally substituted, *i.e.*, unsubstituted ("unsubstituted heteroaryl") or substituted ("substituted heteroaryl") with one or more substituents. In certain embodiments, the heteroaryl group is unsubstituted 5–14 membered heteroaryl. In certain embodiments, the heteroaryl group is substituted 5–14 membered heteroaryl.

[0055] Exemplary 5–membered heteroaryl groups containing one heteroatom include, without limitation, pyrrolyl, furanyl and thiophenyl. Exemplary 5–membered heteroaryl groups containing two heteroatoms include, without limitation, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, and isothiazolyl. Exemplary 5–membered heteroaryl groups containing three heteroatoms include, without limitation, triazolyl, oxadiazolyl, and thiadiazolyl. Exemplary 5–membered heteroaryl groups containing four heteroatoms include, without limitation, tetrazolyl. Exemplary 6–membered heteroaryl groups containing one heteroatom include, without limitation, pyridinyl. Exemplary 6–membered heteroaryl groups containing two heteroatoms include, without limitation, pyridazinyl, pyrimidinyl, and pyrazinyl. Exemplary 6–membered heteroaryl groups containing three or four heteroatoms include, without limitation, triazinyl and tetrazinyl, respectively. Exemplary 7–membered heteroaryl groups containing one heteroatom include, without limitation, azepinyl, oxepinyl, and thiepinyl. Exemplary 5,6–bicyclic heteroaryl groups include, without limitation, indolyl, isoindolyl, indazolyl, benzotriazolyl, benzothiophenyl, isobenzothiophenyl, benzofuranyl, benzoisofuranyl, benzimidazolyl, benzoxazolyl, benzisoxazolyl, benzoxadiazolyl, benzthiazolyl, benzisothiazolyl, benzthiadiazolyl, indolizinyl, and purinyl. Exemplary 6,6–bicyclic heteroaryl groups include, without limitation, naphthyridinyl, pteridinyl, quinolinyl, isoquinolinyl, cinnolinyl, quinoxalinyl, phthalazinyl, and quinazolinyl.

[0056] The term "acyl," as used herein, refers to a group having the general formula $-C(=O)R^{X5}$, $-C(=O)OR^{X5}$, $-C(=O)SR^{X5}$, $-C(=O)N(R^{X6})_2$, $-C(=NR^{X6})R^{X1}$, $-C(=NR^{X6})OR^{X5}$, $-C(=NR^{X6})SR^{X5}$, $-C(=NR^{X6})N(R^{X6})_2$, $-C(=S)R^{X5}$, $-C(=S)OR^{X5}$, $-C(=S)SR^{X5}$, and $-C(=S)N(R^{X6})_2$, wherein each occurrence of R^{X5} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and each occurrence of R^{X6} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or a nitrogen protecting group, or two R^{X6} groups are joined to form an substituted or unsubstituted heterocyclic ring.

[0057] The term "oxo," as used herein, refers to a group of the formula (=O). The term "thiooxo," as used herein, refers to a group of the formula (=S).

[0058] Aliphatic (alkyl, alkenyl, alkynyl, carbocyclyl), heteroaliphatic (heteroalkyl, heteroalkenyl, heteroalkynyl, heterocyclyl), aryl, and heteroaryl groups, as defined herein, are optionally substituted. "Optionally substituted" refers to a group which may be substituted or unsubstituted. In general, the term "substituted" means that at least one hydrogen present on a group (*e.g.,* a carbon or nitrogen atom) is replaced with a permissible substituent, *e.g.,* a substituent which upon substitution results in a stable moiety or compound, *e.g.,* a compound which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, or other reaction, and preferably possess stability sufficient to allow manufacture, and which maintains its integrity for a sufficient period of time to be useful for the purposes detailed herein. Unless otherwise indicated, a "substituted" group has a substituent at one or more substitutable positions of the group, and when more than one position in any given structure is substituted, the substituent is either the same or different at each position. The term "substituted" is contemplated to include substitution with all permissible substituents of organic compounds, any of the substituents described herein that results in the formation of a stable compound. The present invention contemplates any and all such combinations in order to arrive at a stable compound. For purposes of this invention, heteroatoms may have hydrogen substituents and/or any substituent as described herein which satisfy the valencies of the heteroatom and results in the formation of a stable moiety.

[0059] Exemplary substituents include, but are not limited to, any of the substituents described herein, that result in the formation of a stable moiety (*e.g.*, aliphatic, alkyl, alkenyl,

alkynyl, heteroaliphatic, heterocyclic, aryl, heteroaryl, acyl, oxo, imino, thiooxo, cyano, isocyano, amino, azido, nitro, hydroxyl, thiol, halo, and combinations thereof, *e.g.*, aliphaticamino, heteroaliphaticamino, alkylamino, heteroalkylamino, arylamino, heteroarylamino, alkylaryl, arylalkyl, aliphaticoxy, heteroaliphaticoxy, alkyloxy, heteroalkyloxy, aryloxy, heteroaryloxy, aliphaticthioxy, heteroaliphaticthioxy, alkylthioxy, heteroalkylthioxy, arylthioxy, heteroarylthioxy, acyloxy, and the like, each of which may or may not be further substituted). Other exemplary subsitutents are further described herein.

[0060] Exemplary carbon atom substituents include, but are not limited to, halogen, –CN, $-NO_2$, $-N_3$, $-SO_2H$, $-SO_3H$, –OH, $-OR^{aa}$, $-ON(R^{bb})_2$, $-N(R^{bb})_2$, $-N(R^{bb})_3^+X^-$, $-N(OR^{cc})R^{bb}$, –SH, $-SR^{aa}$, $-SSR^{cc}$, $-C(=O)R^{aa}$, $-CO_2H$, –CHO, $-C(OR^{cc})_2$, $-CO_2R^{aa}$, $-OC(=O)R^{aa}$, $-OCO_2R^{aa}$, $-C(=O)N(R^{bb})_2$, $-OC(=O)N(R^{bb})_2$, $-NR^{bb}C(=O)R^{aa}$, $-NR^{bb}CO_2R^{aa}$, $-NR^{bb}C(=O)N(R^{bb})_2$, $-C(=NR^{bb})R^{aa}$, $-C(=NR^{bb})OR^{aa}$, $-OC(=NR^{bb})R^{aa}$, $-OC(=NR^{bb})OR^{aa}$, $-C(=NR^{bb})N(R^{bb})_2$, $-OC(=NR^{bb})N(R^{bb})_2$, $-NR^{bb}C(=NR^{bb})N(R^{bb})_2$, $-C(=O)NR^{bb}SO_2R^{aa}$, $-NR^{bb}SO_2R^{aa}$, $-SO_2N(R^{bb})_2$, $-SO_2R^{aa}$, $-SO_2OR^{aa}$, $-OSO_2R^{aa}$, $-S(=O)R^{aa}$, $-OS(=O)R^{aa}$, $-Si(R^{aa})_3$, $-OSi(R^{aa})_3$ $-C(=S)N(R^{bb})_2$, $-C(=O)SR^{aa}$, $-C(=S)SR^{aa}$, $-SC(=S)SR^{aa}$, $-SC(=O)SR^{aa}$, $-OC(=O)SR^{aa}$, $-SC(=O)OR^{aa}$, $-SC(=O)R^{aa}$, $-P(=O)_2R^{aa}$, $-OP(=O)_2R^{aa}$, $-P(=O)(R^{aa})_2$, $-OP(=O)(R^{aa})_2$, $-OP(=O)(OR^{cc})_2$, $-P(=O)_2N(R^{bb})_2$, $-OP(=O)_2N(R^{bb})_2$, $-P(=O)(NR^{bb})_2$, $-OP(=O)(NR^{bb})_2$, $-NR^{bb}P(=O)(OR^{cc})_2$, $-NR^{bb}P(=O)(NR^{bb})_2$, $-P(R^{cc})_2$, $-P(R^{cc})_3$, $-OP(R^{cc})_2$, $-OP(R^{cc})_3$, $-B(R^{aa})_2$, $-B(OR^{cc})_2$, $-BR^{aa}(OR^{cc})$, C_{1-10} alkyl, C_{1-10} perhaloalkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, C_{3-10} carbocyclyl, 3–14 membered heterocyclyl, C_{6-14} aryl, and 5–14 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups;

or two geminal hydrogens on a carbon atom are replaced with the group =O, =S, $=NN(R^{bb})_2$, $=NNR^{bb}C(=O)R^{aa}$, $=NNR^{bb}C(=O)OR^{aa}$, $=NNR^{bb}S(=O)_2R^{aa}$, $=NR^{bb}$, or $=NOR^{cc}$;

each instance of R^{aa} is, independently, selected from C_{1-10} alkyl, C_{1-10} perhaloalkyl, C_{2-10} alkenyl, C_{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, 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, $-C(=O)R^{aa}$, $-C(=O)N(R^{cc})_2$, $-CO_2R^{aa}$, $-SO_2R^{aa}$, $-C(=NR^{cc})OR^{aa}$, $-C(=NR^{cc})N(R^{cc})_2$, $-SO_2N(R^{cc})_2$, $-SO_2R^{cc}$, $-SO_2OR^{cc}$, $-SOR^{aa}$, $-C(=S)N(R^{cc})_2$, $-C(=O)SR^{cc}$, $-C(=S)SR^{cc}$, $-P(=O)_2R^{aa}$, $-P(=O)(R^{aa})_2$, $-P(=O)_2N(R^{cc})_2$, $-P(=O)(NR^{cc})_2$, C_{1-10} alkyl, C_{1-10}

perhaloalkyl, C_{2-10} alkenyl, C_{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, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups;

each instance of R^{cc} is, independently, selected from hydrogen, C_{1-10} alkyl, C_{1-10} perhaloalkyl, C_{2-10} alkenyl, C_{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, 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, $-CN$, $-NO_2$, $-N_3$, $-SO_2H$, $-SO_3H$, $-OH$, $-OR^{ee}$, $-ON(R^{ff})_2$, $-N(R^{ff})_2$, $-N(R^{ff})_3^+X^-$, $-N(OR^{ee})R^{ff}$, $-SH$, $-SR^{ee}$, $-SSR^{ee}$, $-C(=O)R^{ee}$, $-CO_2H$, $-CO_2R^{ee}$, $-OC(=O)R^{ee}$, $-OCO_2R^{ee}$, $-C(=O)N(R^{ff})_2$, $-OC(=O)N(R^{ff})_2$, $-NR^{ff}C(=O)R^{ee}$, $-NR^{ff}CO_2R^{ee}$, $-NR^{ff}C(=O)N(R^{ff})_2$, $-C(=NR^{ff})OR^{ee}$, $-OC(=NR^{ff})R^{ee}$, $-OC(=NR^{ff})OR^{ee}$, $-C(=NR^{ff})N(R^{ff})_2$, $-OC(=NR^{ff})N(R^{ff})_2$, $-NR^{ff}C(=NR^{ff})N(R^{ff})_2$, $-NR^{ff}SO_2R^{ee}$, $-SO_2N(R^{ff})_2$, $-SO_2R^{ee}$, $-SO_2OR^{ee}$, $-OSO_2R^{ee}$, $-S(=O)R^{ee}$, $-Si(R^{ee})_3$, $-OSi(R^{ee})_3$, $-C(=S)N(R^{ff})_2$, $-C(=O)SR^{ee}$, $-C(=S)SR^{ee}$, $-SC(=S)SR^{ee}$, $-P(=O)_2R^{ee}$, $-P(=O)(R^{ee})_2$, $-OP(=O)(R^{ee})_2$, $-OP(=O)(OR^{ee})_2$, C_{1-6} alkyl, C_{1-6} perhaloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} carbocyclyl, 3–10 membered heterocyclyl, C_{6-10} aryl, 5–10 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, 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;

each instance of R^{ee} is, independently, selected from C_{1-6} alkyl, C_{1-6} perhaloalkyl, C_{2-6} alkenyl, C_{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, 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} perhaloalkyl, C_{2-6} alkenyl, C_{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 3–14 membered heterocyclyl or 5–14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, 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, $–P(=O)_2(C_{1–6}$ alkyl), $–P(=O)(C_{1–6}$ alkyl$)_2$, $–OP(=O)(C_{1–6}$ alkyl$)_2$, –$OP(=O)(OC_{1–6}$ alkyl$)_2$, $C_{1–6}$ alkyl, $C_{1–6}$ perhaloalkyl, $C_{2–6}$ alkenyl, $C_{2–6}$ 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.

[0061] A "counterion" or "anionic counterion" is a negatively charged group associated with a cationic quaternary amino group in order to maintain electronic neutrality. Exemplary counterions include halide ions (*e.g.,* $F^–$, $Cl^–$, $Br^–$, $I^–$), $NO_3^–$, $ClO_4^–$, $OH^–$, $H_2PO_4^–$, $HSO_4^–$, 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), and carboxylate ions (*e.g.,* acetate, ethanoate, propanoate, benzoate, glycerate, lactate, tartrate, glycolate, and the like).

[0062] "Halo" or "halogen" refers to fluorine (fluoro, –F), chlorine (chloro, –Cl), bromine (bromo, –Br), or iodine (iodo, –I).

[0063] As used herein, the term "unsubstituted hydroxyl" or "unsubstituted hydroxy" refers to the group –OH. The term "substituted hydroxyl" or "substituted hydroxyl," by extension, refers to a hydroxyl group wherein the oxygen atom directly attached to the parent molecule is substituted with a group other than hydrogen, and includes groups selected from $–OR^{aa}$, $–ON(R^{bb})_2$, $–OC(=O)SR^{aa}$, $–OC(=O)R^{aa}$, $–OCO_2R^{aa}$, $–OC(=O)N(R^{bb})_2$, –$OC(=NR^{bb})R^{aa}$, $–OC(=NR^{bb})OR^{aa}$, $–OC(=NR^{bb})N(R^{bb})_2$, $–OS(=O)R^{aa}$, $–OSO_2R^{aa}$, $–OSi(R^{aa})_3$, $–OP(R^{cc})_2$, $–OP(R^{cc})_3$, $–OP(=O)_2R^{aa}$, $–OP(=O)(R^{aa})_2$, $–OP(=O)(OR^{cc})_2$, $–OP(=O)_2N(R^{bb})_2$,

and –OP(=O)(NR^{bb})$_2$, wherein R^{aa}, R^{bb}, and R^{cc} are as defined herein. In the case wherein "substituted hydroxyl" is a ligand L_1 or L_2, "substituted hydroxyl" also refers to the group (R^{aa})$_2$O, wherein R^{aa} is as defined herein.

[0064] As used herein, the term "unsubstituted thiol" or "unsubstituted thio" refers to the group –SH. The term "substituted thiol" or "substituted thio," by extension, refers to a thiol group wherein the sulfur atom directly attached to the parent molecule is substituted with a group other than hydrogen, and includes groups selected from –SR^{aa}, –S=SR^{cc}, –SC(=S)SR^{aa}, –SC(=O)SR^{aa}, –SC(=O)OR^{aa}, and –SC(=O)R^{aa}, wherein R^{aa} and R^{cc} are as defined herein.

[0065] As used herein, the term "unsubstituted amino" or "amino" refers to the group –NH_2. The term "substituted amino," by extension, refers to a monosubstituted, disubstituted, or trisubstituted amino group.

[0066] As used herein, the term "monosubstituted amino" refers to an amino group wherein the nitrogen atom directly attached to the parent molecule is substituted with one hydrogen and one group other than hydrogen. Exemplary monosubstituted amino groups include, but are not limited to, –NH(R^{bb}), –NHC(=O)R^{aa}, –NHCO_2R^{aa}, –NHC(=O)N(R^{bb})$_2$, –NHC(=NR^{bb})N(R^{bb})$_2$, –NHSO_2R^{aa}, –NHP(=O)(OR^{cc})$_2$, and –NHP(=O)(NR^{bb})$_2$, wherein R^{aa}, R^{bb} and R^{cc} are as defined herein, and wherein R^{bb} of the group –NH(R^{bb}) is not hydrogen.

[0067] As used herein, the term "disubstituted amino" refers to an amino group wherein the nitrogen atom directly attached to the parent molecule is substituted with two groups other than hydrogen. Exemplary disubstituted amino groups include, but are not limited to, –N(R^{bb})$_2$, –NR^{bb} C(=O)R^{aa}, –$NR^{bb}CO_2R^{aa}$, –NR^{bb}C(=O)N(R^{bb})$_2$, –NR^{bb}C(=NR^{bb})N(R^{bb})$_2$, –$NR^{bb}SO_2R^{aa}$, –NR^{bb}P(=O)(OR^{cc})$_2$, and –NR^{bb}P(=O)(NR^{bb})$_2$, wherein R^{aa}, R^{bb}, and R^{cc} are as defined herein, with the proviso that the nitrogen atom directly attached to the parent molecule is not substituted with hydrogen.

[0068] As used herein, the term "trisubstituted amino" refers to an amino group wherein the nitrogen atom directly attached to the parent molecule is substituted with three groups. Exemplary trisubstituted amino groups include, but are not limited to,–N(R^{bb})$_3$ and –N(R^{bb})$_3^+X^-$, wherein R^{bb} and X^- are as defined herein.

[0069] Nitrogen atoms can be substituted or unsubstituted as valency permits, and include primary, secondary, tertiary, and quarternary nitrogen atoms. Exemplary nitrogen atom substitutents include, but are not limited to, 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^{bb})R^{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}, –

$C(=S)SR^{cc}$, $-P(=O)_2R^{aa}$, $-P(=O)(R^{aa})_2$, $-P(=O)_2N(R^{cc})_2$, $-P(=O)(NR^{cc})_2$, C_{1-10} alkyl, C_{1-10} perhaloalkyl, C_{2-10} alkenyl, C_{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 attached to a nitrogen atom are joined to form a 3–14 membered heterocyclyl or 5–14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups, and wherein R^{aa}, R^{bb}, R^{cc} and R^{dd} are as defined above.

[0070] The term "protecting group" as used herein, refers to a chemical modification of a functional group of a compound that prevents the functional group to take part in an undesired chemical reaction. Protecting groups play an important role in multi-step organic compound synthesis, and suitable protecting groups for various functional groups and chemical environments are well known in the art. Examples of protecting groups are nitrogen protecting groups, oxygen protecting groups, sulfur protecting groups, and carboxylic acid protecting groups are described in more detail herein.

[0071] In certain embodiments, the substituent present on a nitrogen atom is a nitrogen protecting group (also referred to as an amino protecting group). Nitrogen protecting groups include, but are not limited to, –OH, $-OR^{aa}$, $-N(R^{cc})_2$, $-C(=O)R^{aa}$, $-C(=O)N(R^{cc})_2$, $-CO_2R^{aa}$, $-SO_2R^{aa}$, $-C(=NR^{cc})R^{aa}$, $-C(=NR^{cc})OR^{aa}$, $-C(=NR^{cc})N(R^{cc})_2$, $-SO_2N(R^{cc})_2$, $-SO_2R^{cc}$, $-SO_2OR^{cc}$, $-SOR^{aa}$, $-C(=S)N(R^{cc})_2$, $-C(=O)SR^{cc}$, $-C(=S)SR^{cc}$, C_{1-10} alkyl (*e.g.,* aralkyl, heteroaralkyl), C_{2-10} alkenyl, C_{2-10} alkynyl, C_{3-10} carbocyclyl, 3–14 membered heterocyclyl, C_{6-14} aryl, and 5–14 membered heteroaryl groups, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aralkyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups, and wherein R^{aa}, R^{bb}, R^{cc}, and R^{dd} are as defined herein. Nitrogen 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.

[0072] Amide nitrogen protecting groups *(e.g.,* $-C(=O)R^{aa}$) include, but are not limited to, formamide, acetamide, chloroacetamide, trichloroacetamide, trifluoroacetamide, phenylacetamide, 3–phenylpropanamide, picolinamide, 3–pyridylcarboxamide, *N*–benzoylphenylalanyl derivative, benzamide, *p*–phenylbenzamide, *o*–nitophenylacetamide, *o*–nitrophenoxyacetamide, acetoacetamide, (*N'*–dithiobenzyloxyacylamino)acetamide, 3–(*p*–hydroxyphenyl)propanamide, 3–(*o*–nitrophenyl)propanamide, 2–methyl–2–(*o*–nitrophenoxy)propanamide, 2–methyl–2–(*o*–phenylazophenoxy)propanamide, 4–

chlorobutanamide, 3–methyl–3–nitrobutanamide, *o*–nitrocinnamide, *N*–acetylmethionine, *o*–nitrobenzamide, and *o*–(benzoyloxymethyl)benzamide.

[0073] Carbamate nitrogen protecting groups (*e.g.*, –C(=O)OR^{aa}) include, but are not limited to, methyl carbamate, ethyl carbamante, 9–fluorenylmethyl carbamate (Fmoc), 9–(2–sulfo)fluorenylmethyl carbamate, 9–(2,7–dibromo)fluoroenylmethyl carbamate, 2,7–di–*t*–butyl–[9–(10,10–dioxo–10,10,10,10–tetrahydrothioxanthyl)]methyl carbamate (DBD–Tmoc), 4–methoxyphenacyl carbamate (Phenoc), 2,2,2–trichloroethyl carbamate (Troc), 2–trimethylsilylethyl carbamate (Teoc), 2–phenylethyl carbamate (hZ), 1–(1–adamantyl)–1–methylethyl carbamate (Adpoc), 1,1–dimethyl–2–haloethyl carbamate, 1,1–dimethyl–2,2–dibromoethyl carbamate (DB–*t*–BOC), 1,1–dimethyl–2,2,2–trichloroethyl carbamate (TCBOC), 1–methyl–1–(4–biphenylyl)ethyl carbamate (Bpoc), 1–(3,5–di–*t*–butylphenyl)–1–methylethyl carbamate (*t*–Bumeoc), 2–(2'– and 4'–pyridyl)ethyl carbamate (Pyoc), 2–(*N,N*–dicyclohexylcarboxamido)ethyl carbamate, *t*–butyl carbamate (BOC), 1–adamantyl carbamate (Adoc), vinyl carbamate (Voc), allyl carbamate (Alloc), 1–isopropylallyl carbamate (Ipaoc), cinnamyl carbamate (Coc), 4–nitrocinnamyl carbamate (Noc), 8–quinolyl carbamate, *N*–hydroxypiperidinyl carbamate, alkyldithio carbamate, benzyl carbamate (Cbz), *p*–methoxybenzyl carbamate (Moz), *p*–nitobenzyl carbamate, *p*–bromobenzyl carbamate, *p*–chlorobenzyl carbamate, 2,4–dichlorobenzyl carbamate, 4–methylsulfinylbenzyl carbamate (Msz), 9–anthrylmethyl carbamate, diphenylmethyl carbamate, 2–methylthioethyl carbamate, 2–methylsulfonylethyl carbamate, 2–(*p*–toluenesulfonyl)ethyl carbamate, [2–(1,3–dithianyl)]methyl carbamate (Dmoc), 4–methylthiophenyl carbamate (Mtpc), 2,4–dimethylthiophenyl carbamate (Bmpc), 2–phosphonioethyl carbamate (Peoc), 2–triphenylphosphonioisopropyl carbamate (Ppoc), 1,1–dimethyl–2–cyanoethyl carbamate, *m*–chloro–*p*–acyloxybenzyl carbamate, *p*–(dihydroxyboryl)benzyl carbamate, 5–benzisoxazolylmethyl carbamate, 2–(trifluoromethyl)–6–chromonylmethyl carbamate (Tcroc), *m*–nitrophenyl carbamate, 3,5–dimethoxybenzyl carbamate, *o*–nitrobenzyl carbamate, 3,4–dimethoxy–6–nitrobenzyl carbamate, phenyl(*o*–nitrophenyl)methyl carbamate, *t*–amyl carbamate, *S*–benzyl thiocarbamate, *p*–cyanobenzyl carbamate, cyclobutyl carbamate, cyclohexyl carbamate, cyclopentyl carbamate, cyclopropylmethyl carbamate, *p*–decyloxybenzyl carbamate, 2,2–dimethoxyacylvinyl carbamate, *o*–(*N,N*–dimethylcarboxamido)benzyl carbamate, 1,1–dimethyl–3–(*N,N*–dimethylcarboxamido)propyl carbamate, 1,1–dimethylpropynyl carbamate, di(2–pyridyl)methyl carbamate, 2–furanylmethyl carbamate, 2–iodoethyl carbamate, isoborynl carbamate, isobutyl carbamate, isonicotinyl carbamate, *p*–(*p'*–methoxyphenylazo)benzyl carbamate, 1–methylcyclobutyl

carbamate, 1–methylcyclohexyl carbamate, 1–methyl–1–cyclopropylmethyl carbamate, 1–methyl–1–(3,5–dimethoxyphenyl)ethyl carbamate, 1–methyl–1–(*p*–phenylazophenyl)ethyl carbamate, 1–methyl–1–phenylethyl carbamate, 1–methyl–1–(4–pyridyl)ethyl carbamate, phenyl carbamate, *p*–(phenylazo)benzyl carbamate, 2,4,6–tri–*t*–butylphenyl carbamate, 4–(trimethylammonium)benzyl carbamate, and 2,4,6–trimethylbenzyl carbamate.

[0074] Sulfonamide nitrogen protecting groups (*e.g.*, $-S(=O)_2R^{aa}$) include, but are not limited to, *p*–toluenesulfonamide (Ts), benzenesulfonamide, 2,3,6,–trimethyl–4–methoxybenzenesulfonamide (Mtr), 2,4,6–trimethoxybenzenesulfonamide (Mtb), 2,6–dimethyl–4–methoxybenzenesulfonamide (Pme), 2,3,5,6–tetramethyl–4–methoxybenzenesulfonamide (Mte), 4–methoxybenzenesulfonamide (Mbs), 2,4,6–trimethylbenzenesulfonamide (Mts), 2,6–dimethoxy–4–methylbenzenesulfonamide (iMds), 2,2,5,7,8–pentamethylchroman–6–sulfonamide (Pmc), methanesulfonamide (Ms), β–trimethylsilylethanesulfonamide (SES), 9–anthracenesulfonamide, 4–(4',8'–dimethoxynaphthylmethyl)benzenesulfonamide (DNMBS), benzylsulfonamide, trifluoromethylsulfonamide, and phenacylsulfonamide.

[0075] Other nitrogen protecting groups include, but are not limited to, phenothiazinyl–(10)–acyl derivative, *N'*–*p*–toluenesulfonylaminoacyl derivative, *N'*–phenylaminothioacyl derivative, *N*–benzoylphenylalanyl derivative, *N*–acetylmethionine derivative, 4,5–diphenyl–3–oxazolin–2–one, *N*–phthalimide, *N*–dithiasuccinimide (Dts), *N*–2,3–diphenylmaleimide, *N*–2,5–dimethylpyrrole, *N*–1,1,4,4–tetramethyldisilylazacyclopentane adduct (STABASE), 5–substituted 1,3–dimethyl–1,3,5–triazacyclohexan–2–one, 5–substituted 1,3–dibenzyl–1,3,5–triazacyclohexan–2–one, 1–substituted 3,5–dinitro–4–pyridone, *N*–methylamine, *N*–allylamine, *N*–[2–(trimethylsilyl)ethoxy]methylamine (SEM), *N*–3–acetoxypropylamine, *N*–(1–isopropyl–4–nitro–2–oxo–3–pyroolin–3–yl)amine, quaternary ammonium salts, *N*–benzylamine, *N*–di(4–methoxyphenyl)methylamine, *N*–5–dibenzosuberylamine, *N*–triphenylmethylamine (Tr), *N*–[(4–methoxyphenyl)diphenylmethyl]amine (MMTr), *N*–9–phenylfluorenylamine (PhF), *N*–2,7–dichloro–9–fluorenylmethyleneamine, *N*–ferrocenylmethylamino (Fcm), *N*–2–picolylamino *N'*–oxide, *N*–1,1–dimethylthiomethyleneamine, *N*–benzylideneamine, *N*–*p*–methoxybenzylideneamine, *N*–diphenylmethyleneamine, *N*–[(2–pyridyl)mesityl]methyleneamine, *N*–(*N',N'*–dimethylaminomethylene)amine, *N,N'*–isopropylidenediamine, *N*–*p*–nitrobenzylideneamine, *N*–salicylideneamine, *N*–5–chlorosalicylideneamine, *N*–(5–chloro–2–hydroxyphenyl)phenylmethyleneamine, *N*–cyclohexylideneamine, *N*–(5,5–dimethyl–3–oxo–1–cyclohexenyl)amine, *N*–borane

derivative, *N*–diphenylborinic acid derivative, *N*–[phenyl(pentaacylchromium– or tungsten)acyl]amine, *N*–copper chelate, *N*–zinc chelate, *N*–nitroamine, *N*–nitrosoamine, amine *N*–oxide, diphenylphosphinamide (Dpp), dimethylthiophosphinamide (Mpt), diphenylthiophosphinamide (Ppt), dialkyl phosphoramidates, dibenzyl phosphoramidate, diphenyl phosphoramidate, benzenesulfenamide, *o*–nitrobenzenesulfenamide (Nps), 2,4–dinitrobenzenesulfenamide, pentachlorobenzenesulfenamide, 2–nitro–4–methoxybenzenesulfenamide, triphenylmethylsulfenamide, and 3–nitropyridinesulfenamide (Npys).

[0076] In certain embodiments, the substituent present on an oxygen atom is an oxygen protecting group (also referred to as a hydroxyl protecting group). Oxygen protecting groups include, but are not limited to, $–R^{aa}$, $–N(R^{bb})_2$, $–C(=O)SR^{aa}$, $–C(=O)R^{aa}$, $–CO_2R^{aa}$, $–C(=O)N(R^{bb})_2$, $–C(=NR^{bb})R^{aa}$, $–C(=NR^{bb})OR^{aa}$, $–C(=NR^{bb})N(R^{bb})_2$, $–S(=O)R^{aa}$, $–SO_2R^{aa}$, $–Si(R^{aa})_3$, $–P(R^{cc})_2$, $–P(R^{cc})_3$, $–P(=O)_2R^{aa}$, $–P(=O)(R^{aa})_2$, $–P(=O)(OR^{cc})_2$, $–P(=O)_2N(R^{bb})_2$, and $–P(=O)(NR^{bb})_2$, wherein R^{aa}, R^{bb}, and R^{cc} are as defined herein. 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.

[0077] 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–benzodisulfuran–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), alkyl methyl carbonate, 9–fluorenylmethyl carbonate (Fmoc), alkyl ethyl carbonate, alkyl 2,2,2–trichloroethyl carbonate (Troc), 2–(trimethylsilyl)ethyl carbonate (TMSEC), 2–(phenylsulfonyl) ethyl carbonate (Psec), 2–(triphenylphosphonio) ethyl carbonate (Peoc), alkyl isobutyl carbonate, alkyl vinyl carbonate alkyl allyl carbonate, alkyl *p*–nitrophenyl carbonate, alkyl benzyl carbonate, alkyl *p*–methoxybenzyl carbonate, alkyl 3,4–dimethoxybenzyl carbonate, alkyl *o*–nitrobenzyl carbonate, alkyl *p*–nitrobenzyl carbonate, alkyl *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).

[0078] In certain embodiments, the substituent present on a sulfur atom is a sulfur protecting group (also referred to as a thiol protecting group). Sulfur protecting groups include, but are not limited to, $-R^{aa}$, $-N(R^{bb})_2$, $-C(=O)SR^{aa}$, $-C(=O)R^{aa}$, $-CO_2R^{aa}$, $-C(=O)N(R^{bb})_2$, $-C(=NR^{bb})R^{aa}$, $-C(=NR^{bb})OR^{aa}$, $-C(=NR^{bb})N(R^{bb})_2$, $-S(=O)R^{aa}$, $-SO_2R^{aa}$, $-Si(R^{aa})_3$, $-P(R^{cc})_2$, $-P(R^{cc})_3$, $-P(=O)_2R^{aa}$, $-P(=O)(R^{aa})_2$, $-P(=O)(OR^{cc})_2$, $-P(=O)_2N(R^{bb})_2$, and $-P(=O)(NR^{bb})_2$, wherein R^{aa}, R^{bb}, and R^{cc} are as defined herein. Sulfur 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.

[0079] A "carboxylic acid protecting group" or "protected carboxylic acid," as used herein, are well known in the art and include those described in detail in Greene (1999). Examples of protected carboxylic acids further include, but are not limited to, silyl–, alkyl–, alkenyl–, aryl–, and arylalkyl–protected carboxylic acids. Examples of suitable silyl groups include trimethylsilyl, triethylsilyl, t–butyldimethylsilyl, t–butyldiphenylsilyl, triisopropylsilyl, and the like. Examples of suitable alkyl groups include methyl, benzyl, p–methoxybenzyl, 3,4–dimethoxybenzyl, trityl, t–butyl, tetrahydropyran–2–yl. Examples of suitable alkenyl groups include allyl. Examples of suitable aryl groups include optionally substituted phenyl, biphenyl, or naphthyl. Examples of suitable arylalkyl groups include optionally substituted benzyl (*e.g.,* p–methoxybenzyl (MPM), 3,4–dimethoxybenzyl, O–nitrobenzyl, p–nitrobenzyl, p–halobenzyl, 2,6–dichlorobenzyl, p–cyanobenzyl), and 2– and 4–picolyl.

[0080] These and other exemplary substituents and protecting groups are described in more detail in the Detailed Description, Examples, and claims. The invention is not intended to be limited in any manner by the above exemplary listing of substituents and protecting groups.

Other definitions

[0081] As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, immunological response, and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, Berge *et al.* describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 1977, *66*, 1–19, incorporated herein by reference. Pharmaceutically acceptable salts of the compounds

of this invention include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2–hydroxy–ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2–naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3–phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p–toluenesulfonate, undecanoate, valerate salts, and the like. Salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and $N^+(C_{1-4}alkyl)_4$ salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate, and aryl sulfonate.

[0082] A "subject" to which administration is contemplated includes, but is not limited to, humans (*i.e.,* a male or female of any age group, *e.g.,* a pediatric subject (*e.g,* infant, child, adolescent) or adult subject (*e.g.,* young adult, middle–aged adult or senior adult)) and/or other non–human animals, for example mammals (*e.g.*, primates (*e.g.,* cynomolgus monkeys, rhesus monkeys); commercially relevant mammals such as cattle, pigs, horses, sheep, goats, cats, and/or dogs), birds (*e.g.*, commercially relevant birds such as chickens, ducks, geese, and/or turkeys), reptiles, amphibians, and fish. In certain embodiments, the non–human animal is a mammal. The non–human animal may be a male or female at any stage of development. A non–human animal may be a transgenic animal.

[0083] The terms "administer," "administering," or "administration," as used herein refers to implanting, absorbing, ingesting, injecting, or inhaling a substance, for example, a compound as described herein.

[0084] As used herein the term "inhibit" or "inhibition" in the context of enzymes, for example, in the context of kinases, refers to a reduction in the level of kinase activity. In

some embodiments, the term refers to a reduction of the level of kinase activity to a level that is statistically significantly lower than an initial level, which may, for example, be a baseline level of kinase activity.

[0085] As used herein, the term "activate" or "activation" in the context of enzymes, for example, in the context of kinases, refers to an increase in the level of kinase activity. In some embodiments, the term refers to an increase of the level of kinase activity to a level that is statistically significantly higher than an initial level, which may, for example, be a baseline level of kinase activity.

[0086] As used herein, the terms "treatment," "treat," and "treating," refer to a clinical intervention aimed to reverse, alleviate, delay the onset of, or inhibit the progress of a disease or disorder, or one or more symptoms thereof, as described herein. As used herein, the terms "treatment," "treat," and "treating" refer to a clinical intervention aimed to reverse, alleviate, delay the onset of, or inhibit the progress of a disease or disorder, or one or more symptoms thereof, as described herein. In some embodiments, treatment may be administered after one or more symptoms have developed and/or after a disease has been diagnosed. In other embodiments, treatment may be administered in the absence of symptoms. For example, treatment may be administered to a susceptible individual prior to the onset of symptoms (*e.g.*, in light of a history of symptoms and/or in light of genetic or other susceptibility factors). Treatment may also be continued after symptoms have resolved, for example to prevent or delay their recurrence.

[0087] The terms "effective amount" and "therapeutically effective amount," as used herein, refer to the amount or concentration of an inventive compound, that, when administered to a subject, is effective to at least partially treat a condition from which the subject is suffering. In some embodiments, an effective amount of a kinase inhibitor is an amount the administration of which results in inhibition of at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, at least about 99.5%, or about 100% of target kinase activity as compared to a baseline level, for example, a level of target kinase activity in the absence of the inhibitor. In some embodiments, an effective amount of a kinase activator is an amount the administration of which results in an increase of target kinase activity of at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 7.5-fold, at least about 10-fold, at least about 20-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 200-fold, or at

least about 500-fold as compared to a baseline level of target kinase activity, for example, a level in the absence of the activator.

[0088] As used herein, the term "kinase-associated disease or disorder" refers to, but is not limited to diseases and disorders in which there is abnormal kinase activity. As used herein, the term "kinase activity" refers to the enzymatic activity of a kinase, for example to the phosphotransferase activity of a kinase. Abnormal kinase activity may, in some embodiments, be determined to be an abnormal level of kinase activity. In some embodiments, abnormal kinase activity may be dysregulated kinase activity, for example, constitutive activity of a kinase that is normally regulated. An abnormal level of kinase activity may be a level that is higher than a normal level or may be a level that is lower than a normal level, wherein a "normal" level is, in some embodiments, the level in a cell, tissue, or subject that is not characterized by or that does not have a disease or disorder associated with aberrant kinase activity. For example, a normal activity of Src kinase in a tissue, for example, colonic tissue, is, in some embodiments, the average level of Src kinase activity that can be detected in subjects with healthy colonic tissue, for example, in subjects that do not manifest any clinical symptoms or any clinical symptoms associated with a colonic tissue dysfunction. In some embodiments, a kinase-associated disease or disorder is characterized by a higher-than-normal level of a specific kinase activity, a suitable therapeutic intervention, in some embodiments, may be the administration of an effective amount of an inhibitor of the respective kinase. Similarly, if a kinase-associated disease or disorder is characterized by a lower-than-normal level of a specific kinase activity, a suitable therapeutic intervention, in some embodiments, may be the administration of an effective amount of an activator of the respective kinase. Examples of kinase-activity-associated diseases and disorders include, but are not limited to proliferative diseases, benign neoplasms, cancer, inflammatory diseases, autoimmune diseases, developmental defects, neurodegenerative disorders (*e.g.*, Alzheimer's disease); metabolic syndrome, diabetes mellitus, and insulin resistance.

[0089] The term "cancer" as used herein refers to an uncontrolled growth of cells. In some embodiments, a cancer is an uncontrolled growth that interferes with the normal functioning of the bodily organs and systems. Cancers which migrate from their original location and seed vital organs can eventually lead to the death of the subject through the functional deterioration of the affected organs. Some exemplary subtypes of cancers are sarcoma, carcinoma, and hematopoietic cancers. The term "carcinoma" refers to a malignant cancer originating from epithelial cells and includes adenocarcinoma and squamous cell carcinoma. The term "sarcoma" refers to a cancer of the connective or supportive tissue and

includes osteosarcoma, chondrosarcoma and gastrointestinal stromal tumors. The term "hematopoietic cancer", refers to a cancer of the blood cell lineages, such as leukemia. Hematopoietic cancers are able to outcompete the normal hematopoietic compartments in a subject, thereby leading to hematopoietic failure (*e.g.*, in the form of anemia, thrombocytopenia and neutropenia) and can ultimately cause death. A person of ordinary skill in the art will appreciate that other classifications of cancers are known and will be able to classify a cancer according to the known classification schemes.

[0090] As used herein, the term "resin" refers to a resin useful for solid phase synthesis. Solid phase synthesis is a well–known synthetic technique; see generally, Atherton, E., Sheppard, R.C. *Solid Phase Peptide Synthesis: A Practical Approach*, IRL Press, Oxford, England, 1989, and Stewart J.M., Young, J.D. *Solid Phase Peptide Synthesis*, 2nd edition, Pierce Chemical Company, Rockford, 1984, the entire contents of each of which are hereby incorporated herein by reference.

[0091] As used herein, a "therapeutic agent" refers to any substance used as a medicine for treatment, prevention, delay, reduction or amelioration of a disease, condition, or disorder, and refers to a substance that is useful for therapy, including prophylactic and therapeutic treatment.

[0092] As used herein, a "label" refers to a moiety that has at least one element, isotope, or functional group incorporated into the moiety which enables detection of the compound to which the label is attached. In general, a label can fall into any one (or more) of five classes: a) a label which contains isotopic moieties, which may be radioactive or heavy isotopes, including, but not limited to, ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{31}P, ^{32}P, ^{35}S, ^{67}Ga, ^{99m}Tc (Tc-99m), ^{111}In, ^{123}I, ^{125}I, ^{169}Yb, and ^{186}Re; b) a label which contains an immune moiety, which may be antibodies or antigens, which may be bound to enzymes (*e.g.,* such as horseradish peroxidase); c) a label which is a colored, luminescent, phosphorescent, or fluorescent moieties (*e.g.,* such as the fluorescent label FITC); d) a label which has one or more photoaffinity moieties; and e) a label which has a ligand moiety with one or more known binding partners (such as biotin-streptavidin, FK506-FKBP, *etc.*). Any of these type of labels as described above may also be referred to as "diagnostic agents" as defined herein.

[0093] In certain embodiments, such as in the identification of a biological target, label comprises a radioactive isotope, preferably an isotope which emits detectable particles, such as β particles. In certain embodiments, the label comprises one or more photoaffinity moieties for the direct elucidation of intermolecular interactions in biological systems. A variety of known photophores can be employed, most relying on photoconversion of diazo

compounds, azides, or diazirines to nitrenes or carbenes (*see*, Bayley, H., Photogenerated Reagents in Biochemistry and Molecular Biology (1983), Elsevier, Amsterdam, the entire contents of which are incorporated herein by reference). In certain embodiments of the invention, the photoaffinity labels employed are o-, m- and p-azidobenzoyls, substituted with one or more halogen moieties, including, but not limited to 4-azido-2,3,5,6-tetrafluorobenzoic acid. In certain embodiments, the label comprises one or more fluorescent moieties. In certain embodiments, the label is the fluorescent label FITC. In certain embodiments, the label comprises a ligand moiety with one or more known binding partners. In certain embodiments, the label comprises the ligand moiety biotin.

[0094] In certain embodiments, the label is an imaging agent. Exemplary imaging agents include, but are not limited to, those used in positron emissions tomography (PET), computer assisted tomography (CAT), single photon emission computerized tomography, x-ray, fluoroscopy, and magnetic resonance imaging (MRI); anti-emetics; and contrast agents. Exemplary diagnostic agents include but are not limited to, fluorescent moieties, luminescent moieties, magnetic moieties; gadolinium chelates (e.g., gadolinium chelates with DTPA, DTPA-BMA, DOTA and HP-DO3A), iron chelates, magnesium chelates, manganese chelates, copper chelates, chromium chelates, iodine-based materials useful for CAT and x-ray imaging, and radionuclides. Suitable radionuclides include, but are not limited to, ^{123}I, ^{125}I, ^{130}I, ^{131}I, ^{133}I, ^{135}I, ^{47}Sc, ^{72}As, ^{72}Se, ^{90}Y, ^{88}Y, ^{97}Ru, ^{100}Pd, ^{101}mRh, ^{119}Sb, ^{128}Ba, ^{197}Hg, ^{211}At, ^{212}Bi, ^{212}Pb, ^{109}Pd, ^{111}In, ^{67}Ga, ^{68}Ga, ^{67}Cu, ^{75}Br, ^{77}Br, ^{99}mTc, ^{14}C, ^{13}N, ^{15}O, ^{32}P, ^{33}P, and ^{18}F. Fluorescent and luminescent moieties include, but are not limited to, a variety of different organic or inorganic small molecules commonly referred to as "dyes," "labels," or "indicators." Examples include, but are not limited to, fluorescein, rhodamine, acridine dyes, Alexa dyes, cyanine dyes, *etc.* Fluorescent and luminescent moieties may include a variety of naturally occurring proteins and derivatives thereof, *e.g.*, genetically engineered variants. For example, fluorescent proteins include green fluorescent protein (GFP), enhanced GFP, red, blue, yellow, cyan, and sapphire fluorescent proteins, reef coral fluorescent protein, *etc.* Luminescent proteins include luciferase, aequorin and derivatives thereof. Numerous fluorescent and luminescent dyes and proteins are known in the art (see, *e.g.*, U.S. Patent Publication 2004/0067503; Valeur, B., "Molecular Fluorescence: Principles and Applications," John Wiley and Sons, 2002; and *Handbook of Fluorescent Probes and Research Products*, Molecular Probes, 9th edition, 2002).

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[0095] Some aspects of the invention provide macrocyclic compounds that bind and/or modulate the activity of specific kinases. In some embodiments, a macrocyclic compound is provided that modulates the activity of a kinase it binds. In some embodiments, a macrocyclic compound is provided that inhibits the activity of a kinase. In some embodiments, a macrocyclic compound is provided that activates a specific kinase. In some embodiments, a macrocyclic compound is provided that selectively inhibits Src, Akt3, AMPK, ERBB4, MK2, p38α, MKK6, and/or Pim1. In some embodiments, a macrocyclic compound is provided that activates VEGFR2.

[0096] In some embodiments, a macrocyclic compound is provided that specifically binds and inhibits a Src kinase. Src kinases are a family of proto-oncogenic tyrosine kinases. The human c-Src gene is similar to the v-Src gene of Rous Sarcoma Virus (RSV). Without wishing to be bound by theory, it is believed that c-Src may play a role in the regulation of embryonic development and cell growth. In normal cells, Src activity is regulated, at least in part, by phosphorylation. Without wishing to be bound by theory, it is believed that phosphorylation of a specific C-terminal residue inhibits Src activity. Mutations resulting in truncation of the Src protein, *e.g.*, a loss of the C-terminal phosphorylation site, can lead to aberrant activity of the Src kinase, which, in turn, is thought to be causally associated with increased cell proliferation and survival, and, thus, with the formation of various cancers. In humans, two c-Src transcript variants have been described. Src protein and nucleotide sequences are well known to those of skill in the art and can be retrieved from publicly available databases (*e.g.*, the NCBI and Ensemble databases at www.ncbi.nlm,nih.gov or www.ensembl.org, respectively). Representative sequences for Src are provided below:

[0097] >gi|38202215|ref|NM_005417.3| Homo sapiens v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian) (SRC), transcript variant 1, mRNA

CAAACAAGTGCGGCCATTTCACCAGCCCAGGCTGGCTTCTGCTGTTGACTGGCTG
TGGCACCTCAAGCAGCCCCTTTCCCCTCTAGCCTCAGTTTATCACCGCAAGAGCT
ACCATTCATCTAGCACAACCTGACCATCCTCACACTGGTCAGTTCCAACCTTCCC
AGGAATCTTCTGTGGCCATGTTCACTCCGGTTTTACAGAACAGAGAACAGAAGCT
CAGAGAAGTGAAGCAACTTGCCCAGCTATGAGAGACAGAGCCAGGATTTGAAAC
CAGATGAGGACGCTGAGGCCCAGAGAGGGAAAGCCACTTGCCTAGGGACACAC
AGCGGGGAGAGGTGGAGCAGGGCCTCTATTTCGAGACCCCTGACTCCACACCTG
GTGTTTGTGCCAAGACCCCAGGCTGCCTCCCAGGTCCTCTGGGACAGCCCCTGCC
TTCTACCAGGACCATGGGTAGCAACAAGAGCAAGCCCAAGGATGCCAGCCAGCG

GCGCCGCAGCCTGGAGCCCGCCGAGAACGTGCACGGCGCTGGCGGGGGCGCTTT
CCCCGCCTCGCAGACCCCCAGCAAGCCAGCCTCGGCCGACGGCCACCGCGGCCC
CAGCGCGGCCTTCGCCCCCGCGGCCGCCGAGCCCAAGCTGTTCGGAGGCTTCAA
CTCCTCGGACACCGTCACCTCCCCGCAGAGGGCGGGCCCGCTGGCCGGTGGAGT
GACCACCTTTGTGGCCCTCTATGACTATGAGTCTAGGACGGAGACAGACCTGTCC
TTCAAGAAAGGCGAGCGGCTCCAGATTGTCAACAACACAGAGGGAGACTGGTGG
CTGGCCCACTCGCTCAGCACAGGACAGACAGGCTACATCCCCAGCAACTACGTG
GCGCCCTCCGACTCCATCCAGGCTGAGGAGTGGTATTTTGGCAAGATCACCAGAC
GGGAGTCAGAGCGGTTACTGCTCAATGCAGAGAACCCGAGAGGGACCTTCCTCG
TGCGAGAAAGTGAGACCACGAAAGGTGCCTACTGCCTCTCAGTGTCTGACTTCGA
CAACGCCAAGGGCCTCAACGTGAAGCACTACAAGATCCGCAAGCTGGACAGCGG
CGGCTTCTACATCACCTCCCGCACCCAGTTCAACAGCCTGCAGCAGCTGGTGGCC
TACTACTCCAAACACGCCGATGGCCTGTGCCACCGCCTCACCACCGTGTGCCCCA
CGTCCAAGCCGCAGACTCAGGGCCTGGCCAAGGATGCCTGGGAGATCCCTCGGG
AGTCGCTGCGGCTGGAGGTCAAGCTGGGCCAGGGCTGCTTTGGCGAGGTGTGGA
TGGGGACCTGGAACGGTACCACCAGGGTGGCCATCAAAACCCTGAAGCCTGGCA
CGATGTCTCCAGAGGCCTTCCTGCAGGAGGCCCAGGTCATGAAGAAGCTGAGGC
ATGAGAAGCTGGTGCAGTTGTATGCTGTGGTTTCAGAGGAGCCCATTTACATCGT
CACGGAGTACATGAGCAAGGGGAGTTTGCTGGACTTTCTCAAGGGGGAGACAGG
CAAGTACCTGCGGCTGCCTCAGCTGGTGGACATGGCTGCTCAGATCGCCTCAGGC
ATGGCGTACGTGGAGCGGATGAACTACGTCCACCGGGACCTTCGTGCAGCCAAC
ATCCTGGTGGGAGAGAACCTGGTGTGCAAAGTGGCCGACTTTGGGCTGGCTCGG
CTCATTGAAGACAATGAGTACACGGCGCGGCAAGGTGCCAAATTCCCCATCAAG
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CCCCAGTACCAGCCCGGGGAGAACCTCTAGGCACAGGCGGGCCCAGACCGGCTT
CTCGGCTTGGATCCTGGGCTGGGTGGCCCCTGTCTCGGGGCTTGCCCCACTCTGC
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CCTCTTTGGTGGCATGGAAGGGGCTTCTGGACCTAGGGTGGCCTGAGAGGGCGG
TGGGTATGCGAGACCAGCACGGTGACTCTGTCCAGCTCCCGCTGTGGCCGCACGC

CTCTCCCTGCACTCCCTCCTGGAGCTCTGTGGGTCTCTGGAAGAGGAACCAGGAG
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AAGAGCCTTTCCAAAGAGGAGCGATGGGCCCCTGGCCCCGCCTGCCTGCCACCC
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GAGCGGGGATGTGACATGCCCAAGGCCACGGAGCAGTTCAGAGTGGAGGCGGG
CTTGGAACCCGGTGCTCCCTCTGTCATCCTCAGGAACCAACAATTCGTCGGAGGC
ATCATGGAAAGACTGGGACAGCCCAGGAAACAAGGGGTCTGAGGATGCATTCGA
GATGGCAGATTCCCACTGCCGCTGCCCGCTCAGCCCAGCTGTTGGGAACAGCATG
GAGGCAGATGTGGGGCTGAGCTGGGGAATCAGGGTAAAAGGTGCAGGTGTGGA
GAGAGAGGCTTCAATCGGCTTGTGGGTGATGTTTGACCTTCAGAGCCAGCCGGCT
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GCCAAGAGTCCAGGAGGCCCTGGTCCTGGCCTCCTTCCCCGTACTTTGTCCCGTG
GCATTTCAATTCCTGGCCCTGTTCTCCTCCCCAAGTCGGCACCCTTTAACTCATGA
GGAGGGAAAAGAGTGCCTAAGCGGGGGTGAAAGAGGACGTGTTACCCACTGCC
ATGCACCAGGACTGGCTGTGTAACCTTGGGTGGCCCCTGCTGTCTCTCTGGGCTG
CAGAGTCTGCCCCACATGTGGCCATGGCCTCTGCAACTGCTCAGCTCTGGTCCAG
GCCCTGTGGCAGGACACACATGGTGAGCCTAGCCCTGGGACATCAGGAGACTGG
GCTCTGGCTCTGTTCGGCCTTTGGGTGTGTGGTGGATTCTCCCTGGGCCTCAG
TGTGCCCATCTGTAAAGGGGCAGCTGACAGTTTGTGGCATCTTGCCAAGGGTCCC
TGTGTGTGTGTATGTGTGTGCATGTGTGCGTGTCTCCATGTGCGTCCATATTTAAC
ATGTAAAAATGTCCCCCCCGCTCCGTCCCCCAAACATGTTGTACATTTCACCATG
GCCCCCTCATCATAGCAATAACATTCCCACTGCCAGGGGTTCTTGAGCCAGCCAG
GCCCTGCCAGTGGGGAAGGAGGCCAAGCAGTGCCTGCCTATGAAATTTCAACTTT
TCCTTTCATACGTCTTTATTACCCAAGTCTTCTCCCGTCCATTCCAGTCAAATCTG
GGCTCACTCACCCCAGCGAGCTCTCAAATCCCTCTCCAACTGCCTAAGGCCCTTT
GTGTAAGGTGTCTTAATACTGTCCTTTTTTTTTTTTTAACAGTGTTTTGTAGATTTC
AGATGACTATGCAGAGGCCTGGGGGACCCCTGGCTCTGGGCCGGGCCTGGGGCT
CCGAAATTCCAAGGCCCAGACTTGCGGGGGGTGGGGGGGTATCCAGAATTGGTT
GTAAATACTTTGCATATTGTCTGATTAAACACAAACAGACCTCAGAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO: 1).

[0098] >gi|38202216|ref|NM_198291.1| Homo sapiens v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian) (SRC), transcript variant 2, mRNA

GCCGGAGCGGCCAGGCCGCCGTCTGCCCGTCCCGCTGGACGTCCCGCGGTCCGC
CCTCCCGTGCGTCCGTCTGCCGGTGAGCCCGCCCGCCCGCCGGCCCAGAACAGA
GAACAGAAGCTCAGAGAAGTGAAGCAACTTGCCCAGCTATGAGAGACAGAGCC
AGGATTTGAAACCAGATGAGGACGCTGAGGCCCAGAGAGGGAAAGCCACTTGCC
TAGGGACACACAGCGGGGAGAGGTGGAGCAGGGCCTCTATTTCGAGACCCCTGA
CTCCACACCTGGTGTTTGTGCCAAGACCCCAGGCTGCCTCCCAGGTCCTCTGGGA
CAGCCCCTGCCTTCTACCAGGACCATGGGTAGCAACAAGAGCAAGCCCAAGGAT
GCCAGCCAGCGGCGCCGCAGCCTGGAGCCCGCCGAGAACGTGCACGGCGCTGGC
GGGGGCGCTTTCCCCGCCTCGCAGACCCCCAGCAAGCCAGCCTCGGCCGACGGC
CACCGCGGCCCCAGCGCGGCCTTCGCCCCCGCGGCCGCCGAGCCCAAGCTGTTC
GGAGGCTTCAACTCCTCGGACACCGTCACCTCCCCGCAGAGGGCGGGCCCGCTG
GCCGGTGGAGTGACCACCTTTGTGGCCCTCTATGACTATGAGTCTAGGACGGAGA
CAGACCTGTCCTTCAAGAAAGGCGAGCGGCTCCAGATTGTCAACAACACAGAGG
GAGACTGGTGGCTGGCCCACTCGCTCAGCACAGGACAGACAGGCTACATCCCCA
GCAACTACGTGGCGCCCTCCGACTCCATCCAGGCTGAGGAGTGGTATTTTGGCAA
GATCACCAGACGGGAGTCAGAGCGGTTACTGCTCAATGCAGAGAACCCGAGAGG
GACCTTCCTCGTGCGAGAAAGTGAGACCACGAAAGGTGCCTACTGCCTCTCAGTG
TCTGACTTCGACAACGCCAAGGGCCTCAACGTGAAGCACTACAAGATCCGCAAG
CTGGACAGCGGCGGCTTCTACATCACCTCCCGCACCCAGTTCAACAGCCTGCAGC
AGCTGGTGGCCTACTACTCCAAACACGCCGATGGCCTGTGCCACCGCCTCACCAC
CGTGTGCCCCACGTCCAAGCCGCAGACTCAGGGCCTGGCCAAGGATGCCTGGGA
GATCCCTCGGGAGTCGCTGCGGCTGGAGGTCAAGCTGGGCCAGGGCTGCTTTGG
CGAGGTGTGGATGGGGACCTGGAACGGTACCACCAGGGTGGCCATCAAAACCCT
GAAGCCTGGCACGATGTCTCCAGAGGCCTTCCTGCAGGAGGCCCAGGTCATGAA
GAAGCTGAGGCATGAGAAGCTGGTGCAGTTGTATGCTGTGGTTTCAGAGGAGCC
CATTTACATCGTCACGGAGTACATGAGCAAGGGGAGTTTGCTGGACTTTCTCAAG
GGGGAGACAGGCAAGTACCTGCGGCTGCCTCAGCTGGTGGACATGGCTGCTCAG
ATCGCCTCAGGCATGGCGTACGTGGAGCGGATGAACTACGTCCACCGGGACCTT
CGTGCAGCCAACATCCTGGTGGGAGAGAACCTGGTGTGCAAAGTGGCCGACTTT
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GCTACCGGATGCCCTGCCCGCCGGAGTGTCCCGAGTCCCTGCACGACCTCATGTG
CCAGTGCTGGCGGAAGGAGCCTGAGGAGCGGCCCACCTTCGAGTACCTGCAGGC
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GGCCCCTGTCTCGGGGCTTGCCCCACTCTGCCTGCCTGCTGTTGGTCCTCTCTCTG
TGGGGCTGAATTGCCAGGGGCGAGGCCCTTCCTCTTTGGTGGCATGGAAGGGGCT
TCTGGACCTAGGGTGGCCTGAGAGGGCGGTGGGTATGCGAGACCAGCACGGTGA
CTCTGTCCAGCTCCCGCTGTGGCCGCACGCCTCTCCCTGCACTCCCTCCTGGAGCT
CTGTGGGTCTCTGGAAGAGGAACCAGGAGAAGGGCTGGGGCCGGGGCTGAGGGT
GCCCTTTTCCAGCCTCAGCCTACTCCGCTCACTGAACTCCTTCCCCACTTCTGTGC
CACCCCCGGTCTATGTCGAGAGCTGGCCAAAGAGCCTTTCCAAAGAGGAGCGAT
GGGCCCCTGGCCCCGCCTGCCTGCCACCCTGCCCCTTGCCATCCATTCTGGAAAC
ACCTGTAGGCAGAGGCTGCCGAGACAGACCCTCTGCCGCTGCTTCCAGGCTGGG
CAGCACAAGGCCTTGCCTGGCCTGATGATGGTGGGTGGGTGGGATGAGTACCCC
CTCAAACCCTGCCCTCCTTAGACCTGAGGGACCCTTCGAGATCATCACTTCCTTG
CCCCCATTTCACCCATGGGGAGACAGTTGAGAGCGGGGATGTGACATGCCCAAG
GCCACGGAGCAGTTCAGAGTGGAGGCGGGCTTGGAACCCGGTGCTCCCTCTGTC
ATCCTCAGGAACCAACAATTCGTCGGAGGCATCATGGAAAGACTGGGACAGCCC
AGGAAACAAGGGGTCTGAGGATGCATTCGAGATGGCAGATTCCCACTGCCGCTG
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GGGAATCAGGGTAAAAGGTGCAGGTGTGGAGAGAGAGGCTTCAATCGGCTTGTG
GGTGATGTTTGACCTTCAGAGCCAGCCGGCTATGAAAGGGAGCGAGCCCCTCGG
CTCTGGAGGCAATCAAGCAGACATAGAAGAGCCAAGAGTCCAGGAGGCCCTGGT
CCTGGCCTCCTTCCCCGTACTTTGTCCCGTGGCATTTCAATTCCTGGCCCTGTTCT
CCTCCCCAAGTCGGCACCCTTTAACTCATGAGGAGGGAAAAGAGTGCCTAAGCG
GGGGTGAAAGAGGACGTGTTACCCACTGCCATGCACCAGGACTGGCTGTGTAAC
CTTGGGTGGCCCCTGCTGTCTCTCTGGGCTGCAGAGTCTGCCCCACATGTGGCCA
TGGCCTCTGCAACTGCTCAGCTCTGGTCCAGGCCCTGTGGCAGGACACACATGGT
GAGCCTAGCCCTGGGACATCAGGAGACTGGGCTCTGGCTCTGTTCGGCCTTTGGG
TGTGTGGTGGATTCTCCCTGGGCCTCAGTGTGCCCATCTGTAAAGGGGCAGCTGA
CAGTTTGTGGCATCTTGCCAAGGGTCCCTGTGTGTGTGTATGTGTGTGCATGTGTG
CGTGTCTCCATGTGCGTCCATATTTAACATGTAAAAATGTCCCCCCCGCTCCGTCC
CCCAAACATGTTGTACATTTCACCATGGCCCCCTCATCATAGCAATAACATTCCC

ACTGCCAGGGGTTCTTGAGCCAGCCAGGCCCTGCCAGTGGGGAAGGAGGCCAAG
CAGTGCCTGCCTATGAAATTTCAACTTTTCCTTTCATACGTCTTTATTACCCAAGT
CTTCTCCCGTCCATTCCAGTCAAATCTGGGCTCACTCACCCCAGCGAGCTCTCAA
ATCCCTCTCCAACTGCCTAAGGCCCTTTGTGTAAGGTGTCTTAATACTGTCCTTTT
TTTTTTTTTAACAGTGTTTTGTAGATTTCAGATGACTATGCAGAGGCCTGGGGGAC
CCCTGGCTCTGGGCCGGGCCTGGGGCTCCGAAATTCCAAGGCCCAGACTTGCGG
GGGGTGGGGGGGTATCCAGAATTGGTTGTAAATACTTTGCATATTGTCTGATTAA
ACACAAACAGACCTCAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAA (SEQ ID NO: 2).

[0099] >gi|4885609|ref|NP_005408.1| proto-oncogene tyrosine-protein kinase Src [Homo sapiens] (See also NP_938033.1)

MGSNKSKPKDASQRRRSLEPAENVHGAGGGAFPASQTPSKPASADGHRGPSAAFAP
AAAEPKLFGGFNSSDTVTSPQRAGPLAGGVTTFVALYDYESRTETDLSFKKGERLQI
VNNTEGDWWLAHSLSTGQTGYIPSNYVAPSDSIQAEEWYFGKITRRESERLLLNAEN
PRGTFLVRESETTKGAYCLSVSDFDNAKGLNVKHYKIRKLDSGGFYITSRTQFNSLQ
QLVAYYSKHADGLCHRLTTVCPTSKPQTQGLAKDAWEIPRESLRLEVKLGQGCFGE
VWMGTWNGTTRVAIKTLKPGTMSPEAFLQEAQVMKKLRHEKLVQLYAVVSEEPIYI
V<u>T</u>EYMSKGSLLDFLKGETGKYLRLPQLVDMAAQIASGMAYVERMNYVHRDLRAA
NILVGENLVCKVADFGLARLIEDNEYTARQGAKFPIKWTAPEAALYGRFTIKSDVWS
FGILLTELTTKGRVPYPGMVNREVLDQVERGYRMPCPPECPESLHDLMCQCWRKEP
EERPTFEYLQAFLEDYFTSTEPQYQPGENL (SEQ ID NO: 3).

[00100] The underlined residue, threonine 341 in the representative sequence above, is referred to herein as the "gatekeeper residue" or "gatekeeper threonine". Depending on the numbering scheme, the gatekeeper residue may be assigned a different residue number. Those of skill in the art will, however, be able to ascertain the gatekeeper residue, e.g, the gatekeeper threonine of src, for example, by identifying the residue that is homologous with the T341 in the above described sequence, or with a gatekeeper residue or residue position described in the art. The src kinase gatekeeper threonine is also often referred to as threonine 338, Thr338, or T338, and the terms "T338" and "T341," as used in connection with src kinase herein, both refer to the gatekeeper threonine residue of src kinase. Mutations of the gatekeeper residue, for example, T338I or T341I mutations in src kinases, typically results in resistance of the mutated kinase to kinase inhibitors. Since aberrant src kinase activation can lead to disease, e.g., certain types of cancer, as described in more detail elsewhere herein, a gatekeeper mutation can hamper the treatment of such a disease with a src kinase inhibitor.

Gatekeeper residues and gatekeeper residue mutations in kinases, for example, in src kinases, are well known in the art (see, e.g., Figure 1a and related text in Azam et al., Nat Struct Mol Biol. 2008 October; 15(10): 1109–1118, the entire contents of which are incorporated herein by reference).

[00101] Mutated forms, for example, truncated forms of Src kinases have been described and sequences related to such mutated forms are well known in the art. In some instances, mutation, for example, truncation of a Src kinase can lead to aberrant activity, for example increased or constitutive activity of the kinase. Such aberrant Src kinase activity has been linked to various forms of cancer. For example, a truncated, activated form of Src has been found in colon cancers and in sarcomas (*e.g.*, Sugimura M, Kobayashi K, Sagae S, Nishioka Y, Ishioka S, Terasawa K, Tokino T, Kudo R. *Mutation of the SRC gene in endometrial carcinoma*. Jpn J Cancer Res. 2000 Apr;91(4):395-8; Irby RB, Mao W, Coppola D, Kang J, Loubeau JM, Trudeau W, Karl R, Fujita DJ, Jove R, Yeatman TJ. *Activating SRC mutation in a subset of advanced human colon cancers*. Nat Genet. 1999 Feb;21(2):187-90; all references incorporated herein by reference). Further, it has been reported that Src expression and Src kinase activity are frequently increased in a wide array of cancers, including tumors from breast, colon, pancreas, lung, ovary, and CNS (*e.g.*, Irby RB, Mao W, Coppola D, Kang J, Loubeau JM, Trudeau W, Karl R, Fujita DJ, Jove R, Yeatman TJ. *Activating SRC mutation in a subset of advanced human colon cancers*. Nat Genet. 1999 Feb;21(2):187-90; incorporated herein by reference). Accordingly, inhibition of Src kinase activity is desirable in certain clinical scenarios characterized by aberrant Src kinase activity, for example, but not limited to cancers exhibiting constitutive or increased Src expression or expressing mutated (*e.g.*, truncated) forms of a Src kinase.

[00102] In some embodiments, compounds are provided that bind Akt3, MAPKAPK2, Pim1, or VEGFR2. In some embodiments, compounds are provided that inhibit Akt3, MAPKAPK2, Pim1, and p38α-MAPKAPK2. In some embodiments, compounds are provided that activate VEGFR2. In some embodiments, a compound provided herein modulates (*e.g.*, inhibits or activates) the activity of a clinically relevant target kinase, for example, of a Abl1, ACK, ACTR2B, Akt1, Akt2, ALK, ALK1, ALK2, ALK4, ANKK1, ANPα, ANPβ, ARG, ATM, ATR, AurA, AurB, Axl, BARK1, Bcr, BMPR1A, BMPR1B, BMPR2, BRAF, BRD4, BRK, BTK, BUB1, BUBR1, CAMK2δ, CDC2, CDK2, CDK4, CDK5, CDK6, CDK9, CDKL5, CHK1, CHK2, CK1δ,, CK1α, CK1ε, CK2α1,, CK2α2, COT/TPL2, CTK/MATK, CYGD, CYGF, DAPK1, DCAMKL1, DMPK1, DNAPK, DYRK1A, eEF2K, EGFR, Eph, family, EphA1, EphA2, EphA3, EphB2, EphB4, Erk5, FAK,

FER, FES, FGFR1, FGFR2, FGFR3, FGFR4, FGR, FLT1, FLT3, FLT4, FMS, Fyn, GRK4, GSK3, HER2, HER3, HER4, HGK, HIPK1, HIPK2, IGF1R, IKKα,, IKKβ, IKKε, ILK, INSR, IRAK2, IRAK4, ITK, Jak1, Jak2, Jak3, JNK1, JNK3, KDR, Kit, LATS1, LATS2, LCK, LIMK1, LKB1, LRRK2, LTK, LYN, MASTL, MEK1, MEK2, MELK, Mer, Met, MISR2, MKK3, MKK4, MLK4, skMLCK, smMLCK, Mst4, mTOR, MusK, MYO3A, NEK1, NEK2, NEK8, p38, p70S6K, PAK3, PAK4, PDGFRα, PDGFRβ, PEK, PHKγ2, Pim-1, Pim-2, Pim-3, PINK1, PKACα, PKC, PKCα, PKCβ, PKCγ, PKCδ, PKCε, PKCη, PKCθ, PKR, PLK1, PRKX, PRKY, PYK2, Raf1, Ret, RHOK, RNAseL, ROCK1,, ROCK2, RON, ROR2, ROS, RSK2, SGK1, SRC, Syk, TGFβR1, TGFβR2, Tie2, TITIN, TrkA, TrkB, TrkC, Tyk2, TYRO3, Wnk1, Wnk4, Yes, or Zap-70 kinase.

[00103] In some embodiments, a compound provided herein that inhibits or activates a kinase is useful for clinical applications, for example, for the inhibition or activation of a kinase implicated in a human disease or condition. In some embodiments, a compound provided herein that inhibits or activates a kinase is useful for basic research applications, for example, for characterizing a kinase, and/or for studying the role or function of a target kinase in a molecular pathway, a cell, a tissue, or an organism.

[00104] Akt 3 is a member of the AKT, also called PKB, serine/threonine protein kinase family. AKT kinases are known to be regulators of cell signaling in response to insulin and growth factors. They are involved in a wide variety of biological processes including cell proliferation, differentiation, apoptosis, tumorigenesis, as well as glycogen synthesis and glucose uptake. Akt3 has been shown to be stimulated by platelet-derived growth factor (PDGF), insulin, and insulin-like growth factor 1 (IGF1). Alternatively splice transcript variants encoding distinct isoforms have been described. Increased Akt3 activity has been reported in various cancers, including melanomas (*e.g.*, Sharma *et al.*, *Targeting Akt3 signaling in malignant melanoma using isoelenocyanates*, Clinical Cancer Research, 2009, 15:1647, incorporated herein by reference). Representative AKT3 sequences are given below:

[00105] >gi|32307164|ref|NM_005465.3| Homo sapiens v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma) (AKT3), transcript variant 1, mRNA

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AAGTTGGGGGCTCAGAGGGGAGTCATCATGAGCGATGTTACCATTGTGAAAGAA
GGTTGGGTTCAGAAGAGGGGAGAATATATAAAAAACTGGAGGCCAAGATACTT
CCTTTTGAAGACAGATGGCTCATTCATAGGATATAAAGAGAAACCTCAAGATGT
GGATTTACCTTATCCCCTCAACAACTTTTCAGTGGCAAAATGCCAGTTAATGAAA

ACAGAACGACCAAAGCCAAACACATTTATAATCAGATGTCTCCAGTGGACTACT
GTTATAGAGAGAACATTTCATGTAGATACTCCAGAGGAAAGGGAAGAATGGAC
AGAAGCTATCCAGGCTGTAGCAGACAGACTGCAGAGGCAAGAAGAGGAGAGAA
TGAATTGTAGTCCAACTTCACAAATTGATAATATAGGAGAGGAAGAGATGGATG
CCTCTACAACCCATCATAAAAGAAAGACAATGAATGATTTTGACTATTTGAAAC
TACTAGGTAAAGGCACTTTTGGGAAAGTTATTTTGGTTCGAGAGAAGGCAAGTG
GAAAATACTATGCTATGAAGATTCTGAAGAAAGAAGTCATTATTGCAAAGGATG
AAGTGGCACACACTCTAACTGAAAGCAGAGTATTAAAGAACACTAGACATCCCT
TTTTAACATCCTTGAAATATTCCTTCCAGACAAAAGACCGTTTGTGTTTTGTGAT
GGAATATGTTAATGGGGGCGAGCTGTTTTTCCATTTGTCGAGAGAGCGGGTGTTC
TCTGAGGACCGCACACGTTTCTATGGTGCAGAAATTGTCTCTGCCTTGGACTATC
TACATTCCGGAAAGATTGTGTACCGTGATCTCAAGTTGGAGAATCTAATGCTGG
ACAAAGATGGCCACATAAAAATTACAGATTTTGGACTTTGCAAAGAAGGGATCA
CAGATGCAGCCACCATGAAGACATTCTGTGGCACTCCAGAATATCTGGCACCAG
AGGTGTTAGAAGATAATGACTATGGCCGAGCAGTAGACTGGTGGGGCCTAGGG
GTTGTCATGTATGAAATGATGTGTGGGAGGTTACCTTTCTACAACCAGGACCATG
AGAAACTTTTTGAATTAATATTAATGGAAGACATTAAATTTCCTCGAACACTCTC
TTCAGATGCAAAATCATTGCTTTCAGGGCTCTTGATAAAGGATCCAAATAAACG
CCTTGGTGGAGGACCAGATGATGCAAAAGAAATTATGAGACACAGTTTCTTCTC
TGGAGTAAACTGGCAAGATGTATATGATAAAAAGCTTGTACCTCCTTTTAAACCT
CAAGTAACATCTGAGACAGATACTAGATATTTTGATGAAGAATTTACAGCTCAG
ACTATTACAATAACACCACCTGAAAAATATGATGAGGATGGTATGGACTGCATG
GACAATGAGAGGCGGCCGCATTTCCCTCAATTTTCCTACTCTGCAAGTGGACGA
GAATAAGTCTCTTTCATTCTGCTACTTCACTGTCATCTTCAATTTATTACTGAAAA
TGATTCCTGGACATCACCAGTCCTAGCTCTTACACATAGCAGGGGCACCTTCCGA
CATCCCAGACCAGCCAAGGGTCCTCACCCCTCGCCACCTTTCACCCTCATGAAAA
CACACATACACGCAAATACACTCCAGTTTTTGTTTTTGCATGAAATTGTATCTCA
GTCTAAGGTCTCATGCTGTTGCTGCTACTGTCTTACTATTATAGCAACTTTAAGA
AGTAATTTTCCAACCTTTGGAAGTCATGAGCCCACCATTGTTCATTTGTGCACCA
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CTTCTTGACATTTATCACCTCTGATGGCTGAAGAATGTAGACAGGTATAATGATA
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GTCCTTTTATGATTTTGTTTATATTGCAGTAGTATTTATTTTTTAGTGATGAGAAT
TGTATGTCATGTTAGCAAACGCAGCTCCAACTTATATAAAATAGACTTACTGCAG
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CATTAATGATGATCTTCAGATTAAACCCTTTGGTGCTAGGAGCTGACAATTTCCA
AAGCAGCCTGTGAAGTCCTAGGGGCTGGGGGCCACTCTTGCGGCAAGCAGAAG
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GAGCAAGTGACTTTCTAAGTAGGATGAATCATATTCATATGCAGATGTCTTAGCC
TCTTGACGCTGGAAGTGTGGATTTATAGCTATGAAACCACTGCTGGCAGTGGGT
GGGCCACTGGGACTGACGGGGGTTAAAGGGCATTTTACTAAGGCAGCTAAGACA
TATTCAGACATCAACGTTATCCTTCTTTTTCATATTTCTACCTGAGTGAAG (SEQ
ID NO: 4).

[00106] >gi|32307162|ref|NM_181690.1| Homo sapiens v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma) (AKT3), transcript variant 2, mRNA

GCAGCAGCAGAGAATCCAAACCCTAAAGCTGATATCACAAAGTACCATTTCTCC
AAGTTGGGGGCTCAGAGGGGAGTCATCATGAGCGATGTTACCATTGTGAAAGAA
GGTTGGGTTCAGAAGAGGGGAGAATATATAAAAAACTGGAGGCCAAGATACTT
CCTTTTGAAGACAGATGGCTCATTCATAGGATATAAAGAGAAACCTCAAGATGT

GGATTTACCTTATCCCCTCAACAACTTTTCAGTGGCAAAATGCCAGTTAATGAAA
ACAGAACGACCAAAGCCAAACACATTTATAATCAGATGTCTCCAGTGGACTACT
GTTATAGAGAGAACATTTCATGTAGATACTCCAGAGGAAAGGGAAGAATGGAC
AGAAGCTATCCAGGCTGTAGCAGACAGACTGCAGAGGCAAGAAGAGGAGAGAA
TGAATTGTAGTCCAACTTCACAAATTGATAATATAGGAGAGGAAGAGATGGATG
CCTCTACAACCCATCATAAAAGAAAGACAATGAATGATTTTGACTATTTGAAAC
TACTAGGTAAAGGCACTTTTGGGAAAGTTATTTTGGTTCGAGAGAAGGCAAGTG
GAAAATACTATGCTATGAAGATTCTGAAGAAAGAAGTCATTATTGCAAAGGATG
AAGTGGCACACACTCTAACTGAAAGCAGAGTATTAAAGAACACTAGACATCCCT
TTTTAACATCCTTGAAATATTCCTTCCAGACAAAAGACCGTTTGTGTTTTGTGAT
GGAATATGTTAATGGGGGCGAGCTGTTTTTCCATTTGTCGAGAGAGCGGGTGTTC
TCTGAGGACCGCACACGTTTCTATGGTGCAGAAATTGTCTCTGCCTTGGACTATC
TACATTCCGGAAAGATTGTGTACCGTGATCTCAAGTTGGAGAATCTAATGCTGG
ACAAAGATGGCCACATAAAAATTACAGATTTTGGACTTTGCAAAGAAGGGATCA
CAGATGCAGCCACCATGAAGACATTCTGTGGCACTCCAGAATATCTGGCACCAG
AGGTGTTAGAAGATAATGACTATGGCCGAGCAGTAGACTGGTGGGGCCTAGGG
GTTGTCATGTATGAAATGATGTGTGGGAGGTTACCTTTCTACAACCAGGACCATG
AGAAACTTTTTGAATTAATATTAATGGAAGACATTAAATTTCCTCGAACACTCTC
TTCAGATGCAAAATCATTGCTTTCAGGGCTCTTGATAAAGGATCCAAATAAACG
CCTTGGTGGAGGACCAGATGATGCAAAAGAAATTATGAGACACAGTTTCTTCTC
TGGAGTAAACTGGCAAGATGTATATGATAAAAAGCTTGTACCTCCTTTTAAACCT
CAAGTAACATCTGAGACAGATACTAGATATTTTGATGAAGAATTTACAGCTCAG
ACTATTACAATAACACCACCTGAAAAATGTCAGCAATCAGATTGTGGCATGCTG
GGTAACTGGAAAAAATAATAAAAATCGGCTTCCTACAGCCAGCAGCACAGTCAC
CCATGGAACTGTTGGCTTTGGATTAAATGTGGAATTGAACGACTACCCAGAAGT
GTTCTGGAAAGAAGCGAGATGTGTGGCCTGCCTCACCGTCCTCACCCATCAAAA
GCACCAGCAGGCACGTTAACTCGAATTCTCACAAGGAAAAGGCCATTAAAGCTC
AAGGTGCATTTCAAACTCCAGGCTAC (SEQ ID NO: 5).

[00107] >gi|4885549|ref|NP_005456.1| RAC-gamma serine/threonine-protein kinase isoform 1 [Homo sapiens]

MSDVTIVKEGWVQKRGEYIKNWRPRYFLLKTDGSFIGYKEKPQDVDLPYPLNNFSV
AKCQLMKTERPKPNTFIIRCLQWTTVIERTFHVDTPEEREEWTEAIQAVADRLQRQE
EERMNCSPTSQIDNIGEEEMDASTTHHKRKTMNDFDYLKLLGKGTFGKVILVREKA
SGKYYAMKILKKEVIIAKDEVAHTLTESRVLKNTRHPFLTSLKYSFQTKDRLCFVME

YVNGGELFFHLSRERVFSEDRTRFYGAEIVSALDYLHSGKIVYRDLKLENLMLDKD
GHIKITDFGLCKEGITDAATMKTFCGTPEYLAPEVLEDNDYGRAVDWWGLGVVMY
EMMCGRLPFYNQDHEKLFELILMEDIKFPRTLSSDAKSLLSGLLIKDPNKRLGGGPD
DAKEIMRHSFFSGVNWQDVYDKKLVPPFKPQVTSETDTRYFDEEFTAQTITITPPEK
YDEDGMDCMDNERRPHFPQFSYSASGRE (SEQ ID NO: 6).

[00108] >gi|32307163|ref|NP_859029.1| RAC-gamma serine/threonine-protein kinase isoform 2 [Homo sapiens]
MSDVTIVKEGWVQKRGEYIKNWRPRYFLLKTDGSFIGYKEKPQDVDLPYPLNNFSV
AKCQLMKTERPKPNTFIIRCLQWTTVIERTFHVDTPEEREEWTEAIQAVADRLQRQE
EERMNCSPTSQIDNIGEEEMDASTTHHKRKTMNDFDYLKLLGKGTFGKVILVREKA
SGKYYAMKILKKEVIIAKDEVAHTLTESRVLKNTRHPFLTSLKYSFQTKDRLCFVME
YVNGGELFFHLSRERVFSEDRTRFYGAEIVSALDYLHSGKIVYRDLKLENLMLDKD
GHIKITDFGLCKEGITDAATMKTFCGTPEYLAPEVLEDNDYGRAVDWWGLGVVMY
EMMCGRLPFYNQDHEKLFELILMEDIKFPRTLSSDAKSLLSGLLIKDPNKRLGGGPD
DAKEIMRHSFFSGVNWQDVYDKKLVPPFKPQVTSETDTRYFDEEFTAQTITITPPEKC
QQSDCGMLGNWKK (SEQ ID NO: 7).

[00109] MAPKAP2 is a member of the serine/threonine protein kinase family. MAPKAP2 is regulated through direct phosphorylation by p38 MAP kinase. In conjunction with p38 MAP kinase, MAPKAP2 has been reported to be involved in many cellular processes including stress and inflammatory responses, nuclear export, gene expression regulation and cell proliferation. Two transcript variants encoding two different isoforms have been reported. Representative sequences are given below:

[00110] >gi|32481207|ref|NM_004759.3| Homo sapiens mitogen-activated protein kinase-activated protein kinase 2 (MAPKAPK2), transcript variant 1, mRNA
GCGGCCGCTTCCCCCCGGCCGGGCCCCCGCCGCCCCGCGGTCCCCAGAGCGCCA
GGCCCCCGGGGGGAGGGAGGGAGGGCGCCGGGCCGGTGGGAGCCAGCGGCGCG
CGGTGGGACCCACGGAGCCCCGCGACCCGCCGAGCCTGGAGCCGGGCCGGGTC
GGGGAAGCCGGCTCCAGCCCGGAGCGAACTTCGCAGCCCGTCGGGGGGCGGCG
GGGAGGGGGCCCGGAGCCGGAGGAGGGGGCGGCCGCGGGCACCCCCGCCTGTG
CCCCGGCGTCCCCGGGCACCATGCTGTCCAACTCCCAGGGCCAGAGCCCGCCGG
TGCCGTTCCCCGCCCCGGCCCCGCCGCCGCAGCCCCCCACCCCTGCCCTGCCGCA
CCCCCCGGCGCAGCCGCCGCCGCCGCCCCCGCAGCAGTTCCCGCAGTTCCACGT
CAAGTCCGGCCTGCAGATCAAGAAGAACGCCATCATCGATGACTACAAGGTCAC
CAGCCAGGTCCTGGGGCTGGGCATCAACGGCAAAGTTTTGCAGATCTTCAACAA

GAGGACCCAGGAGAAATTCGCCCTCAAAATGCTTCAGGACTGCCCCAAGGCCCG
CAGGGAGGTGGAGCTGCACTGGCGGGCCTCCCAGTGCCCGCACATCGTACGGAT
CGTGGATGTGTACGAGAATCTGTACGCAGGGAGGAAGTGCCTGCTGATTGTCAT
GGAATGTTTGGACGGTGGAGAACTCTTTAGCCGAATCCAGGATCGAGGAGACCA
GGCATTCACAGAAAGAGAAGCATCCGAAATCATGAAGAGCATCGGTGAGGCCA
TCCAGTATCTGCATTCAATCAACATTGCCCATCGGGATGTCAAGCCTGAGAATCT
CTTATACACCTCCAAAAGGCCCAACGCCATCCTGAAACTCACTGACTTTGGCTTT
GCCAAGGAAACCACCAGCCACAACTCTTTGACCACTCCTTGTTATACACCGTACT
ATGTGGCTCCAGAAGTGCTGGGTCCAGAGAAGTATGACAAGTCCTGTGACATGT
GGTCCCTGGGTGTCATCATGTACATCCTGCTGTGTGGGTATCCCCCCTTCTACTC
CAACCACGGCCTTGCCATCTCTCCGGGCATGAAGACTCGCATCCGAATGGGCCA
GTATGAATTTCCCAACCCAGAATGGTCAGAAGTATCAGAGGAAGTGAAGATGCT
CATTCGGAATCTGCTGAAAACAGAGCCCACCCAGAGAATGACCATCACCGAGTT
TATGAACCACCCTTGGATCATGCAATCAACAAAGGTCCCTCAAACCCCACTGCA
CACCAGCCGGGTCCTGAAGGAGGACAAGGAGCGGTGGGAGGATGTCAAGGGGT
GTCTTCATGACAAGAACAGCGACCAGGCCACTTGGCTGACCAGGTTGTGAGCAG
AGGATTCTGTGTTCCTGTCCAAACTCAGTGCTGTTTCTTAGAATCCTTTTATTCCC
TGGGTCTCTAATGGGACCTTAAAGACCATCTGGTATCATCTTCTCATTTTGCAGA
AGAGAAACTGAGGCCCAGAGGCGGAGGGCAGTCTGCTCAAGGTCACGCAGCTG
GTGACTGGTTGGGGCAGACCGGACCCAGGTTTCCTGACTCCTGGCCCAAGTCTCT
TCCTCCTATCCTGCGGGATCACTGGGGGGCTCTCAGGGAACAGCAGCAGTGCCA
TAGCCAGGCTCTCTGCTGCCCAGCGCTGGGGTGAGGCTGCCGTTGTCAGCGTGG
ACCACTAACCAGCCCGTCTTCTCTCTCTGCTCCCACCCCTGCCGCCCTCACCCTG
CCCTTGTTGTCTCTGTCTCTCACGTCTCTCTTCTGCTGTCTCTCCTACCTGTCTTCT
GGCTCTCTCTGTACCCTTCCTGGTGCTGCCGTGCCCCCAGGAGGAGATGACCAGT
GCCTTGGCCACAATGCGCGTTGACTACGAGCAGATCAAGATAAAAAAGATTGAA
GATGCATCCAACCCTCTGCTGCTGAAGAGGCGGAAGAAAGCTCGGGCCCTGGAG
GCTGCGGCTCTGGCCCACTGAGCCACCGCGCCCTCCTGCCCACGGGAGGACAAG
CAATAACTCTCTACAGGAATATATTTTTTAAACGAAGAGACAGAACTGTCCACA
TCTGCCTCCTCTCCTCCTCAGCTGCATGGAGCCTGGAACTGCATCAGTGACTGAA
TTCTGCCTTGGTTCTGGCCACCCCAGAGTGGGAGAGGCTGGGAGGTTGGGAGGC
TGTGGAGAGAAGTGAGCAAGGTGCTCTTGAACCTGTGCTCATTTTGCAATTTTAT
CAGTAATTTGACTTAGAGTTTTTACGAAACCTCTTTTGTTGTCCTTGCCCCACTCC
TCTCCACCAGACGCCTTCCTCTCTGGATACTGCAAAGGCTTGTGGTTTGTTAGAG

GGTATTTGTGGAAACTGTCATAGGGATTGTCCCTGTGTTGTCCCATCTGCCCTCC
CTGTTTCTCCACAACAGCCTGGGGTTGTCCCCGCTGGCTCACGCGTTCTGGGAGC
TCAAGGCCACCTTGGAGGAGGATGCCACGCACTTCCTCTCTCGGAGCCCTCAGA
CATCTCCAGTGTGCCAGACAAATAGGAGTGAGTGTATGTGTGTGTGTGTGTGTGT
GTGTGTGCACACGTGTGTATGAGTGCGCAGATCTGTGCCTGGGATCGTGCATTTG
AGGGGCCAGGGGCAGGCAGGGCTGCAGAGGGAGACGGCCCTGCTGGGGCTTAG
GAACCTTCTCCCTTCTTGGGTCTGCCCTGCCCATACTGAGCCTGCCAAAGTGCCT
GGGAAGCCCACCCAGATTCTGAAACAGGCCCTCTGTGGCCTGTCTCTATTAGCTG
GGTTCCGGGAGGCAGAGAGGAGTGACCGGGCACTGGCACTGCGATCAGGAAGA
CTGGACCCCCAGCCCCCAGGGCCCCCCTCCCCCCACTTAGTGCTGGTCCTAGGTC
CTCTGAGGCACTCATCTACTGAATGACCTCTCTACTTCCCCTTCTTGCCATTATTA
ACCCATTTTTGTTTATTTTCCTTAAATTTTTAGCCATTTCTCCATGGGCCACCGCC
CAGCTCATGTAGGTGAGCCTGGGCAGCTTCTGTTGGCAGAGCTTTTGCATTTCCT
GTGTTTGTCCTGGGTTCTGGGGCATCAGCCAGCTACCCCTTGTGGGCAAAGGCA
GGGCCACTTTTGAAGTCTTCCCTCAGATTTCCATTGTGTGGCCTGGTGGGTCAGG
GGGAGTCTTTGCACCAAAGATGTCCTGACTTTGCCCCCTTGCCCATCAGCCATTT
GCCATCACCCCAAACAACTCAGCTTCGGGGCCGGTGAGGGGAGGGGCCTCCCCC
AGCACAGATGAGGAGCAGCTGGGGTAGGCTGTCTGTGCCATGGCCCCCCACTCC
CCCTTCCCTTGGAGGGAGAGGTGGCAGGAATACTTCACCTTTCCTCTCCCTCAGG
GGCAGGTGGTGGAGGGGCGCCCAGGGTCGTCTTTGTGTATGGGGGAAGGCGCTG
GGTGCCTGCAGCGCCTCCCTTGTCTCAGATGGTGTGTCCAGCACTCGATTGTTGT
AAACTGTTGTTTTGTATGAGCGAAATTGTCTTTACTAAACAGATTTAATAGTTAA
AAAAAAAAAAAAAAAAA (SEQ ID NO: 8).

[00111] >gi|32481208|ref|NM_032960.2| Homo sapiens mitogen-activated protein kinase-activated protein kinase 2 (MAPKAPK2), transcript variant 2, mRNA

GCGGCCGCTTCCCCCCGGCCGGGCCCCCGCCGCCCCGCGGTCCCCAGAGCGCCA
GGCCCCCGGGGGGAGGGAGGGAGGGCGCCGGGCCGGTGGGAGCCAGCGGCGCG
CGGTGGGACCCACGGAGCCCCGCGACCCGCCGAGCCTGGAGCCGGGCCGGGTC
GGGGAAGCCGGCTCCAGCCCGGAGCGAACTTCGCAGCCCGTCGGGGGGCGGCG
GGGAGGGGGCCCGGAGCCGGAGGAGGGGGCGGCCGCGGGCACCCCCGCCTGTG
CCCCGGCGTCCCCGGGCACCATGCTGTCCAACTCCCAGGGCCAGAGCCCGCCGG
TGCCGTTCCCCGCCCCGGCCCCGCCGCCGCAGCCCCCCACCCCTGCCCTGCCGCA
CCCCCCGGCGCAGCCGCCGCCGCCCCCGCAGCAGTTCCCGCAGTTCCACGT
CAAGTCCGGCCTGCAGATCAAGAAGAACGCCATCATCGATGACTACAAGGTCAC

CAGCCAGGTCCTGGGGCTGGGCATCAACGGCAAAGTTTTGCAGATCTTCAACAA
GAGGACCCAGGAGAAATTCGCCCTCAAAATGCTTCAGGACTGCCCCAAGGCCCG
CAGGGAGGTGGAGCTGCACTGGCGGGCCTCCCAGTGCCCGCACATCGTACGGAT
CGTGGATGTGTACGAGAATCTGTACGCAGGGAGGAAGTGCCTGCTGATTGTCAT
GGAATGTTTGGACGGTGGAGAACTCTTTAGCCGAATCCAGGATCGAGGAGACCA
GGCATTCACAGAAAGAGAAGCATCCGAAATCATGAAGAGCATCGGTGAGGCCA
TCCAGTATCTGCATTCAATCAACATTGCCCATCGGGATGTCAAGCCTGAGAATCT
CTTATACACCTCCAAAAGGCCCAACGCCATCCTGAAACTCACTGACTTTGGCTTT
GCCAAGGAAACCACCAGCCACAACTCTTTGACCACTCCTTGTTATACACCGTACT
ATGTGGCTCCAGAAGTGCTGGGTCCAGAGAAGTATGACAAGTCCTGTGACATGT
GGTCCCTGGGTGTCATCATGTACATTCTGCTGTGTGGGTATCCCCCCTTCTACTCC
AACCACGGCCTTGCCATCTCTCCGGGCATGAAGACTCGCATCCGAATGGGCCAG
TATGAATTTCCCAACCCAGAATGGTCAGAAGTATCAGAGGAAGTGAAGATGCTC
ATTCGGAATCTGCTGAAAACAGAGCCCACCCAGAGAATGACCATCACCGAGTTT
ATGAACCACCCTTGGATCATGCAATCAACAAAGGTCCCTCAAACCCCACTGCAC
ACCAGCCGGGTCCTGAAGGAGGACAAGGAGCGGTGGGAGGATGTCAAGGAGGA
GATGACCAGTGCCTTGGCCACAATGCGCGTTGACTACGAGCAGATCAAGATAAA
AAAGATTGAAGATGCATCCAACCCTCTGCTGCTGAAGAGGCGGAAGAAAGCTCG
GGCCCTGGAGGCTGCGGCTCTGGCCCACTGAGCCACCGCGCCCTCCTGCCCACG
GGAGGACAAGCAATAACTCTCTACAGGAATATATTTTTTAAACGAAGAGACAGA
ACTGTCCACATCTGCCTCCTCTCCTCCTCAGCTGCATGGAGCCTGGAACTGCATC
AGTGACTGAATTCTGCCTTGGTTCTGGCCACCCCAGAGTGGGAGAGGCTGGGAG
GTTGGGAGGCTGTGGAGAGAAGTGAGCAAGGTGCTCTTGAACCTGTGCTCATTT
TGCAATTTTATCAGTAATTTGACTTAGAGTTTTTACGAAACCTCTTTTGTTGTCCT
TGCCCCACTCCTCTCCACCAGACGCCTTCCTCTCTGGATACTGCAAAGGCTTGTG
GTTTGTTAGAGGGTATTTGTGGAAACTGTCATAGGGATTGTCCCTGTGTTGTCCC
ATCTGCCCTCCCTGTTTCTCCACAACAGCCTGGGGTTGTCCCCGCTGGCTCACGC
GTTCTGGGAGCTCAAGGCCACCTTGGAGGAGGATGCCACGCACTTCCTCTCTCG
GAGCCCTCAGACATCTCCAGTGTGCCAGACAAATAGGAGTGAGTGTATGTGTGT
GTGTGTGTGTGTGTGTGTGCACACGTGTGTATGAGTGCGCAGATCTGTGCCTGGG
ATCGTGCATTTGAGGGGCCAGGGGCAGGCAGGGCTGCAGAGGGAGACGGCCCT
GCTGGGGCTTAGGAACCTTCTCCCTTCTTGGGTCTGCCCTGCCCATACTGAGCCT
GCCAAAGTGCCTGGGAAGCCCACCCAGATTCTGAAACAGGCCCTCTGTGGCCTG
TCTCTATTAGCTGGGTTCCGGGAGGCAGAGAGGAGTGACCGGGCACTGGCACTG

CGATCAGGAAGACTGGACCCCCAGCCCCCAGGGCCCCCCTCCCCCCCACTTAGTG
CTGGTCCTAGGTCCTCTGAGGCACTCATCTACTGAATGACCTCTCTACTTCCCCTT
CTTGCCATTATTAACCCATTTTTGTTTATTTTCCTTAAATTTTTAGCCATTTCTCCA
TGGGCCACCGCCCAGCTCATGTAGGTGAGCCTGGGCAGCTTCTGTTGGCAGAGC
TTTTGCATTTCCTGTGTTTGTCCTGGGTTCTGGGGCATCAGCCAGCTACCCCTTGT
GGGCAAAGGCAGGGCCACTTTTGAAGTCTTCCCTCAGATTTCCATTGTGTGGCCT
GGTGGGTCAGGGGGAGTCTTTGCACCAAAGATGTCCTGACTTTGCCCCCTTGCCC
ATCAGCCATTTGCCATCACCCCAAACAACTCAGCTTCGGGGCCGGTGAGGGGAG
GGGCCTCCCCCAGCACAGATGAGGAGCAGCTGGGGTAGGCTGTCTGTGCCATGG
CCCCCCACTCCCCCTTCCCTTGGAGGGAGAGGTGGCAGGAATACTTCACCTTTCC
TCTCCCTCAGGGGCAGGTGGTGGAGGGGCGCCCAGGGTCGTCTTTGTGTATGGG
GGAAGGCGCTGGGTGCCTGCAGCGCCTCCCTTGTCTCAGATGGTGTGTCCAGCA
CTCGATTGTTGTAAACTGTTGTTTTGTATGAGCGAAATTGTCTTTACTAAACAGA
TTTAATAGTTAAAAAAAAAAAAAAAAAA (SEQ ID NO: 9).

[00112] >gi|10863901|ref|NP_004750.1| MAP kinase-activated protein kinase 2 isoform 1 [Homo sapiens]
MLSNSQGQSPPVPFPAPAPPPQPPTPALPHPPAQPPPPPPQQFPQFHVKSGLQIKKNAII
DDYKVTSQVLGLGINGKVLQIFNKRTQEKFALKMLQDCPKARREVELHWRASQCP
HIVRIVDVYENLYAGRKCLLIVMECLDGGELFSRIQDRGDQAFTEREASEIMKSIGEA
IQYLHSINIAHRDVKPENLLYTSKRPNAILKLTDFGFAKETTSHNSLTTPCYTPYYVA
PEVLGPEKYDKSCDMWSLGVIMYILLCGYPPFYSNHGLAISPGMKTRIRMGQYEFPN
PEWSEVSEEVKMLIRNLLKTEPTQRMTITEFMNHPWIMQSTKVPQTPLHTSRVLKED
KERWEDVKGCLHDKNSDQATWLTRL (SEQ ID NO: 10).

[00113] >gi|32481209|ref|NP_116584.2| MAP kinase-activated protein kinase 2 isoform 2 [Homo sapiens]
MLSNSQGQSPPVPFPAPAPPPQPPTPALPHPPAQPPPPPPQQFPQFHVKSGLQIKKNAII
DDYKVTSQVLGLGINGKVLQIFNKRTQEKFALKMLQDCPKARREVELHWRASQCP
HIVRIVDVYENLYAGRKCLLIVMECLDGGELFSRIQDRGDQAFTEREASEIMKSIGEA
IQYLHSINIAHRDVKPENLLYTSKRPNAILKLTDFGFAKETTSHNSLTTPCYTPYYVA
PEVLGPEKYDKSCDMWSLGVIMYILLCGYPPFYSNHGLAISPGMKTRIRMGQYEFPN
PEWSEVSEEVKMLIRNLLKTEPTQRMTITEFMNHPWIMQSTKVPQTPLHTSRVLKED
KERWEDVKEEMTSALATMRVDYEQIKIKKIEDASNPLLLKRRKKARALEAAALAH
(SEQ ID NO: 11).

[00114] Pim1 is a proto-oncogenic kinase that is believed to be regulated by the

Jak/Stat pathway. Representative Pim1 sequences are given below:

[00115] >gi|208431772|ref|NM_002648.3| Homo sapiens pim-1 oncogene (PIM1), mRNA

CCCTTTACTCCTGGCTGCGGGGCGAGCCGGGCGTCTGCTGCAGCGGCCGCGGTGG
CTGAGGAGGCCCGAGAGGAGTCGGTGGCAGCGGCGGCGGCGGGACCGGCAGCA
GCAGCAGCAGCAGCAGCAGCAGCAACCACTAGCCTCCTGCCCCGCGGCGCTGCC
GCACGAGCCCCACGAGCCGCTCACCCCGCCGTTCTCAGCGCTGCCCGACCCCGCT
GGCGCGCCCTCCCGCCGCCAGTCCCGGCAGCGCCCTCAGTTGTCCTCCGACTCGC
CCTCGGCCTTCCGCGCCAGCCGCAGCCACAGCCGCAACGCCACCCGCAGCCACA
GCCACAGCCACAGCCCCAGGCATAGCCTTCGGCACAGCCCCGGCTCCGGCTCCT
GCGGCAGCTCCTCTGGGCACCGTCCCTGCGCCGACATCCTGGAGGTTGGGATGCT
CTTGTCCAAAATCAACTCGCTTGCCCACCTGCGCGCCGCGCCCTGCAACGACCTG
CACGCCACCAAGCTGGCGCCCGGCAAGGAGAAGGAGCCCCTGGAGTCGCAGTAC
CAGGTGGGCCCGCTACTGGGCAGCGGCGGCTTCGGCTCGGTCTACTCAGGCATCC
GCGTCTCCGACAACTTGCCGGTGGCCATCAAACACGTGGAGAAGGACCGGATTT
CCGACTGGGGAGAGCTGCCTAATGGCACTCGAGTGCCCATGGAAGTGGTCCTGC
TGAAGAAGGTGAGCTCGGGTTTCTCCGGCGTCATTAGGCTCCTGGACTGGTTCGA
GAGGCCCGACAGTTTCGTCCTGATCCTGGAGAGGCCCGAGCCGGTGCAAGATCT
CTTCGACTTCATCACGGAAAGGGGAGCCCTGCAAGAGGAGCTGGCCCGCAGCTT
CTTCTGGCAGGTGCTGGAGGCCGTGCGGCACTGCCACAACTGCGGGGTGCTCCAC
CGCGACATCAAGGACGAAAACATCCTTATCGACCTCAATCGCGGCGAGCTCAAG
CTCATCGACTTCGGGTCGGGGGCGCTGCTCAAGGACACCGTCTACACGGACTTCG
ATGGGACCCGAGTGTATAGCCCTCCAGAGTGGATCCGCTACCATCGCTACCATGG
CAGGTCGGCGGCAGTCTGGTCCCTGGGGATCCTGCTGTATGATATGGTGTGTGGA
GATATTCCTTTCGAGCATGACGAAGAGATCATCAGGGGCCAGGTTTTCTTCAGGC
AGAGGGTCTCTTCAGAATGTCAGCATCTCATTAGATGGTGCTTGGCCCTGAGACC
ATCAGATAGGCCAACCTTCGAAGAAATCCAGAACCATCCATGGATGCAAGATGT
TCTCCTGCCCCAGGAAACTGCTGAGATCCACCTCCACAGCCTGTCGCCGGGGCCC
AGCAAATAGCAGCCTTTCTGGCAGGTCCTCCCCTCTCTTGTCAGATGCCCGAGGG
AGGGGAAGCTTCTGTCTCCAGCTTCCCGAGTACCAGTGACACGTCTCGCCAAGCA
GGACAGTGCTTGATACAGGAACAACATTTACAACTCATTCCAGATCCCAGGCCCC
TGGAGGCTGCCTCCCAACAGTGGGGAAGAGTGACTCTCCAGGGGTCCTAGGCCT
CAACTCCTCCCATAGATACTCTCTTCTTCTCATAGGTGTCCAGCATTGCTGGACTC
TGAAATATCCCGGGGGTGGGGGGTGGGGGTGGGTCAGAACCCTGCCATGGAACT

GTTTCCTTCATCATGAGTTCTGCTGAATGCCGCGATGGGTCAGGTAGGGGGGAAA
CAGGTTGGGATGGGATAGGACTAGCACCATTTTAAGTCCCTGTCACCTCTTCCGA
CTCTTTCTGAGTGCCTTCTGTGGGGACTCCGGCTGTGCTGGGAGAAATACTTGAA
CTTGCCTCTTTTACCTGCTGCTTCTCCAAAAATCTGCCTGGGTTTTGTTCCCTATTT
TTCTCTCCTGTCCTCCCTCACCCCCTCCTTCATATGAAAGGTGCCATGGAAGAGGC
TACAGGGCCAAACGCTGAGCCACCTGCCCTTTTTTCTGCCTCCTTTAGTAAAACTC
CGAGTGAACTGGTCTTCCTTTTTGGTTTTTACTTAACTGTTTCAAAGCCAAGACCT
CACACACACAAAAAATGCACAAACAATGCAATCAACAGAAAAGCTGTAAATGTG
TGTACAGTTGGCATGGTAGTATACAAAAAGATTGTAGTGGATCTAATTTTTAAGA
AATTTTGCCTTTAAGTTATTTTACCTGTTTTTGTTTCTTGTTTTGAAAGATGCGCAT
TCTAACCTGGAGGTCAATGTTATGTATTTATTTATTTATTTATTTGGTTCCCTTCCT
ATTCCAAGCTTCCATAGCTGCTGCCCTAGTTTTCTTTCCTCCTTTCCTCCTCTGACT
TGGGGACCTTTTGGGGGAGGGCTGCGACGCTTGCTCTGTTTGTGGGGTGACGGGA
CTCAGGCGGGACAGTGCTGCAGCTCCCTGGCTTCTGTGGGGCCCCTCACCTACTT
ACCCAGGTGGGTCCCGGCTCTGTGGGTGATGGGGAGGGGCATTGCTGACTGTGT
ATATAGGATAATTATGAAAAGCAGTTCTGGATGGTGTGCCTTCCAGATCCTCTCT
GGGGCTGTGTTTTGAGCAGCAGGTAGCCTGCTGGTTTTATCTGAGTGAAATACTG
TACAGGGGAATAAAAGAGATCTTATTTTTTTTTTTATACTTGGCGTTTTTTGAATA
AAAACCTTTTGTCTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAA (SEQ ID NO: 12).

[00116] >gi|4505811|ref|NP_002639.1| proto-oncogene serine/threonine-protein kinase pim-1 [Homo sapiens]
MLLSKINSLAHLRAAPCNDLHATKLAPGKEKEPLESQYQVGPLLGSGGFGSVYSGIR
VSDNLPVAIKHVEKDRISDWGELPNGTRVPMEVVLLKKVSSGFSGVIRLLDWFERPD
SFVLILERPEPVQDLFDFITERGALQEELARSFFWQVLEAVRHCHNCGVLHRDIKDEN
ILIDLNRGELKLIDFGSGALLKDTVYTDFDGTRVYSPPEWIRYHRYHGRSAAVWSLGI
LLYDMVCGDIPFEHDEEIIRGQVFFRQRVSSECQHLIRWCLALRPSDRPTFEEIQNHPW
MQDVLLPQETAEIHLHSLSPGPSK (SEQ ID NO: 13).

[00117] Without wishing to be bound by any particular theory, Pim1 expression has been reported to be increased in certain prostate cancers where it may lead to genomic instability (*e.g.*, Roh M, Gary B, Song C, Said-Al-Naief N, Tousson A, Kraft A, Eltoum IE, Abdulkadir SA, *Overexpression of the oncogenic kinase Pim-1 leads to genomic instability. Cancer Res.* 2003 Dec 1;63(23):8079-84; incorporated herein by reference) and reduce androgen receptor-dependent transcription (*e.g.*, Thompson J, Peltola KJ, Koskinen PJ, Jänne

OA, Palvimo JJ. *Attenuation of androgen receptor-dependent transcription by the serine/threonine kinase Pim-1. Lab Invest.* 2003 Sep;83(9):1301-9; incorporated herein by reference). Pim1 expression level has been reported to carry prognostic value. Increased Pim1 activity or expression has also been observed in hematopoietic malignancies and in diffuse large-cell lymphomas. T cell lymphomas have been reported in Pim-1 overexpressing mice and can be induced by viral insertions at the Pim-1 locus.

[00118] VEGFR2 is a tyrosine kinase, also known as kinase insert domain receptor (KDR), a type III receptor tyrosine kinase or CD309 (cluster of differentiation 309). Aberrant VEGFR2 activity is implicated in numerous diseases (*e.g.*, Holmes K, Roberts OL, Thomas AM, Cross MJ. *Vascular endothelial growth factor receptor-2: structure, function, intracellular signaling and therapeutic inhibition. Cell Signal.* 2007 Oct;19(10):2003-12; incorporated herein by reference). The activation of VEGFR2 is envisioned as a clinically useful means to heal, for example, bone and tissue. Further, the activation of VEGFR2 is expected to be a useful research tool to study, for example, the effects of VEGFR2 upregulation in the cell (*e.g.*, to better characterize the molecular details of cancer). Representative sequences of VEGFR2 are given below.

[00119] >gi|195546779|ref|NM_002253.2| Homo sapiens kinase insert domain receptor (a type III receptor tyrosine kinase) (KDR), mRNA

ACTGAGTCCCGGGACCCCGGGAGAGCGGTCAATGTGTGGTCGCTGCGTTTCCTCT
GCCTGCGCCGGGCATCACTTGCGCGCCGCAGAAAGTCCGTCTGGCAGCCTGGAT
ATCCTCTCCTACCGGCACCCGCAGACGCCCCTGCAGCCGCGGTCGGCGCCCGGGC
TCCCTAGCCCTGTGCGCTCAACTGTCCTGCGCTGCGGGGTGCCGCGAGTTCCACC
TCCGCGCCTCCTTCTCTAGACAGGCGCTGGGAGAAAGAACCGGCTCCCGAGTTCT
GGGCATTTCGCCCGGCTCGAGGTGCAGGATGCAGAGCAAGGTGCTGCTGGCCGT
CGCCCTGTGGCTCTGCGTGGAGACCCGGGCCGCCTCTGTGGGTTTGCCTAGTGTT
TCTCTTGATCTGCCCAGGCTCAGCATACAAAAAGACATACTTACAATTAAGGCTA
ATACAACTCTTCAAATTACTTGCAGGGGACAGAGGGACTTGGACTGGCTTTGGCC
CAATAATCAGAGTGGCAGTGAGCAAAGGGTGGAGGTGACTGAGTGCAGCGATGG
CCTCTTCTGTAAGACACTCACAATTCCAAAAGTGATCGGAAATGACACTGGAGCC
TACAAGTGCTTCTACCGGGAAACTGACTTGGCCTCGGTCATTTATGTCTATGTTCA
AGATTACAGATCTCCATTTATTGCTTCTGTTAGTGACCAACATGGAGTCGTGTAC
ATTACTGAGAACAAAAACAAAACTGTGGTGATTCCATGTCTCGGGTCCATTTCAA
ATCTCAACGTGTCACTTTGTGCAAGATACCCAGAAAAGAGATTTGTTCCTGATGG
TAACAGAATTTCCTGGGACAGCAAGAAGGGCTTTACTATTCCCAGCTACATGATC

AGCTATGCTGGCATGGTCTTCTGTGAAGCAAAAATTAATGATGAAAGTTACCAGT
CTATTATGTACATAGTTGTCGTTGTAGGGTATAGGATTTATGATGTGGTTCTGAGT
CCGTCTCATGGAATTGAACTATCTGTTGGAGAAAAGCTTGTCTTAAATTGTACAG
CAAGAACTGAACTAAATGTGGGGATTGACTTCAACTGGGAATACCCTTCTTCGAA
GCATCAGCATAAGAAACTTGTAAACCGAGACCTAAAAACCCAGTCTGGGAGTGA
GATGAAGAAATTTTTGAGCACCTTAACTATAGATGGTGTAACCCGGAGTGACCA
AGGATTGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAGAAGAACAGCAC
ATTTGTCAGGGTCCATGAAAAACCTTTTGTTGCTTTTGGAAGTGGCATGGAATCT
CTGGTGGAAGCCACGGTGGGGGAGCGTGTCAGAATCCCTGCGAAGTACCTTGGT
TACCCACCCCCAGAAATAAAATGGTATAAAAATGGAATACCCCTTGAGTCCAAT
CACACAATTAAAGCGGGGCATGTACTGACGATTATGGAAGTGAGTGAAAGAGAC
ACAGGAAATTACACTGTCATCCTTACCAATCCCATTTCAAAGGAGAAGCAGAGC
CATGTGGTCTCTCTGGTTGTGTATGTCCCACCCCAGATTGGTGAGAAATCTCTAAT
CTCTCCTGTGGATTCCTACCAGTACGGCACCACTCAAACGCTGACATGTACGGTC
TATGCCATTCCTCCCCCGCATCACATCCACTGGTATTGGCAGTTGGAGGAAGAGT
GCGCCAACGAGCCCAGCCAAGCTGTCTCAGTGACAAACCCATACCCTTGTGAAG
AATGGAGAAGTGTGGAGGACTTCCAGGGAGGAAATAAAATTGAAGTTAATAAAA
ATCAATTTGCTCTAATTGAAGGAAAAAACAAAACTGTAAGTACCCTTGTTATCCA
AGCGGCAAATGTGTCAGCTTTGTACAAATGTGAAGCGGTCAACAAAGTCGGGAG
AGGAGAGAGGGTGATCTCCTTCCACGTGACCAGGGGTCCTGAAATTACTTTGCAA
CCTGACATGCAGCCCACTGAGCAGGAGAGCGTGTCTTTGTGGTGCACTGCAGAC
AGATCTACGTTTGAGAACCTCACATGGTACAAGCTTGGCCCACAGCCTCTGCCAA
TCCATGTGGGAGAGTTGCCCACACCTGTTTGCAAGAACTTGGATACTCTTTGGAA
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AAGACCAAGAAAAGACATTGCGTGGTCAGGCAGCTCACAGTCCTAGAGCGTGTG
GCACCCACGATCACAGGAAACCTGGAGAATCAGACGACAAGTATTGGGGAAAGC
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GGAACCTCACTATCCGCAGAGTGAGGAAGGAGGACGAAGGCCTCTACACCTGCC
AGGCATGCAGTGTTCTTGGCTGTGCAAAAGTGGAGGCATTTTTCATAATAGAAGG
TGCCCAGGAAAAGACGAACTTGGAAATCATTATTCTAGTAGGCACGGCGGTGAT
TGCCATGTTCTTCTGGCTACTTCTTGTCATCATCCTACGGACCGTTAAGCGGGCCA
ATGGAGGGGAACTGAAGACAGGCTACTTGTCCATCGTCATGGATCCAGATGAAC

TCCCATTGGATGAACATTGTGAACGACTGCCTTATGATGCCAGCAAATGGGAATT
CCCCAGAGACCGGCTGAAGCTAGGTAAGCCTCTTGGCCGTGGTGCCTTTGGCCAA
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GCAGTCAAAATGTTGAAAGAAGGAGCAACACACAGTGAGCATCGAGCTCTCATG
TCTGAACTCAAGATCCTCATTCATATTGGTCACCATCTCAATGTGGTCAACCTTCT
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AAGACCAAAGGGGCACGATTCCGTCAAGGGAAAGACTACGTTGGAGCAATCCCT
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TCTGGATTTGTGGAGGAGAAGTCCCTCAGTGATGTAGAAGAAGAGGAAGCTCCT
GAAGATCTGTATAAGGACTTCCTGACCTTGGAGCATCTCATCTGTTACAGCTTCC
AAGTGGCTAAGGGCATGGAGTTCTTGGCATCGCGAAAGTGTATCCACAGGGACC
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TGGCTTGGCCCGGGATATTTATAAAGATCCAGATTATGTCAGAAAAGGAGATGCT
CGCCTCCCTTTGAAATGGATGGCCCCAGAAACAATTTTTGACAGAGTGTACACAA
TCCAGAGTGACGTCTGGTCTTTTGGTGTTTTGCTGTGGGAAATATTTTCCTTAGGT
GCTTCTCCATATCCTGGGGTAAAGATTGATGAAGAATTTTGTAGGCGATTGAAAG
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TGCTGGACTGCTGGCACGGGGAGCCCAGTCAGAGACCCACGTTTTCAGAGTTGGT
GGAACATTTGGGAAATCTCTTGCAAGCTAATGCTCAGCAGGATGGCAAAGACTA
CATTGTTCTTCCGATATCAGAGACTTTGAGCATGGAAGAGGATTCTGGACTCTCT
CTGCCTACCTCACCTGTTTCCTGTATGGAGGAGGAGGAAGTATGTGACCCCAAAT
TCCATTATGACAACACAGCAGGAATCAGTCAGTATCTGCAGAACAGTAAGCGAA
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AAGTAAAAGTAATCCCAGATGACAACCAGACGGACAGTGGTATGGTTCTTGCCT
CAGAAGAGCTGAAAACTTTGGAAGACAGAACCAAATTATCTCCATCTTTTGGTGG
AATGGTGCCCAGCAAAAGCAGGGAGTCTGTGGCATCTGAAGGCTCAAACCAGAC
AAGCGGCTACCAGTCCGGATATCACTCCGATGACACAGACACCACCGTGTACTCC
AGTGAGGAAGCAGAACTTTTAAAGCTGATAGAGATTGGAGTGCAAACCGGTAGC
ACAGCCCAGATTCTCCAGCCTGACTCGGGGACCACACTGAGCTCTCCTCCTGTTT
AAAAGGAAGCATCCACACCCCCAACTCCTGGACATCACATGAGAGGTGCTGCTC
AGATTTTCAAGTGTTGTTCTTTCCACCAGCAGGAAGTAGCCGCATTTGATTTTCAT
TTCGACAACAGAAAAAGGACCTCGGACTGCAGGGAGCCAGTCTTCTAGGCATAT
CCTGGAAGAGGCTTGTGACCCAAGAATGTGTCTGTGTCTTCTCCCAGTGTTGACC

TGATCCTCTTTTTCATTCATTTAAAAAGCATTTATCATGCCCCCTGCTGCGGGTCT
CACCATGGGTTTAGAACAAAGACGTTCAAGAAATGGCCCCATCCTCAAAGAAGT
AGCAGTACCTGGGGAGCTGACACTTCTGTAAAACTAGAAGATAAACCAGGCAAT
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AGAGGCTTTGTTTAGGACGTGGGTCCCAAGCCAAGCCTTAAGTGTGGAATTCGGA
TTGATAGAAAGGAAGACTAACGTTACCTTGCTTTGGAGAGTACTGGAGCCTGCA
AATGCATTGTGTTTGCTCTGGTGGAGGTGGGCATGGGGTCTGTTCTGAAATGTAA
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AAAGTAGAGTTCGTTGTGCTGTTTCTGACTCCTAATGAGAGTTCCTTCCAGACCG
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TCTCCCAGGCTGATCCTTTATTCAGAATACCACAAAGAAAGGACATTCAGCTCAA
GGCTCCCTGCCGTGTTGAAGAGTTCTGACTGCACAAACCAGCTTCTGGTTTCTTCT
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AGGTTCAATGTGAAGCTGTGTGTGGTGTCAAAGTTTCAGGAAGGATTTTACCCTT
TTGTTCTTCCCCCTGTCCCCAACCCACTCTCACCCCGCAACCCATCAGTATTTTAG
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TTTTCAAAAAAGAAAATGTGTTTTTTGTTTGGTACCATAGTGTGAAATGCTGGGA
ACAATGACTATAAGACATGCTATGGCACATATATTTATAGTCTGTTTATGTAGAA
ACAAATGTAATATATTAAAGCCTTATATATAATGAACTTTGTACTATTCACATTTT
GTATCAGTATTATGTAGCATAACAAAGGTCATAATGCTTTCAGCAATTGATGTCA
TTTTATTAAAGAACATTGAAAAACTTGAAGGAATCCCTTTGCAAGGTTGCATTAC
TGTACCCATCATTTCTAAAATGGAAGAGGGGGTGGCTGGGCACAGTGGCCGACA
CCTAAAAACCCAGCACTTTGGGGGGCCAAGGTGGGAGGATCGCTTGAGCCCAGG
AGTTCAAGACCAGTCTGGCCAACATGGTCAGATTCCATCTCAAAGAAAAAAGGT
AAAAATAAAATAAAATGGAGAAGAAGGAATCAGA (SEQ ID NO: 14).

[00120] >gi|11321597|ref|NP_002244.1| vascular endothelial growth factor receptor 2 precursor [Homo sapiens]

MQSKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQRD
LDWLWPNNQSGSEQRVEVTECSDGLFCKTLTIPKVIGNDTGAYKCFYRETDLASVIY
VYVQDYRSPFIASVSDQHGVVYITENKNKTVVIPCLGSISNLNVSLCARYPEKRFVPD
GNRISWDSKKGFTIPSYMISYAGMVFCEAKINDESYQSIMYIVVVVGYRIYDVVLSPS
HGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKK

FLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSGMESLVEATV
GERVRIPAKYLGYPPPEIKWYKNGIPLESNHTIKAGHVLTIMEVSERDTGNYTVILTNP
ISKEKQSHVVSLVVYVPPQIGEKSLISPVDSYQYGTTQTLTCTVYAIPPPHHIHWYWQ
LEEECANEPSQAVSVTNPYPCEEWRSVEDFQGGNKIEVNKNQFALIEGKNKTVSTLVI
QAANVSALYKCEAVNKVGRGERVISFHVTRGPEITLQPDMQPTEQESVSLWCTADRS
TFENLTWYKLGPQPLPIHVGELPTPVCKNLDTLWKLNATMFSNSTNDILIMELKNAS
LQDQGDYVCLAQDRKTKKRHCVVRQLTVLERVAPTITGNLENQTTSIGESIEVSCTA
SGNPPPQIMWFKDNETLVEDSGIVLKDGNRNLTIRRVRKEDEGLYTCQACSVLGCAK
VEAFFIIEGAQEKTNLEIIILVGTAVIAMFFWLLLVIILRTVKRANGGELKTGYLSIVMD
PDELPLDEHCERLPYDASKWEFPRDRLKLGKPLGRGAFGQVIEADAFGIDKTATCRT
VAVKMLKEGATHSEHRALMSELKILIHIGHHLNVVNLLGACTKPGGPLMVIVEFCKF
GNLSTYLRSKRNEFVPYKTKGARFRQGKDYVGAIPVDLKRRLDSITSSQSSASSGFVE
EKSLSDVEEEEAPEDLYKDFLTLEHLICYSFQVAKGMEFLASRKCIHRDLAARNILLS
EKNVVKICDFGLARDIYKDPDYVRKGDARLPLKWMAPETIFDRVYTIQSDVWSFGV
LLWEIFSLGASPYPGVKIDEEFCRRLKEGTRMRAPDYTTPEMYQTMLDCWHGEPSQR
PTFSELVEHLGNLLQANAQQDGKDYIVLPISETLSMEEDSGLSLPTSPVSCMEEEEVC
DPKFHYDNTAGISQYLQNSKRKSRPVSVKTFEDIPLEEPEVKVIPDDNQTDSGMVLAS
EELKTLEDRTKLSPSFGGMVPSKSRESVASEGSNQTSGYQSGYHSDDTDTTVYSSEE
AELLKLIEIGVQTGSTAQILQPDSGTTLSSPPV (SEQ ID NO: 15).

Compounds

[00121] Macrocyclic compounds that modulate kinase activity are described herein. In certain embodiments, compounds of the present invention include inhibitors of Src, Akt3, MAPKAP2, AMPK, ERBB4, MK2, p38α, MKK6, or Pim1, and activators of VEGFR2. In certain embodiments, the compounds have an IC_{50} of less than approximately 100 μM, *e.g.*, less than approximately 10 μM, *e.g.*, less than approximately 1 μM, *e.g.*, less than approximately 0.1 μM, or *e.g.*, less than approximately 0.01 μM. In some embodiments, the inventive compounds may be useful in the treatment of a variety of diseases as described in more detail elsewhere herein. In certain embodiments, the compounds are useful in the treatment of proliferative diseases, for example, in the treatment of certain cancers or benign neoplasms. In some embodiments, the inventive compounds are useful in the treatment of metabolic syndrome, diabetes and complications thereof, and insulin resistance. Certain compounds are also useful in treating neurological diseases, such as neurodegenerative diseases. In certain embodiments, the compounds are useful in treating autoimmune diseases

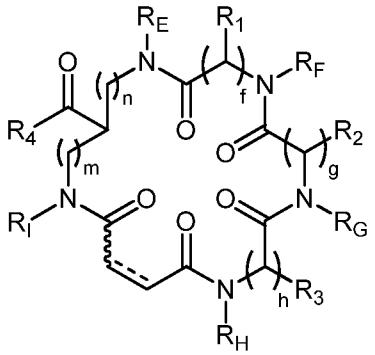
or inflammatory diseases.

[00122] Certain compounds provided herein are useful for the inhibition of kinases, for example, Src kinases, that comprise a gatekeeper mutation, for example, a src kinase T338I or T341I mutation, or a kinase having a homologous gatekeeper mutation. The targeted inhibition of protein tyrosine kinases is an effective therapeutic regimen for the treatment of various neoplastic and malignant diseases, for example, chronic myeloid leukemia (CML) and several solid tumors. Inhibitors employed for such therapies include, for example, GLEEVEC™, IRESSA™, TARCEVA™, and IMATINIB™. Some small-molecule kinase inhibitors exploit a conserved threonine residue within the ATP binding site of the targeted kinase for binding specificity, which controls access of the inhibitors to a hydrophobic pocket deep in the active site that is not contacted by ATP. Accordingly, this threonine residue is often referred to as a gatekeeper residue. Substitution of the gatekeeper threonine residue with an amino acid comprising a bulky side chains can lead to resistance to ATP-competitive kinase inhibitors. See Azam et al., Nat Struct Mol Biol. 2008 October; 15(10): 1109–1118, the entire contents of which are incorporated herein by reference. In some embodiments, a kinase inhibitor is provided that inhibits the activity of a kinase comprising a gatekeeper mutation, for example, of a src kinase comprising a T338I or T341I mutation. For example, macrocycle trans-A10-B1-C5-D6 inhibits both wild-type src kinase and src kinase comprising a gatekeeper mutation, e.g., a T338I or a T341I mutation. Further exemplary macrocycle src kinase inhibitors that inhibit both wild-type src kinase and src kinase comprising a gatekeeper residue mutation include, but are not limited to macrocycles A10-Phe-C5-D6, [4-Me-Phe]-Phe-C5-D6, [4-Cl-Phe]-Phe-C5-D6, [4-Br-Phe]-Phe-C5-D6, [4-CF_3-Phe]-Phe-C5-D6, [4-$CONH_2$-Phe]-Phe-C5-D6, [4-CN-Phe]-Phe-C5-D6, A10-Phe-Cha-D6, [4-Me-Phe]-Phe-Cha-D6, [4-Cl-Phe]-Phe-Cha-D6, [4-Br-Phe]-Phe-Cha-D6, [4-CF_3-Phe]-Phe-Cha-D6, [4-$CONH_2$-Phe]-Phe-Cha-D6, [4-CN-Phe]-Phe-Cha-D6, A10-[4-F-Phe]-Cha-D6, [4-Me-Phe]-[4-F-Phe]-Cha-D6, [4-Cl-Phe]-[4-F-Phe]-Cha-D6, [4-Br-Phe]-[4-F-Phe]-Cha-D6, [4-CF_3-Phe]-[4-F-Phe]-Cha-D6, [4-$CONH_2$-Phe]-[4-F-Phe]-Cha-D6, and [4-CN-Phe]-[4-F-Phe]-Cha-D6, where Phe is phenylalanine, Cha is cyclohexylalanine, 4-Me-Phe is 4-methyl-phenylalanine, 4-F-Phe is 4-fluoro-phenylalanine, etc. (A-B-C-D nomenclature as illustrated in e.g., **Figures 5 and 19**). In some embodiments, the exemplary macrocycle src kinase inhibitors that inhibit both wild-type src kinase and src kinase comprising a gatekeeper residue mutation are in trans-configuration.

[00123] In some embodiments, a macrocyclic kinase-modulating compound as described herein is provided, that comprises a tag. In some embodiments, the tag is a

fluorescent tag, for example, a fluorescent molecule, or moiety, that is conjugated, for example, covalently via a linker, to the macrocycle. In some embodiments, the fluorescent tag is a fluorescent protein tag, for example, a GFP tag, a YFP tag, an RFP tag, a BFP tag, or a tag comprising an enhanced fluorescent protein, such as eGFP. Other fluorescent proteins and protein tags are well known to those of skill in the art. In some embodiments, the tag is a cyane dye, or CyDye tag, for example, a Cy3 or C5 tag. In some embodiments, the tag is a fluorescein tag, for example, a fluorescein tag conjugated to the macrocycle structure via a linker as described in **Figure 21A**. Additional suitable fluorescent tags are known to those of skill in the art and the invention is not limited in this respect. In some embodiments, the tag comprises a binding agent. In some embodiments, the binding agent is an antibody or an antigen-binding antibody fragment, a nanobody, an ScFv, an aptamer, or an adnectin. Other binding agents are known to those of skill in the art and the invention is not limited in this respect. In some embodiments, the binding agent specifically binds an antigen, for example, an antigen immobilized on a solid surface, or a cellular antigen, e.g., a cell-surface antigen. In some embodiments, the tag comprising a binding agent specifically binds to a particular cell or cell type, for example, to a tumor cell. In some embodiments, the binding agent binds to a particular cell-surface antigen of a specific cell type, for example, a tumor cell type. In some embodiments, such binding-agent-tagged macrocycles target a specific site characterised by expressing the antigen bound by the binding agent, for example, after administration to a subject harboring such a target site, e.g., a solid tumor, such as a tumor expressing an aberrant level of src kinase, which allows for the creation of high concentrations of the macrocycle at the target site. Antigens useful for targeting specific cells, cell types, or tissues, for example, malignant cells, cell types, or tissues, are well known to those of skill in the art and the invention is not limited in this respect.

[00124] In some embodiments, a compound that binds and modulates the activity of a kinase, as provided herein, is of Formula (I):



(I)

or a pharmaceutically acceptable salt thereof;

or a pharmaceutically acceptable salt thereof;

wherein:

n is 0 or an integer between 1-4, inclusive;

m is 0 an integer between 1-4, inclusive;

f is an integer between 1-3, inclusive;

g is an integer between 1-3, inclusive;

h is an integer between 1-3, inclusive;

⚌ is a single or double C-C bond, wherein when ⚌ is a double C-C bond, then ∿ indicates that the adjacent C-C double bond is in a *cis* or *trans* configuration;

each instance of R_1 is independently hydrogen; halogen; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; acyl; $-OR_A$; $-N(R_A)_2$; $-SR_A$; =O; -CN; $-NO_2$; -SCN; $-SOR_A$; or $-SO_2R_A$; wherein each occurrence of R_A is independently hydrogen; a protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; acyl; substituted or unsubstituted aryl; or substituted or unsubstituted heteroaryl;

each instance of R_2 is independently hydrogen; halogen; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; acyl; $-OR_B$; $-N(R_B)_2$; $-SR_B$; =O; -CN; $-NO_2$; -SCN; $-SOR_B$; or $-SO_2R_B$; wherein each occurrence of R_B independently hydrogen; a protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; acyl; substituted or unsubstituted aryl; or substituted or unsubstituted heteroaryl;

each instance of R_3 is independently hydrogen; halogen; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; acyl; $-OR_C$; $-N(R_C)_2$; $-SR_C$; =O; -CN; $-NO_2$; -SCN; $-SOR_C$; or $-SO_2R_C$; wherein each occurrence of R_C is independently hydrogen; a protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; acyl; substituted or unsubstituted aryl; or substituted or unsubstituted heteroaryl;

R_4 is substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; $-N(R_D)_2$; $-OR_D$; or $-SR_D$; wherein each occurrence of R_D is independently independently hydrogen; a protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; acyl; substituted or unsubstituted aryl; or substituted or

unsubstituted heteroaryl; or two R_D groups are joined to form a substituted or unsubstituted heterocyclic group; optionally wherein R^4 further comprises a label, resin, or therapeutic agent attached thereto; and

each instance of R_E, R_F, R_G, R_H, and R_I is independently hydrogen; acyl; a nitrogen protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; substitute or unsubstituted hydroxyl; substituted or unsubstituted thiol; substituted or unsubstituted amino; or halogen; optionally wherein an R_1 group and R^F are joined to form a substituted or unsubstituted heterocyclic ring; an R_2 group and R^G are joined to form a substituted or unsubstituted heterocyclic ring; and/or an R_3 group and R^H are joined to form a substituted or unsubstituted heterocyclic ring.

Embodiments of R_1

[00125] As generally defined above, each instance of R_1 is independently hydrogen; halogen; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; acyl; $-OR_A$; $-N(R_A)_2$; $-SR_A$; =O; -CN; $-NO_2$; -SCN; $-SOR_A$; or $-SO_2R_A$; wherein each occurrence of R_A is independently hydrogen; a protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; acyl; substituted or unsubstituted aryl; or substituted or unsubstituted heteroaryl.

[00126] In certain embodiments, R_1 is hydrogen. In certain embodiments, R_1 is halogen, *i.e.*, -F, -Cl, -Br, or –I. In certain embodiments, R_1 is $-OR_A$. In certain embodiments, R_1 is $-N(R_A)_2$. In certain embodiments, R_1 is $-SR_A$. In certain embodiments, R_1 is =O. In certain embodiments, R_1 is –CN. In certain embodiments, R_1 is $-NO_2$. In certain embodiments, R_1 is –SCN. In certain embodiments, R_1 is $-SOR_A$. In certain embodiments, R_1 is $-SO_2R_A$.

[00127] In certain embodiments, R_1 is acyl. For example, in certain embodiments, R_1 is acyl selected from the group consisting of $-C(=O)R^{A5}$, $-C(=O)OR^{A5}$, $-C(=O)SR^{A5}$, $-C(=O)N(R^{A6})_2$, $-C(=NR^{A6})R^{A5}$, $-C(=NR^{A6})OR^{A5}$, $-C(=NR^{A6})SR^{A5}$, $-C(=NR^{A6})N(R^{A6})_2$, $-C(=S)R^{A5}$, $-C(=S)OR^{A5}$, $-C(=S)SR^{A5}$, and $-C(=S)N(R^{A6})_2$, wherein each occurrence of R^{A5} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and each occurrence of R^{A6} is independently hydrogen, substituted

or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or a nitrogen protecting group, or two R^{A6} groups are joined to form an substituted or unsubstituted heterocyclic ring.

[00128] In certain embodiments, R_1 is substituted or unsubstituted aryl, *e.g.*, phenyl, napthyl. In certain embodiments, R_1 is substituted or unsubstituted phenyl. In certain embodiments, R_1 is substituted phenyl, *e.g.*, a monosubstituted, disubstituted, or trisubstituted phenyl.

[00129] In certain embodiments, R_1 is substituted or unsubstituted heteroaryl, *e.g.*, a substituted or unsubstituted 5- to 6-membered heteroaryl. In certain embodiments, R_1 is a substituted or unsubstituted 5-membered heteroaryl, *e.g.*, pyrrolyl, furanyl, thiophenyl, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, triazolyl, oxadiazolyl, thiadiazolyl, or tetrazolyl. In certain embodiments, R_1 is a substituted or unsubstituted 6-membered heteroaryl, *e.g.*, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, or tetrazinyl.

[00130] In certain embodiments, R_1 is substituted or unsubstituted aliphatic. In certain embodiments, R_1 is a substituted or unsubstituted aliphatic group comprising 1 to 10 carbon atoms ("C_{1-10}aliphatic"). In certain embodiments, R_1 is substituted or unsubstituted aliphatic group comprising 1 to 8 carbon atoms ("C_{1-8}aliphatic"). In certain embodiments, R_1 is a substituted or unsubstituted aliphatic group comprising 1 to 6 carbon atoms ("C_{1-6}aliphatic"). In certain embodiments, R_1 is a substituted or unsubstituted aliphatic group comprising 1 to 4 carbon atoms ("C_{1-4}aliphatic"). In certain embodiments, R_1 is a substituted or unsubstituted aliphatic group comprising 1 to 3 carbon atoms ("C_{1-3}aliphatic"). In certain embodiments, R_1 is a substituted or unsubstituted aliphatic group comprising 1 carbon atom ("C_1aliphatic"). In certain embodiments, R_1 is a substituted or unsubstituted aliphatic group comprising 2 carbon atoms ("C_2aliphatic"). In certain embodiments, R_1 is a substituted or unsubstituted aliphatic group comprising 3 carbon atoms ("C_3aliphatic"). In any of the above embodiments, R_1 is an acyclic aliphatic group. In any of the above embodiments, R_1 is an acyclic and substituted aliphatic group. In any of the above embodiments, the aliphatic group is an alkyl, alkenyl, or alkynyl group.

[00131] In certain embodiments, R_1 is substituted or unsubstituted alkyl. In certain embodiments, R_1 is unsubstituted alkyl. In certain embodiments, R_1 is substituted alkyl. In certain embodiments, R_1 is substituted C_{1-10}alkyl. In certain embodiments, R_1 is substituted

C_{1-8}alkyl. In certain embodiments, R_1 is substituted C_{1-6}alkyl. In certain embodiments, R_1 is substituted C_{1-5}alkyl. In certain embodiments, R_1 is substituted C_{1-4}alkyl. In certain embodiments, R_1 is substituted C_{1-3}alkyl. In certain embodiments, R_1 is substituted C_{1-2}alkyl. In certain embodiments, R_1 is substituted C_{2-4}alkyl. In certain embodiments, R_1 is substituted C_1alkyl. In certain embodiments, R_1 is substituted C_2alkyl. In certain embodiments, R_1 is substituted C_3alkyl. In certain embodiments, R_1 is substituted C_4alkyl.

[00132] In certain embodiments, R_1 is substituted or unsubstituted alkenyl. In certain embodiments, R_1 is unsubstituted alkenyl. In certain embodiments, R_1 is substituted alkenyl. In certain embodiments, R_1 is substituted C_{2-10}alkenyl. In certain embodiments, R_1 is substituted C_{2-8}alkenyl. In certain embodiments, R_1 is substituted C_{2-6}alkenyl. In certain embodiments, R_1 is substituted C_{2-5}alkenyl. In certain embodiments, R_1 is substituted C_{2-4}alkenyl. In certain embodiments, R_1 is substituted C_{2-3}alkenyl. In certain embodiments, R_1 is substituted C_1alkenyl. In certain embodiments, R_1 is substituted C_2alkenyl. In certain embodiments, R_1 is substituted C_3alkenyl. In certain embodiments, R_1 is substituted C_4alkenyl.

[00133] In certain embodiments, R_1 is substituted or unsubstituted heteroaliphatic. In certain embodiments, R_1 is substituted or unsubstituted heteroaliphatic group comprising 1 to 10 carbon atoms and 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur ("C_{1-10}heteroaliphatic"). In certain embodiments, the heteroaliphatic group comprises 1 heteroatom selected from oxygen, nitrogen, and sulfur. In certain embodiments, the heteroaliphatic group comprises 2 heteroatoms independently selected from oxygen, nitrogen, and sulfur. In certain embodiments, the heteroaliphatic group comprises 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_1 is a substituted or unsubstituted heteroaliphatic group comprising 1 to 8 carbon atoms and 1-3 heteroatoms selected from oxygen, nitrogen, and sulfur ("C_{1-8}heteroaliphatic"). In certain embodiments, R_1 is a substituted or unsubstituted heteroaliphatic group comprising 1 to 6 carbon atoms and 1-2 heteroatoms selected from oxygen, nitrogen, and sulfur ("C_{1-6}heteroaliphatic"). In certain embodiments, R_1 is a substituted or unsubstituted heteroaliphatic group comprising 1 to 4 carbon atoms and 1-2 heteroatoms selected from oxygen, nitrogen, and sulfur ("C_{1-4}heteroaliphatic"). In certain embodiments, R_1 is a substituted or unsubstituted heteroaliphatic group comprising 1 to 3 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("C_{1-3}heteroaliphatic"). In certain embodiments, R_1 is a substituted or unsubstituted heteroaliphatic group comprising 1 carbon atom and 1 heteroatom selected from oxygen,

nitrogen, and sulfur ("C_1heteroaliphatic"). In certain embodiments, R_1 is a substituted or unsubstituted heteroaliphatic group comprising 2 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("C_2heteroaliphatic"). In certain embodiments, R_1 is a substituted or unsubstituted heteroaliphatic group comprising 3 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("C_3heteroaliphatic"). In any of the above embodiments, R_1 is an acyclic heteroaliphatic group. In any of the above embodiments, R_1 is an acyclic and substituted heteroaliphatic group. In any of the above embodiments, the heteroaliphatic group is an heteroalkyl, heteroalkenyl, or heteroalkynyl group.

[00134] In certain embodiments, R_1 is substituted or unsubstituted heteroalkyl comprising 10 carbon atoms and 1-3 heteroatoms selected from oxygen, nitrogen, and sulfur ("substituted C_{1-10}heteroalkyl"). In certain embodiments, the heteroalkyl group comprises 1 heteroatom selected from oxygen, nitrogen, and sulfur. In certain embodiments, the heteroalkyl group comprises 2 heteroatoms independently selected from oxygen, nitrogen, and sulfur. In certain embodiments, the heteroalkyl group comprises 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_1 is a substituted heteroalkyl comprising 1-8 carbon atoms and 1-3 heteroatoms selected from oxygen, nitrogen, and sulfur ("substituted C_{1-8}heteroalkyl"). In certain embodiments, R_1 is a substituted heteroalkyl comprising 1-6 carbon atoms and 1-2 heteroatoms selected from oxygen, nitrogen, and sulfur ("substituted C_{1-6}heteroalkyl"). In certain embodiments, R_1 is a substituted heteroalkyl comprising 1-4 carbon atoms and 1-2 heteroatoms selected from oxygen, nitrogen, and sulfur ("substituted C_{1-4}heteroalkyl"). In certain embodiments, R_1 is a substituted heteroalkyl comprising 1-3 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("substituted C_{1-3}heteroalkyl"). In certain embodiments, R_1 is a substituted heteroalkyl comprising 1-2 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("substituted C_{1-2}heteroalkyl"). In certain embodiments, R_1 is a substituted heteroalkyl comprising 1 carbon atom and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("substituted C_1heteroalkyl"). In certain embodiments, R_1 is a substituted heteroalkyl comprising 2 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("substituted C_2heteroalkyl"). In certain embodiments, R_1 is a substituted heteroalkyl comprising 3 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("substituted C_3heteroalkyl"). In certain embodiments, R_1 is a substituted heteroalkyl comprising 4 carbon atoms and 1-2 heteroatoms selected from oxygen, nitrogen, and sulfur ("substituted C_4heteroalkyl").

[00135] As generally understood from the above, in certain embodiments, R_1 is a group selected from aliphatic, heteroaliphatic, aryl, or heteroaryl which may be unsubstituted or substituted with one or more substituents. Exemplary R_1 group substituents, collectively referred to herein as "R^A," include, but are not limited to, halogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-OR^{A1}$, $-N(R^{A2})_2$, $-SR^{A1}$, $-C(=O)R^{A1}$, $-C(=O)OR^{A1}$, $-C(=O)SR^{A1}$, $-C(=O)N(R^{A2})_2$, $-OC(=O)R^{A1}$, $-OC(=O)OR^{A1}$, $-OC(=O)SR^{A1}$, $-OC(=O)N(R^{A2})_2$, $-NR^{A2}C(=O)R^{A2}$, $-NR^{A2}C(=O)OR^{A1}$, $-NR^{A2}C(=O)SR^{A1}$, $-NR^{A2}C(=O)N(R^{A2})_2$, $-SC(=O)R^{A1}$, $-SC(=O)OR^{A1}$, $-SC(=O)SR^{A1}$, $-SC(=O)N(R^{A2})_2$, $-C(=NR^{A2})R^{A1}$, $-C(=NR^{A2})OR^{A1}$, $-C(=NR^{A2})SR^{A1}$, $-C(=NR^{A2})N(R^{A2})_2$, $-OC(=NR^{A2})R^{A1}$, $-OC(=NR^{A2})OR^{A1}$, $-OC(=NR^{A2})SR^{A1}$, $-OC(=NR^{A2})N(R^{A2})_2$, $-NR^{A2}C(=NR^{A2})R^{A2}$, $-NR^{A2}C(=NR^{A2})OR^{A1}$, $-NR^{A2}C(=NR^{A2})SR^{A1}$, $-NR^{A2}C(=NR^{A2})N(R^{A2})_2$, $-SC(=NR^{A2})R^{A1}$, $-SC(=NR^{A2})OR^{A1}$, $-SC(=NR^{A2})SR^{A1}$, $-SC(=NR^{A2})N(R^{A2})_2$, $-C(=S)R^{A1}$, $-C(=S)OR^{A1}$, $-C(=S)SR^{A1}$, $-C(=S)N(R^{A2})_2$, $-OC(=S)R^{A1}$, $-OC(=S)OR^{A1}$, $-OC(=S)SR^{A1}$, $-OC(=S)N(R^{A2})_2$, $-NR^{A2}C(=S)R^{A2}$, $-NR^{A2}C(=S)OR^{A1}$, $-NR^{A2}C(=S)SR^{A1}$, $-NR^{A2}C(=S)N(R^{A2})_2$, $-SC(=S)R^{A1}$, $-SC(=S)OR^{A1}$, $-SC(=S)SR^{A1}$, $-SC(=S)N(R^{A2})_2$, $-S(=O)R^{A1}$, $-SO_2R^{A1}$, $-NR^{A2}SO_2R^{A1}$, $-SO_2N(R^{A2})_2$, $-N_3$, -CN, -SCN, and $-NO_2$, wherein each occurrence of R^{A1} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and each occurrence of R^{A2} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or a nitrogen protecting group, or two R^{A2} groups are joined to form an substituted or unsubstituted heterocyclic ring.

[00136] In certain embodiments, R_1 is aliphatic, as defined above and herein, substituted by one or more R^A substituents, as provided above, *e.g.,* 1, 2, 3, or 4 substituents. In certain embodiments, R_1 is alkyl, as defined above and herein, substituted by one or more R^A substituents, as provided above, *e.g.,* 1, 2, 3, or 4 substituents. In certain embodiments, R_1 is a C_{1-4}alkyl substituted by one or more R^A substituents, as provided above, *e.g.,* 1, 2, 3, or 4 substituents. In certain embodiments, R_1 is a C_{2-4}alkenyl substituted by one or more R^A substituents, as provided above, *e.g.,* 1, 2, 3, or 4 substituents.

[00137] For example, in certain embodiments, R_1 is aliphatic substituted by an optionally substituted aryl or optionally substituted heteroaryl group, *e.g.*, an optionally substituted phenyl group or optionally substituted 5- to 6-membered heteroaryl group.

[00138] In certain embodiments, R_1 is aliphatic substituted by an optionally substituted aryl group ("optionally substituted arylaliphatic"). In certain embodiments, the aryl group is an optionally substituted phenyl ("optionally substituted phenylaliphatic"). In certain embodiments, the aryl group is unsubstituted. However, in certain embodiments, the aryl group is substituted. For example, in certain embodiments, the aryl group is a monosubstituted phenyl ring, *e.g.,* substituted at the *ortho*, *meta*, or *para* position of the phenyl ring relative to the point of attachment. In certain embodiments, the aryl group is a disubstituted phenyl ring, *e.g.,* substituted at the 1,2-, the 1,3-, the 1,4-, the 2,3-, the 3,4-, or the 2,4- positions of the phenyl ring relative to the point of attachment. In certain embodiments, the aryl group is a trisubstituted phenyl ring, *e.g.,* substituted at the 1,2,3-, the 1,2,4-, or the 2,3,4-, positions of the phenyl ring relative to the point of attachment. In certain embodiments, the aryl group is a tetrasubstituted phenyl ring. In any of the above embodiments, the aliphatic group is selected from alkyl, alkenyl, or alkynyl. In certain embodiments, the aliphatic group is alkyl. For example, in certain embodiments, R_1 is alkyl substituted by an optionally substituted aryl group ("optionally substituted arylalkyl"), *e.g.*, an optionally substituted phenyl group ("optionally substituted phenylalkyl"). In certain embodiments, R_1 is a C_{1-4}alkyl substituted by an optionally substituted aryl group, *e.g.*, an optionally substituted phenyl group. In certain embodiments, R_1 is a C_1alkyl substituted by an optionally substituted aryl group, *e.g.*, an optionally substituted phenyl group In certain embodiments, the aliphatic group is alkenyl. For example, in certain embodiments, R_1 is a C_{2-4}alkenyl substituted by an optionally substituted aryl group ("optionally substituted arylalkenyl"), *e.g.*, an optionally substituted phenyl group ("optionally substituted phenylalkenyl"). In certain embodiments, R_1 is a C_2alkenyl substituted by an optionally substituted aryl group, *e.g.*, an optionally substituted phenyl group. In certain embodiments, R_1 is a C_3alkenyl substituted by an optionally substituted aryl group, *e.g.*, an optionally substituted phenyl group.

[00139] In certain embodiments, R_1 is aliphatic substituted by an optionally substituted 5- to 6-membered heteroaryl group, *e.g.,* a substituted or unsubstituted 5-membered heteroaryl, *e.g.*, pyrrolyl, furanyl, thiophenyl, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, triazolyl, oxadiazolyl, thiadiazolyl, or tetrazolyl, or a substituted or unsubstituted 6-membered heteroaryl, *e.g.*, pyridinyl, pyridazinyl, pyrimidinyl,

pyrazinyl, triazinyl, or tetrazinyl. In certain embodiments, the heteroaryl group is a substituted 5- to 6-membered heteroaryl ("substituted 5- to 6-membered heteroarylaliphatic"). In any of the above embodiments, the aliphatic group is selected from alkyl, alkenyl, or alkynyl. In certain embodiments, the aliphatic group is alkyl. For example, in certain embodiments, R_1 is alkyl substituted by a substituted by an optionally substituted heteroaryl group ("optionally substituted heteroarylalkyl"), *e.g.*, an optionally substituted 5- to 6-membered heteroaryl group ("optionally substituted 5- to 6-membered heteroarylalkyl"). In certain embodiments, R_1 is a C_{1-4}alkyl substituted by an optionally substituted heteroaryl group, *e.g.*, an optionally substituted 5- to 6-membered heteroaryl group. In certain embodiments, R_1 is a C_1alkyl substituted by an optionally substituted heteroaryl group, *e.g.*, an optionally substituted 5- to 6-membered heteroaryl group. In certain embodiments, R_1 is a C_{2-4}alkenyl substituted by an optionally substituted heteroaryl group, *e.g.*, an optionally substituted 5- to 6-membered heteroaryl group. In certain embodiments, R_1 is a C_2alkenyl substituted by an optionally substituted heteroaryl group, *e.g.*, an optionally substituted 5- to 6-membered heteroaryl group. In certain embodiments, R_1 is a C_3alkenyl substituted by an optionally substituted heteroaryl group, *e.g.*, an optionally substituted 5- to 6-membered heteroaryl group. In any of the above embodiments, the heteroaryl group is substituted. However, in any of the above embodiments, the heteroaryl group is unsubstituted.

[00140] In certain embodiments, R_1 is aliphatic substituted by a substituted or unsubstituted carbocyclyl ("optionally substituted carbocylcyl") or a substituted or unsubstituted heterocyclyl ("optionally substituted heterocyclyl"), *e.g.*, optionally substituted C_{3-8}carbocyclyl or optionally substituted 5- to 8-membered heterocyclyl.

[00141] For example, in certain embodiments, R_1 is aliphatic substituted by an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_1 is aliphatic substituted by an optionally substituted 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, *e.g.,* azocanyl, oxecanyl, or thiocanyl. In certain embodiments, R_1 is aliphatic substituted by an optionally substituted 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, *e.g.*, azepanyl, oxepanyl, or thiepanyl. In certain embodiments, R_1 is aliphatic substituted by an optionally substituted 6-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, *e.g.*, piperazinyl, morpholinyl, dithianyl, dioxanyl, triazinanyl, and 6,6-bicycles such as tetrahydroquinolinyl and tetrahydroisoquinolinyl. In certain embodiments, R_1 is aliphatic

substituted by an optionally substituted 5-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, *e.g.*, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothiophenyl, dihydrothiophenyl, pyrrolidinyl, dihydropyrrolyl, pyrrolyl–2,5–dione, dioxolanyl, oxasulfuranyl, disulfuranyl, oxazolidin-2-one, triazolinyl, oxadiazolinyl, thiadiazolinyl, and 5,6-bicycles such as indolinyl, isoindolinyl, dihydrobenzofuranyl, dihydrobenzothienyl, and benzoxazolinonyl. In any of the above embodiments, the heterocyclyl contains 1 heteroatom. In any of the above embodiments, the heterocyclyl contains 2 heteroatoms. In any of the above embodiments, the heterocyclyl contains 3 heteroatoms. In any of the above embodiments, the aliphatic group is selected from alkyl, alkenyl, or alkynyl. In certain embodiments, the aliphatic group is alkyl. For example, in certain embodiments, R_1 is alkyl substituted by a substituted by an optionally substituted heterocyclyl group ("optionally substituted heterocyclylalkyl"), *e.g.*, an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_1 is a C_{1-4}alkyl substituted by an optionally substituted heterocyclyl group, *e.g.*, an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_1 is a C_1alkyl substituted by an optionally substituted heterocyclyl group, *e.g.*, an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_1 is a C_{2-4}alkenyl substituted by an optionally substituted heterocyclyl group, *e.g.*, an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_1 is a C_2alkenyl substituted by an optionally substituted heterocyclyl group, *e.g.*, an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_1 is a C_3alkenyl substituted by an optionally substituted heterocyclyl group, *e.g.*, an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In any of the above embodiments, the heterocyclyl group is not substituted. However, in any of the above embodiments, the heterocyclyl group is substituted.

[00142] In certain embodiments, R_1 is aliphatic substituted by an optionally substituted C_{3-7}carbocyclyl. In certain embodiments, R_1 is aliphatic substituted by an optionally substituted C_{3-6}carbocyclyl. In certain embodiments, R_1 is aliphatic substituted by an optionally substituted C_{3-5}carbocyclyl. In certain embodiments, R_1 is aliphatic substituted by an optionally substituted C_{3-4}carbocyclyl. In certain embodiments, R_1 is aliphatic substituted

by an optionally substituted C_{4-6}carbocyclyl. In certain embodiments, R_1 is aliphatic substituted by an optionally substituted C_3carbocyclyl, *e.g.*, optionally substituted cyclopropyl or optionally substituted cyclopropenyl. In certain embodiments, R_1 is aliphatic substituted by an optionally substituted C_4carbocyclyl, *e.g.*, optionally substituted cyclobutyl or optionally substituted cyclobutenyl. In certain embodiments, R_1 is aliphatic substituted by an optionally substituted C_5carbocyclyl, *e.g.*, optionally substituted cyclopentyl or optionally substituted cyclopentenyl. In certain embodiments, R_1 is aliphatic substituted by an optionally substituted C_6carbocyclyl, *e.g.*, optionally substituted cyclohexyl, optionally substituted cyclohexenyl, or optionally substituted cyclohexadienyl. In certain embodiments, R_1 is aliphatic substituted by an optionally substituted C_7carbocyclyl, *e.g.*, optionally substituted cycloheptyl, optionally substituted cycloheptenyl, optionally substituted cycloheptadienyl, or optionally substituted cycloheptatrienyl. In any of the above embodiments, the aliphatic group is selected from alkyl, alkenyl, or alkynyl. In certain embodiments, the aliphatic group is alkyl. For example, in certain embodiments, R_1 is alkyl substituted by a substituted by an optionally substituted carbocyclyl group ("optionally substituted carbocyclylalkyl"), *e.g.*, an optionally substituted C_{3-7}carbocyclyl group. In certain embodiments, R_1 is a C_{1-4}alkyl substituted by an optionally substituted carbocyclyl group, *e.g.*, an optionally substituted C_{3-7}carbocyclyl. In certain embodiments, R_1 is a C_1alkyl substituted by an optionally substituted carbocyclyl group, *e.g.*, an optionally substituted C_{3-7}carbocyclyl. In certain embodiments, R_1 is a C_{2-4}alkenyl substituted by an optionally substituted carbocyclyl group, *e.g.*, an optionally substituted C_{3-7}carbocyclyl. In certain embodiments, R_1 is a C_2alkenyl substituted by an optionally substituted carbocyclyl group, *e.g.*, an optionally substituted C_{3-7}carbocyclyl. In certain embodiments, R_1 is a C_3alkenyl substituted by an optionally substituted carbocyclyl group, *e.g.*, an optionally substituted C_{3-7}carbocyclyl. In any of the above embodiments, the carbocyclyl group is not substituted. However, in any of the above embodiments, the carbocyclyl group is substituted.

[00143] As understood from the above, the carbocyclyl, heterocyclyl, aryl, and/or heteroaryl substituents present on the R_1 aliphatic group may be unsubstituted or substituted with one or more substituents. Such substituents, collectively referred to herein as "R^{AA}", include, but are not limited to, halogen, substituted or unsubstituted alkyl (*e.g.*, perhaloalkyl), substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-OR^{A3}$, $-N(R^{A4})_2$, $-SR^{A3}$, $-C(=O)R^{A3}$, $-C(=O)OR^{A3}$, $-C(=O)SR^{A3}$, $-C(=O)N(R^{A4})_2$, $-OC(=O)R^{A3}$, $-OC(=O)OR^{A3}$,

$-OC(=O)SR^{A3}$, $-OC(=O)N(R^{A4})_2$, $-NR^{A4}C(=O)R^{A4}$, $-NR^{A4}C(=O)OR^{A3}$, $-NR^{A4}C(=O)SR^{A3}$, $-NR^{A4}C(=O)N(R^{A4})_2$, $-SC(=O)R^{A3}$, $-SC(=O)OR^{A3}$, $-SC(=O)SR^{A3}$, $-SC(=O)N(R^{A4})_2$, $-C(=NR^{A4})R^{A3}$, $-C(=NR^{A4})OR^{A3}$, $-C(=NR^{A4})SR^{A3}$, $-C(=NR^{A4})N(R^{A4})_2$, $-OC(=NR^{A4})R^{A3}$, $-OC(=NR^{A4})OR^{A3}$, $-OC(=NR^{A4})SR^{A3}$, $-OC(=NR^{A4})N(R^{A4})_2$, $-NR^{A4}C(=NR^{A4})R^{A2}$, $-NR^{A4}C(=NR^{A4})OR^{A3}$, $-NR^{A4}C(=NR^{A4})SR^{A3}$, $-NR^{A4}C(=NR^{A4})N(R^{A4})_2$, $-SC(=NR^{A4})R^{A3}$, $-SC(=NR^{A4})OR^{A3}$, $-SC(=NR^{A4})SR^{A3}$, $-SC(=NR^{A4})N(R^{A4})_2$, $-C(=S)R^{A3}$, $-C(=S)OR^{A3}$, $-C(=S)SR^{A3}$, $-C(=S)N(R^{A4})_2$, $-OC(=S)R^{A3}$, $-OC(=S)OR^{A3}$, $-OC(=S)SR^{A3}$, $-OC(=S)N(R^{A4})_2$, $-NR^{A4}C(=S)R^{A4}$, $-NR^{A4}C(=S)OR^{A3}$, $-NR^{A4}C(=S)SR^{A3}$, $-NR^{A4}C(=S)N(R^{A4})_2$, $-SC(=S)R^{A3}$, $-SC(=S)OR^{A3}$, $-SC(=S)SR^{A3}$, $-SC(=S)N(R^{A4})_2$, $-S(=O)R^{A3}$, $-SO_2R^{A3}$, $-NR^{A4}SO_2R^{A3}$, $-SO_2N(R^{A4})_2$, $-N_3$, -CN, -SCN, and $-NO_2$, wherein each occurrence of R^{A3} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and each occurrence of R^{A4} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or a nitrogen protecting group, or two R^{A4} groups are joined to form an substituted or unsubstituted heterocyclic ring.

[00144] In certain embodiments, at least one R^{AA} substituent (*e.g.*, 1, 2, 3, or 4 R^{AA} substituents) is an electron-withdrawing group, *e.g.*, a substituent which pulls electron density away from the parent molecule (*e.g.*, a ring system) and/or stabilizes anions or electron rich structures. Exemplary electron-withdrawing substituents include, but are not limited to, halogen, perhaloalkyl, $-C(=O)R^{A3}$, $-C(=O)OR^{A3}$, $-C(=O)SR^{A3}$, $-C(=O)N(R^{A4})_2$, $-OC(=O)R^{A3}$, $-OC(=O)OR^{A3}$, $-OC(=O)SR^{A3}$, $-OC(=O)N(R^{A4})_2$, $-NR^{A4}C(=O)R^{A4}$, $-NR^{A4}C(=O)OR^{A3}$, $-NR^{A4}C(=O)SR^{A3}$, $-NR^{A4}C(=O)N(R^{A4})_2$, $-SC(=O)R^{A3}$, $-SC(=O)OR^{A3}$, $-SC(=O)SR^{A3}$, $-SC(=O)N(R^{A4})_2$, $-C(=NR^{A4})R^{A3}$, $-C(=NR^{A4})OR^{A3}$, $-C(=NR^{A4})SR^{A3}$, $-C(=NR^{A4})N(R^{A4})_2$, $-OC(=NR^{A4})R^{A3}$, $-OC(=NR^{A4})OR^{A3}$, $-OC(=NR^{A4})SR^{A3}$, $-OC(=NR^{A4})N(R^{A4})_2$, $-NR^{A4}C(=NR^{A4})R^{A2}$, $-NR^{A4}C(=NR^{A4})OR^{A3}$, $-NR^{A4}C(=NR^{A4})SR^{A3}$, $-NR^{A4}C(=NR^{A4})N(R^{A4})_2$, $-SC(=NR^{A4})R^{A3}$, $-SC(=NR^{A4})OR^{A3}$, $-SC(=NR^{A4})SR^{A3}$, $-SC(=NR^{A4})N(R^{A4})_2$, $-C(=S)R^{A3}$, $-C(=S)OR^{A3}$, $-C(=S)SR^{A3}$, $-C(=S)N(R^{A4})_2$, $-OC(=S)R^{A3}$, $-OC(=S)OR^{A3}$, $-OC(=S)SR^{A3}$, $-OC(=S)N(R^{A4})_2$, $-NR^{A4}C(=S)R^{A4}$, $-NR^{A4}C(=S)OR^{A3}$, $-NR^{A4}C(=S)SR^{A3}$, $-NR^{A4}C(=S)N(R^{A4})_2$, $-SC(=S)R^{A3}$, $-SC(=S)OR^{A3}$, $-SC(=S)SR^{A3}$, $-SC(=S)N(R^{A4})_2$, $-S(=O)R^{A3}$, $-SO_2R^{A3}$, $-NR^{A4}SO_2R^{A3}$, $-SO_2N(R^{A4})_2$, -CN, -SCN, and $-NO_2$,

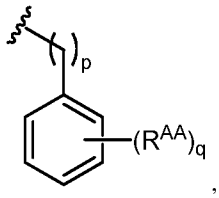
wherein R^{A3} and R^{A4} are as defined herein.

[00145] In certain embodiments, at least one R^{AA} substituent is an electron withdrawing group selected from the group consisting of halogen (*e.g.*, -Br, -Cl), perhaloalkyl (*e.g.*, $-CF_3$), $-C(=O)N(R^{A2})_2$ (*e.g.*, $-C(=O)NH_2$) -CN, -SCN, and $-NO_2$. In certain embodiments, at least one R^{AA} substituent is an electron withdrawing group selected from the group consisting of halogen (*e.g.*, -Br, -Cl), perhaloalkyl (*e.g.*, $-CF_3$), $-C(=O)N(R^{A2})_2$ (*e.g.*, $-C(=O)NH_2$), or -CN. In certain embodiments, at least one R^{AA} substituent is the electron withdrawing group –CN. In certain embodiments, at least one R^{AA} substituent is the electron withdrawing group $-NO_2$. However, in certain embodiments, $-NO_2$ as an aryl group substituent is specifically excluded.

[00146] However, in certain embodiments, at least one R^{AA} substituent (*e.g.*, 1, 2, 3, or 4 R^{AA} substituents) is an electron-donating group, *e.g.*, a substituent which donates electron density toward the parent molecule (*e.g.,* a ring system) and/or stabilizes cations or electron poor structures. Exemplary electron-donating substituents include, but are not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-OR^{A3}$, $-N(R^{A4})_2$, $-SR^{A3}$, wherein R^{A3} and R^{A4} are as defined herein.

[00147] Specific combinations of groups comprising R_1 are further contemplated.

[00148] In certain embodiments, R_1 is an optionally substituted arylalkyl group of the formula:



,

wherein:

p is an integer between 1 and 6, inclusive;

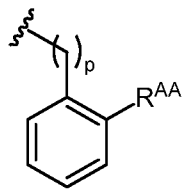
q is 0 or an integer between 1 and 5, inclusive; and

each instance of R^{AA} is independently as defined above and herein.

[00149] In some embodiments, p is 1. In some embodiments, p is 2. In some embodiments, p is 3. In some embodiments, p is 4. In some embodiments, p is 5. In some embodiments, p is 6. In certain embodiments, q is 0. In certain embodiments, q is 1. In certain embodiments, q is 2. In certain embodiments, q is 3. In certain embodiments, q is 4.

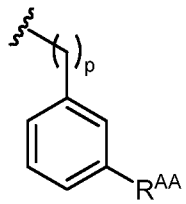
In certain embodiments, q is 5. In certain embodiments, wherein q is an integer between 1 and 5, inclusive, R_1 is a substituted arylalkyl group.

[00150] In certain embodiments, wherein q is 1, and R^{AA} is located in the *ortho* position relative to the point of attachment, R_1 is a monosubstituted arylalkyl group of the formula:



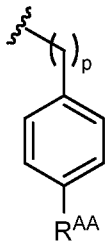
wherein p and R^{AA} are as defined herein.

[00151] In certain embodiments, wherein q is 1, and R^{AA} is located in the *meta* position relative to the point of attachment, R_1 is a monosubstituted arylalkyl group of the formula:



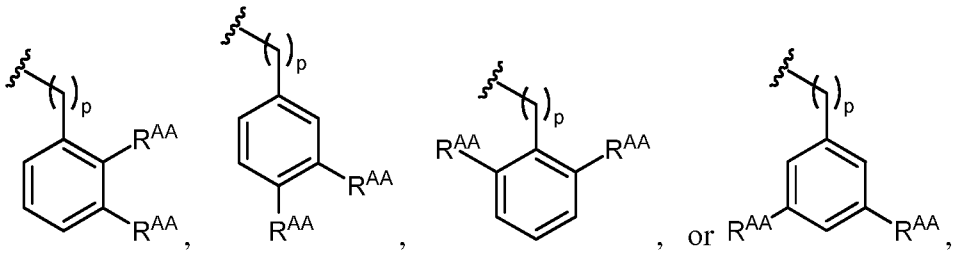
wherein p and R^{AA} are as defined herein.

[00152] In certain embodiments, wherein q is 1, and R^{AA} is located in the *para* position relative to the point of attachment, R_1 is a monosubstituted arylalkyl group of the formula:



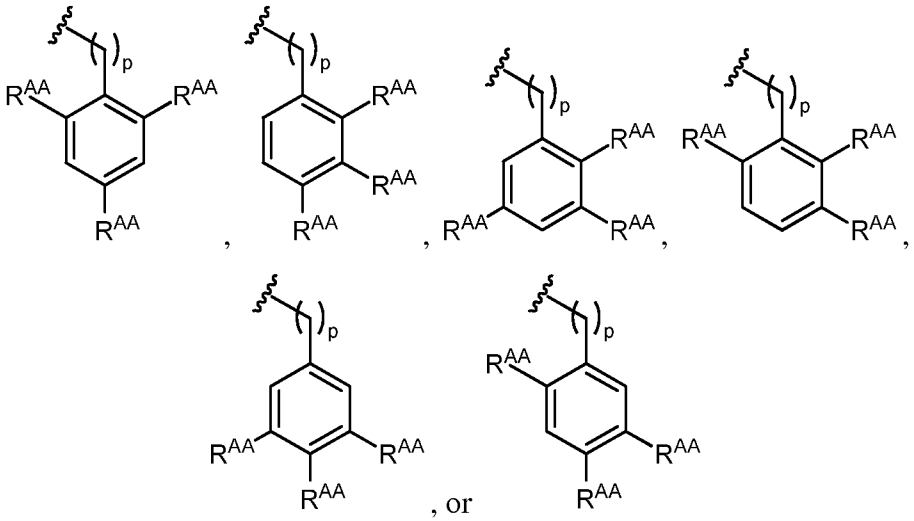
wherein p and R^{AA} are as defined herein.

[00153] In certain embodiments, wherein q is 2, R_1 is a disubstituted arylalkyl group of the formula:



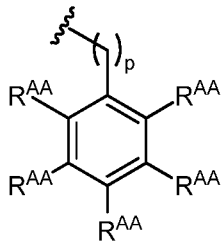
wherein p and R^{AA} are as defined herein.

[00154] In certain embodiments, wherein q is 3, R_1 is a trisubstituted arylalkyl group of the formula:



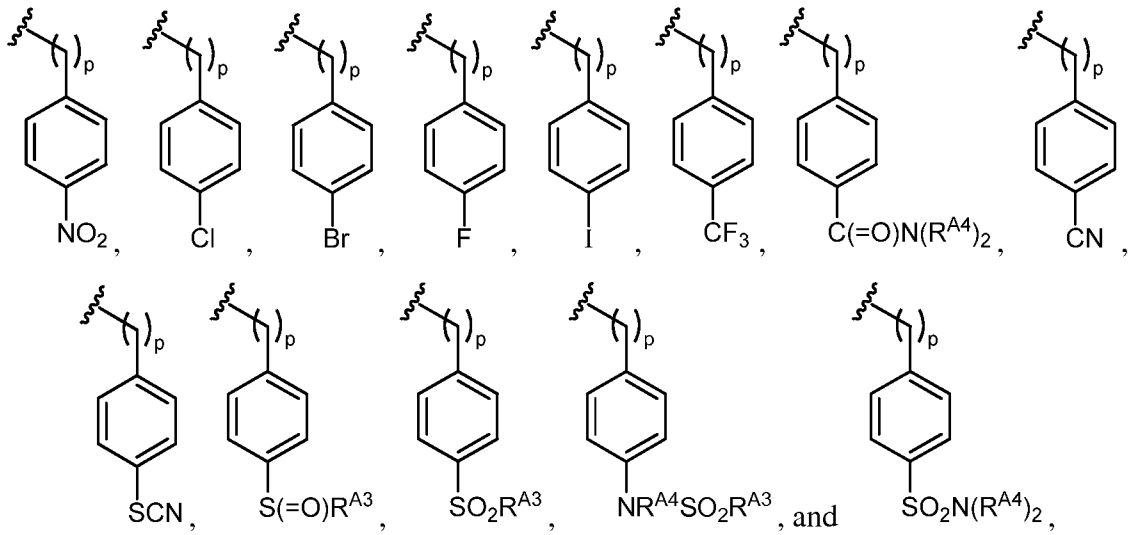
wherein p and R^{AA} are as defined herein.

[00155] In certain embodiments, wherein q is 4, R_1 is a tetrasubstituted arylalkyl group of the formula:



wherein p and R^{AA} are as defined herein.

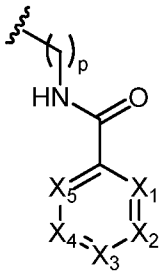
[00156] Exemplary substituted arylalkyl R_1 groups include, but are not limited to,



wherein R^{A3}, R^{A4}, and p is as defined herein. In some embodiments, p is 1. In some embodiments, p is 2. In some embodiments, p is 3. In some embodiments, p is 4. In some embodiments, p is 5. In some embodiments, p is 6.

[00157] In certain embodiments, R_1 is aliphatic substituted by $-NR^{A2}C(=O)R^{A2}$, $-NR^{A2}C(=O)OR^{A1}$, $-NR^{A2}C(=O)SR^{A1}$, or $-NR^{A2}C(=O)N(R^{A2})_2$, wherein each occurrence of R^{A1} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and each occurrence of R^{A2} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or a nitrogen protecting group, or two R^{A2} groups are joined to form an substituted or unsubstituted heterocyclic ring. In certain embodiments, R_1 is aliphatic substituted by $-NR^{A2}C(=O)R^{A2}$. In certain embodiments, R_1 is aliphatic substituted by $-NHC(=O)R^{A2}$, wherein R^{A2} is substituted or unsubstituted aryl (*e.g.*, optionally substituted phenyl) or substituted or unsubstituted heteroaryl (*e.g.*, optionally substituted 5- to 6-membered heteroaryl). In certain embodiments, R_1 is aliphatic substituted by $-NHC(=O)R^{A2}$, wherein R^{A2} is substituted or unsubstituted aryl (*e.g.*, substituted or unsubstituted phenyl). In certain embodiments, R_1 is aliphatic substituted by $-NHC(=O)R^{A2}$, wherein R^{A2} is substituted or unsubstituted heteroaryl (*e.g.*, substituted or unsubstituted 5- to 6-membered heteroaryl). In certain embodiments, R_1 is aliphatic substituted by $-NHC(=O)R^{A2}$, wherein R^{A2} is substituted or unsubstituted 5-membered heteroaryl, *e.g.*, pyrrolyl, furanyl, thiophenyl, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, triazolyl, oxadiazolyl, thiadiazolyl, and tetrazolyl. In certain embodiments, R_1 is aliphatic substituted by $-NHC(=O)R^{A2}$, wherein R^{A2} is substituted or unsubstituted 6-membered heteroaryl, *e.g.*, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, and tetrazinyl. In certain embodiments, R_1 is aliphatic substituted by $-NHC(=O)R^{A2}$, wherein R^{A2} is substituted or unsubstituted pyridinyl. In certain embodiments, R_1 is aliphatic substituted by $-NHC(=O)R^{A2}$, wherein R^{A2} is substituted or unsubstituted pyrazinyl.

[00158] In certain embodiments, wherein R_1 is alkyl substituted by $-NR^{A2}C(=O)R^{A2}$, R_1 is a group the formula:



wherein:

p is an integer between 1 and 6, inclusive;

each instance of X^1, X^2, X^3, X^4, and X^5 is independently selected from N, CH, or CR^{AA}; and

each instance of R^{AA} is independently as defined above and herein.

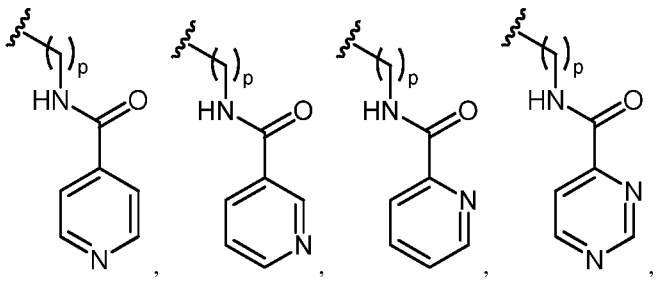
[00159] In certain embodiments, each instance of X^1, X^2, X^3, X^4, and X^5 is independently selected from CH or CR^{AA}.

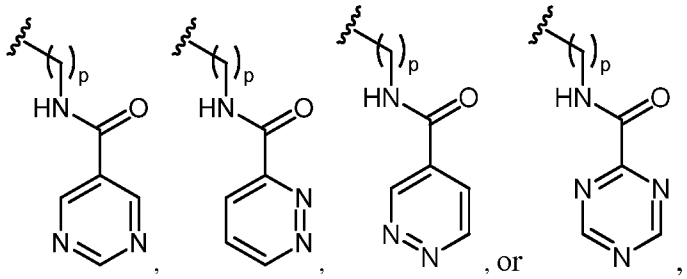
[00160] In certain embodiments, X^1 is N and X^2, X^3, X^4, and X^5 are independently selected from CH or CR^{AA}. In certain embodiments, X^2 is N and X^1, X^3, X^4, and X^5 are independently selected from CH or CR^{AA}. In certain embodiments, X^3 is N and X^1, X^2, X^4, and X^5 are independently selected from CH or CR^{AA}.

[00161] In certain embodiments, X^1 and X^3 are N and X^2, X^4, and X^5 are independently selected from CH or CR^{AA}. In certain embodiments, X^2 and X^4 are N and X^1, X^3, and X^5 are independently selected from CH or CR^{AA}. In certain embodiments, X^1 and X^2 are N and X^3, X^4, and X^5 are independently selected from CH or CR^{AA}. In certain embodiments, X^3 and X^4 are N and X^1, X^2, and X^5 are independently selected from CH or CR^{AA}.

[00162] In certain embodiments, X^1, X^3, and X^5 are N and X^2 and X^4 are independently selected from CH or CR^{AA}.

[00163] In certain embodiments, wherein R_1 is aliphatic substituted by $-NR^{A2}C(=O)R^{A2}$, R_1 is a group the formula:

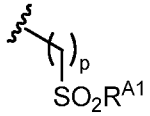




wherein p is as defined herein. In some embodiments, p is 1. In some embodiments, p is 2. In some embodiments, p is 3. In some embodiments, p is 4. In some embodiments, p is 5. In some embodiments, p is 6.

[00164] In certain embodiments, R_1 is aliphatic substituted by $-S(=O)R^{A1}$, $-SO_2R^{A1}$, $-NR^{A2}SO_2R^{A1}$, or $-SO_2N(R^{A2})_2$, wherein each occurrence of R^{A1} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and each occurrence of R^{A2} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or a nitrogen protecting group, or two R^{A2} groups are joined to form an substituted or unsubstituted heterocyclic ring. In certain embodiments, R_1 is aliphatic substituted by $-SO_2R^{A1}$, wherein R^{A1} is substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl ("sulfonyl-substituted aliphatic"). In certain embodiments, R_1 is aliphatic substituted by $-SO_2R^{A1}$, wherein R^{A1} is substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl. In certain embodiments, R_1 is aliphatic substituted by $-SO_2R^{A1}$, wherein R^{A1} is substituted or unsubstituted alkyl (*e.g.*, $-CH_3$, "methyl-sulfonyl-substituted aliphatic"). In certain embodiments, R_1 is alkyl substituted by $-SO_2R^{A1}$, wherein R^{A1} is substituted or unsubstituted alkyl (*e.g.*, $-CH_3$).

[00165] In certain embodiments, wherein R_1 is aliphatic substituted by $-S(=O)R^{A1}$, R^1 is a group of the formula:



wherein p is an integer between 1 and 6, inclusive, and R^{A1} is as defined above and herein. In some embodiments, p is 1. In some embodiments, p is 2. In some embodiments, p is 3. In some embodiments, p is 4. In some embodiments, p is 5. In some embodiments, p is 6. In certain embodiments, R^{A1} is substituted or unsubstituted alkyl (*e.g.*, $-CH_3$).

Embodiments of R_2

[00166] As generally defined above, each instance of R_2 is independently hydrogen; halogen; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; acyl; $-OR_B$; $-N(R_B)_2$; $-SR_B$; =O; -CN; $-NO_2$; -SCN; $-SOR_B$; or $-SO_2R_B$; wherein each occurrence of R_B independently is hydrogen; a protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; acyl; substituted or unsubstituted aryl; or substituted or unsubstituted heteroaryl.

[00167] In certain embodiments, R_2 is hydrogen. In certain embodiments, R_2 is halogen, *i.e.*, -F, -Cl, -Br, or –I. In certain embodiments, R_2 is $–OR_B$. In certain embodiments, R_2 is $-N(R_B)_2$. In certain embodiments, R_2 is $–SR_B$. In certain embodiments, R_2 is =O. In certain embodiments, R_2 is –CN. In certain embodiments, R_2 is $-NO_2$. In certain embodiments, R_2 is –SCN. In certain embodiments, R_2 is $–SOR_B$. In certain embodiments, R_2 is $-SO_2R_B$.

[00168] In certain embodiments, R_2 is acyl. For example, in certain embodiments, R_2 is acyl selected from the group consisting of $-C(=O)R^{B5}$, $-C(=O)OR^{B5}$, $-C(=O)SR^{B5}$, $-C(=O)N(R^{B6})_2$, $-C(=NR^{B6})R^{B5}$, $-C(=NR^{B6})OR^{B5}$, $-C(=NR^{B6})SR^{B5}$, $-C(=NR^{B6})N(R^{B6})_2$, $-C(=S)R^{B5}$, $-C(=S)OR^{B5}$, $-C(=S)SR^{B5}$, and $-C(=S)N(R^{B6})_2$, wherein each occurrence of R^{B5} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and each occurrence of R^{B6} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or a nitrogen protecting group, or two R^{B6} groups are joined to form an substituted or unsubstituted heterocyclic ring.

[00169] In certain embodiments, R_2 is substituted or unsubstituted aryl, *e.g.*, phenyl, napthyl. In certain embodiments, R_2 is substituted or unsubstituted phenyl. In certain

embodiments, R_2 is substituted phenyl, *e.g.*, a monosubstituted, disubstituted, or trisubstituted phenyl.

[00170] In certain embodiments, R_2 is substituted or unsubstituted heteroaryl, *e.g.*, a substituted or unsubstituted 5- to 6-membered heteroaryl. In certain embodiments, R_2 is a substituted or unsubstituted 5-membered heteroaryl, *e.g.*, pyrrolyl, furanyl, thiophenyl, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, triazolyl, oxadiazolyl, thiadiazolyl, or tetrazolyl. In certain embodiments, R_2 is a substituted or unsubstituted 6-membered heteroaryl, *e.g.*, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, or tetrazinyl.

[00171] In certain embodiments, R_2 is substituted or unsubstituted aliphatic. In certain embodiments, R_2 is a substituted or unsubstituted aliphatic group comprising 1 to 10 carbon atoms ("C_{1-10}aliphatic"). In certain embodiments, R_2 is substituted or unsubstituted aliphatic group comprising 1 to 8 carbon atoms ("C_{1-8}aliphatic"). In certain embodiments, R_2 is a substituted or unsubstituted aliphatic group comprising 1 to 6 carbon atoms ("C_{1-6}aliphatic"). In certain embodiments, R_2 is a substituted or unsubstituted aliphatic group comprising 1 to 4 carbon atoms ("C_{1-4}aliphatic"). In certain embodiments, R_2 is a substituted or unsubstituted aliphatic group comprising 1 to 3 carbon atoms ("C_{1-3}aliphatic"). In certain embodiments, R_2 is a substituted or unsubstituted aliphatic group comprising 1 carbon atom ("C_1aliphatic"). In certain embodiments, R_2 is a substituted or unsubstituted aliphatic group comprising 2 carbon atoms ("C_2aliphatic"). In certain embodiments, R_2 is a substituted or unsubstituted aliphatic group comprising 3 carbon atoms ("C_3aliphatic"). In any of the above embodiments, R_2 is an acyclic aliphatic group. In any of the above embodiments, R_2 is an acyclic and substituted aliphatic group. In any of the above embodiments, the aliphatic group is an alkyl, alkenyl, or alkynyl group.

[00172] In certain embodiments, R_2 is substituted or unsubstituted alkyl. In certain embodiments, R_2 is unsubstituted alkyl. In certain embodiments, R_2 is substituted alkyl. In certain embodiments, R_2 is substituted C_{1-10}alkyl. In certain embodiments, R_2 is substituted C_{1-8}alkyl. In certain embodiments, R_2 is substituted C_{1-6}alkyl. In certain embodiments, R_2 is substituted C_{1-5}alkyl. In certain embodiments, R_2 is substituted C_{1-4}alkyl. In certain embodiments, R_2 is substituted C_{1-3}alkyl. In certain embodiments, R_2 is substituted C_{1-2}alkyl. In certain embodiments, R_2 is substituted C_{2-4}alkyl. In certain embodiments, R_2 is substituted C_1alkyl. In certain embodiments, R_2 is substituted C_2alkyl. In certain embodiments, R_2 is substituted C_3alkyl. In certain embodiments, R_2 is substituted C_4alkyl.

[00173] In certain embodiments, R_2 is substituted or unsubstituted alkenyl. In certain

embodiments, R_2 is unsubstituted alkenyl. In certain embodiments, R_2 is substituted alkenyl. In certain embodiments, R_2 is substituted C_{2-10}alkenyl. In certain embodiments, R_2 is substituted C_{2-8}alkenyl. In certain embodiments, R_2 is substituted C_{2-6}alkenyl. In certain embodiments, R_2 is substituted C_{2-5}alkenyl. In certain embodiments, R_2 is substituted C_{2-4}alkenyl. In certain embodiments, R_2 is substituted C_{2-3}alkenyl. In certain embodiments, R_2 is substituted C_1alkenyl. In certain embodiments, R_2 is substituted C_2alkenyl. In certain embodiments, R_2 is substituted C_3alkenyl. In certain embodiments, R_2 is substituted C_4alkenyl.

[00174] In certain embodiments, R_2 is substituted or unsubstituted heteroaliphatic. In certain embodiments, R_2 is substituted or unsubstituted heteroaliphatic group comprising 1 to 10 carbon atoms and 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur ("C_{1-10}heteroaliphatic"). In certain embodiments, the heteroaliphatic group comprises 1 heteroatom selected from oxygen, nitrogen, and sulfur. In certain embodiments, the heteroaliphatic group comprises 2 heteroatoms independently selected from oxygen, nitrogen, and sulfur. In certain embodiments, the heteroaliphatic group comprises 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_2 is a substituted or unsubstituted heteroaliphatic group comprising 1 to 8 carbon atoms and 1-3 heteroatoms selected from oxygen, nitrogen, and sulfur ("C_{1-8}heteroaliphatic"). In certain embodiments, R_2 is a substituted or unsubstituted heteroaliphatic group comprising 1 to 6 carbon atoms and 1-2 heteroatoms selected from oxygen, nitrogen, and sulfur ("C_{1-6}heteroaliphatic"). In certain embodiments, R_2 is a substituted or unsubstituted heteroaliphatic group comprising 1 to 4 carbon atoms and 1-2 heteroatoms selected from oxygen, nitrogen, and sulfur ("C_{1-4}heteroaliphatic"). In certain embodiments, R_2 is a substituted or unsubstituted heteroaliphatic group comprising 1 to 3 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("C_{1-3}heteroaliphatic"). In certain embodiments, R_2 is a substituted or unsubstituted heteroaliphatic group comprising 1 carbon atom and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("C_1heteroaliphatic"). In certain embodiments, R_2 is a substituted or unsubstituted heteroaliphatic group comprising 2 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("C_2heteroaliphatic"). In certain embodiments, R_2 is a substituted or unsubstituted heteroaliphatic group comprising 3 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("C_3heteroaliphatic"). In any of the above embodiments, R_2 is an acyclic heteroaliphatic group. In any of the above embodiments, R_2 is an acyclic and substituted heteroaliphatic group. In any of the above

embodiments, the heteroaliphatic group is an heteroalkyl, heteroalkenyl, or heteroalkynyl group.

[00175] In certain embodiments, R_2 is substituted or unsubstituted heteroalkyl comprising 10 carbon atoms and 1-3 heteroatoms selected from oxygen, nitrogen, and sulfur ("substituted C_{1-10}heteroalkyl"). In certain embodiments, the heteroalkyl group comprises 1 heteroatom selected from oxygen, nitrogen, and sulfur. In certain embodiments, the heteroalkyl group comprises 2 heteroatoms independently selected from oxygen, nitrogen, and sulfur. In certain embodiments, the heteroalkyl group comprises 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_2 is a substituted heteroalkyl comprising 1-8 carbon atoms and 1-3 heteroatoms selected from oxygen, nitrogen, and sulfur ("substituted C_{1-8}heteroalkyl"). In certain embodiments, R_2 is a substituted heteroalkyl comprising 1-6 carbon atoms and 1-2 heteroatoms selected from oxygen, nitrogen, and sulfur ("substituted C_{1-6}heteroalkyl"). In certain embodiments, R_2 is a substituted heteroalkyl comprising 1-4 carbon atoms and 1-2 heteroatoms selected from oxygen, nitrogen, and sulfur ("substituted C_{1-4}heteroalkyl"). In certain embodiments, R_2 is a substituted heteroalkyl comprising 1-3 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("substituted C_{1-3}heteroalkyl"). In certain embodiments, R_2 is a substituted heteroalkyl comprising 1-2 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("substituted C_{1-2}heteroalkyl"). In certain embodiments, R_2 is a substituted heteroalkyl comprising 1 carbon atom and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("substituted C_1heteroalkyl"). In certain embodiments, R_2 is a substituted heteroalkyl comprising 2 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("substituted C_2heteroalkyl"). In certain embodiments, R_2 is a substituted heteroalkyl comprising 3 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("substituted C_3heteroalkyl"). In certain embodiments, R_2 is a substituted heteroalkyl comprising 4 carbon atoms and 1-2 heteroatoms selected from oxygen, nitrogen, and sulfur ("substituted C_4heteroalkyl").

[00176] As generally understood from the above, in certain embodiments, R_2 is a group selected from aliphatic, heteroaliphatic, aryl, or heteroaryl which may be unsubstituted or substituted with one or more substituents. Exemplary R_2 group substituents, collectively referred to as "R^B", include, but are not limited to, halogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-OR^{B1}$, $-N(R^{B2})_2$, -

SR^{B1}, $-C(=O)R^{B1}$, $-C(=O)OR^{B1}$, $-C(=O)SR^{B1}$, $-C(=O)N(R^{B2})_2$, $-OC(=O)R^{B1}$, $-OC(=O)OR^{B1}$, $-OC(=O)SR^{B1}$, $-OC(=O)N(R^{B2})_2$, $-NR^{B2}C(=O)R^{B2}$, $-NR^{B2}C(=O)OR^{B1}$, $-NR^{B2}C(=O)SR^{B1}$, $-NR^{B2}C(=O)N(R^{B2})_2$, $-SC(=O)R^{B1}$, $-SC(=O)OR^{B1}$, $-SC(=O)SR^{B1}$, $-SC(=O)N(R^{B2})_2$, $-C(=NR^{B2})R^{B1}$, $-C(=NR^{B2})OR^{B1}$, $-C(=NR^{B2})SR^{B1}$, $-C(=NR^{B2})N(R^{B2})_2$, $-OC(=NR^{B2})R^{B1}$, $-OC(=NR^{B2})OR^{B1}$, $-OC(=NR^{B2})SR^{B1}$, $-OC(=NR^{B2})N(R^{B2})_2$, $-NR^{B2}C(=NR^{B2})R^{B2}$, $-NR^{B2}C(=NR^{B2})OR^{B1}$, $-NR^{B2}C(=NR^{B2})SR^{B1}$, $-NR^{B2}C(=NR^{B2})N(R^{B2})_2$, $-SC(=NR^{B2})R^{B1}$, $-SC(=NR^{B2})OR^{B1}$, $-SC(=NR^{B2})SR^{B1}$, $-SC(=NR^{B2})N(R^{B2})_2$, $-C(=S)R^{B1}$, $-C(=S)OR^{B1}$, $-C(=S)SR^{B1}$, $-C(=S)N(R^{B2})_2$, $-OC(=S)R^{B1}$, $-OC(=S)OR^{B1}$, $-OC(=S)SR^{B1}$, $-OC(=S)N(R^{B2})_2$, $-NR^{B2}C(=S)R^{B2}$, $-NR^{B2}C(=S)OR^{B1}$, $-NR^{B2}C(=S)SR^{B1}$, $-NR^{B2}C(=S)N(R^{B2})_2$, $-SC(=S)R^{B1}$, $-SC(=S)OR^{B1}$, $-SC(=S)SR^{B1}$, $-SC(=S)N(R^{B2})_2$, $-S(=O)R^{B1}$, $-SO_2R^{B1}$, $-NR^{B2}SO_2R^{B1}$, $-SO_2N(R^{B2})_2$, $-N_3$, -CN, -SCN, and $-NO_2$, wherein each occurrence of R^{B1} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and each occurrence of R^{B2} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or a nitrogen protecting group, or two R^{B2} groups are joined to form an substituted or unsubstituted heterocyclic ring.

[00177] In certain embodiments, R_2 is aliphatic, as defined above and herein, substituted by one or more R^B substituents, as provided above, *e.g.,* 1, 2, 3, or 4 substituents. In certain embodiments, R_2 is alkyl, as defined above and herein, substituted by one or more R^B substituents, as provided above, *e.g.,* 1, 2, 3, or 4 substituents. In certain embodiments, R_2 is a C_{1-4}alkyl substituted by one or more R^B substituents, as provided above, *e.g.,* 1, 2, 3, or 4 substituents. In certain embodiments, R_2 is a C_{2-4}alkenyl substituted by one or more R^B substituents, as provided above, *e.g.,* 1, 2, 3, or 4 substituents.

[00178] For example, in certain embodiments, R_2 is aliphatic substituted by an optionally substituted aryl or optionally substituted heteroaryl group, *e.g.*, an optionally substituted phenyl group or optionally substituted 5- to 6-membered heteroaryl group.

[00179] In certain embodiments, R_2 is aliphatic substituted by an optionally substituted aryl group ("optionally substituted arylaliphatic"). In certain embodiments, the aryl group is an optionally substituted phenyl ("optionally substituted phenylaliphatic"). In certain embodiments, the aryl group is unsubstituted. However, in certain embodiments, the

aryl group is substituted. For example, in certain embodiments, the aryl group is a monosubstituted phenyl ring, *e.g.,* substituted at the *ortho*, *meta*, or *para* position of the phenyl ring relative to the point of attachment. In certain embodiments, the aryl group is a disubstituted phenyl ring, *e.g.,* substituted at the 1,2-, the 1,3-, the 1,4-, the 2,3-, the 3,4-, or the 2,4- positions of the phenyl ring relative to the point of attachment. In certain embodiments, the aryl group is a trisubstituted phenyl ring, *e.g.,* substituted at the 1,2,3-, the 1,2,4-, or the 2,3,4-, positions of the phenyl ring relative to the point of attachment. In certain embodiments, the aryl group is a tetrasubstituted phenyl ring. In any of the above embodiments, the aliphatic group is selected from alkyl, alkenyl, or alkynyl. In certain embodiments, the aliphatic group is alkyl. For example, in certain embodiments, R_2 is alkyl substituted by an optionally substituted aryl group ("optionally substituted arylalkyl"), *e.g.*, an optionally substituted phenyl group ("optionally substituted phenylalkyl"). In certain embodiments, R_2 is a C_{1-4}alkyl substituted by an optionally substituted aryl group, *e.g.*, an optionally substituted phenyl group. In certain embodiments, R_2 is a C_1alkyl substituted by an optionally substituted aryl group, *e.g.*, an optionally substituted phenyl group. In certain embodiments, the aliphatic group is alkenyl. For example, in certain embodiments, R_2 is a C_{2-4}alkenyl substituted by an optionally substituted aryl group ("optionally substituted arylalkenyl"), *e.g.*, an optionally substituted phenyl group ("optionally substituted phenylalkenyl"). In certain embodiments, R_2 is a C_2alkenyl substituted by an optionally substituted aryl group, *e.g.*, an optionally substituted phenyl group. In certain embodiments, R_2 is a C_3alkenyl substituted by an optionally substituted aryl group, *e.g.*, an optionally substituted phenyl group.

[00180] In certain embodiments, R_2 is aliphatic substituted by an optionally substituted 5- to 6-membered heteroaryl group, *e.g.,* a substituted or unsubstituted 5-membered heteroaryl, *e.g.*, pyrrolyl, furanyl, thiophenyl, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, triazolyl, oxadiazolyl, thiadiazolyl, or tetrazolyl, or a substituted or unsubstituted 6-membered heteroaryl, *e.g.*, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, or tetrazinyl. In certain embodiments, the heteroaryl group is a substituted 5- to 6-membered heteroaryl ("substituted 5- to 6-membered heteroarylaliphatic"). In any of the above embodiments, the aliphatic group is selected from alkyl, alkenyl, or alkynyl. In certain embodiments, the aliphatic group is alkyl. For example, in certain embodiments, R_2 is alkyl substituted by a substituted by an optionally substituted heteroaryl group ("optionally substituted heteroarylalkyl"), *e.g.*, an optionally substituted 5- to 6-membered heteroaryl group ("optionally substituted 5- to 6-membered

heteroarylalkyl"). In certain embodiments, R_2 is a C_{1-4}alkyl substituted by an optionally substituted heteroaryl group, *e.g.*, an optionally substituted 5- to 6-membered heteroaryl group. In certain embodiments, R_2 is a C_1alkyl substituted by an optionally substituted heteroaryl group, *e.g.*, an optionally substituted 5- to 6-membered heteroaryl group. In certain embodiments, R_2 is a C_{2-4}alkenyl substituted by an optionally substituted heteroaryl group, *e.g.*, an optionally substituted 5- to 6-membered heteroaryl group. In certain embodiments, R_2 is a C_2alkenyl substituted by an optionally substituted heteroaryl group, *e.g.*, an optionally substituted 5- to 6-membered heteroaryl group. In certain embodiments, R_2 is a C_3alkenyl substituted by an optionally substituted heteroaryl group, *e.g.*, an optionally substituted 5- to 6-membered heteroaryl group. In any of the above embodiments, the heteroaryl group is substituted. However, in any of the above embodiments, the heteroaryl group is unsubstituted.

[00181] In certain embodiments, R_2 is aliphatic substituted by a substituted or unsubstituted carbocyclyl ("optionally substituted carbocylcyl") or a substituted or unsubstituted heterocyclyl ("optionally substituted heterocyclyl"), *e.g.*, optionally substituted C_{3-8}carbocyclyl or optionally substituted 5- to 8-membered heterocyclyl.

[00182] For example, in certain embodiments, R_2 is aliphatic substituted by an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_2 is aliphatic substituted by an optionally substituted 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, *e.g.,* azocanyl, oxecanyl, or thiocanyl. In certain embodiments, R_2 is aliphatic substituted by an optionally substituted 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, *e.g.*, azepanyl, oxepanyl, or thiepanyl. In certain embodiments, R_2 is aliphatic substituted by an optionally substituted 6-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, *e.g.*, piperazinyl, morpholinyl, dithianyl, dioxanyl, triazinanyl, and 6,6-bicycles such as tetrahydroquinolinyl and tetrahydroisoquinolinyl. In certain embodiments, R_2 is aliphatic substituted by an optionally substituted 5-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, *e.g.*, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothiophenyl, dihydrothiophenyl, pyrrolidinyl, dihydropyrrolyl, pyrrolyl–2,5–dione, dioxolanyl, oxasulfuranyl, disulfuranyl, oxazolidin-2-one, triazolinyl, oxadiazolinyl, thiadiazolinyl, and 5,6-bicycles such as indolinyl, isoindolinyl, dihydrobenzofuranyl, dihydrobenzothienyl, and benzoxazolinonyl. In any of the above embodiments, the heterocyclyl contains 1 heteroatom. In any of the above embodiments, the

heterocyclyl contains 2 heteroatoms. In any of the above embodiments, the heterocyclyl contains 3 heteroatoms. In any of the above embodiments, the aliphatic group is selected from alkyl, alkenyl, or alkynyl. In certain embodiments, the aliphatic group is alkyl. For example, in certain embodiments, R_2 is alkyl substituted by a substituted by an optionally substituted heterocyclyl group ("optionally substituted heterocyclylalkyl"), *e.g.*, an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_2 is a C_{1-4}alkyl substituted by an optionally substituted heterocyclyl group, *e.g.*, an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_2 is a C_1alkyl substituted by an optionally substituted heterocyclyl group, *e.g.*, an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_2 is a C_{2-4}alkenyl substituted by an optionally substituted heterocyclyl group, *e.g.*, an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_2 is a C_2alkenyl substituted by an optionally substituted heterocyclyl group, *e.g.*, an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_2 is a C_3alkenyl substituted by an optionally substituted heterocyclyl group, *e.g.*, an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In any of the above embodiments, the heterocyclyl group is not substituted. However, in any of the above embodiments, the heterocyclyl group is substituted.

[00183] In certain embodiments, R_2 is aliphatic substituted by an optionally substituted C_{3-7}carbocyclyl. In certain embodiments, R_2 is aliphatic substituted by an optionally substituted C_{3-6}carbocyclyl. In certain embodiments, R_2 is aliphatic substituted by an optionally substituted C_{3-5}carbocyclyl. In certain embodiments, R_2 is aliphatic substituted by an optionally substituted C_{3-4}carbocyclyl. In certain embodiments, R_2 is aliphatic substituted by an optionally substituted C_{4-6}carbocyclyl. In certain embodiments, R_2 is aliphatic substituted by an optionally substituted C_3carbocyclyl, *e.g.*, optionally substituted cyclopropyl or optionally substituted cyclopropenyl. In certain embodiments, R_2 is aliphatic substituted by an optionally substituted C_4carbocyclyl, *e.g.*, optionally substituted cyclobutyl or optionally substituted cyclobutenyl. In certain embodiments, R_2 is aliphatic substituted by an optionally substituted C_5carbocyclyl, *e.g.*, optionally substituted cyclopentyl or optionally substituted cyclopentenyl. In certain embodiments, R_2 is aliphatic substituted by an optionally

substituted C_6carbocyclyl, *e.g.*, optionally substituted cyclohexyl, optionally substituted cyclohexenyl, or optionally substituted cyclohexadienyl. In certain embodiments, R_1 is aliphatic substituted by an optionally substituted C_7carbocyclyl, *e.g.*, optionally substituted cycloheptyl, optionally substituted cycloheptenyl, optionally substituted cycloheptadienyl, or optionally substituted cycloheptatrienyl. In any of the above embodiments, the aliphatic group is selected from alkyl, alkenyl, or alkynyl. In certain embodiments, the aliphatic group is alkyl. For example, in certain embodiments, R_2 is alkyl substituted by a substituted by an optionally substituted carbocyclyl group ("optionally substituted carbocyclylalkyl"), *e.g.*, an optionally substituted C_{3-7}carbocyclyl group. In certain embodiments, R_2 is a C_{1-4}alkyl substituted by an optionally substituted carbocyclyl group, *e.g.*, an optionally substituted C_{3-7}carbocyclyl. In certain embodiments, R_2 is a C_1alkyl substituted by an optionally substituted carbocyclyl group, *e.g.*, an optionally substituted C_{3-7}carbocyclyl. In certain embodiments, R_2 is a C_{2-4}alkenyl substituted by an optionally substituted carbocyclyl group, *e.g.*, an optionally substituted C_{3-7}carbocyclyl. In certain embodiments, R_2 is a C_2alkenyl substituted by an optionally substituted carbocyclyl group, *e.g.*, an optionally substituted C_{3-7}carbocyclyl. In certain embodiments, R_2 is a C_3alkenyl substituted by an optionally substituted carbocyclyl group, *e.g.*, an optionally substituted C_{3-7}carbocyclyl. In any of the above embodiments, the carbocyclyl group is not substituted. However, in any of the above embodiments, the carbocyclyl group is substituted.

[00184] As understood from the above, the carbocyclyl, heterocyclyl, aryl, and/or heteroaryl substituents present on the aliphatic group of R_2 may be unsubstituted or substituted with one or more substituents. Such substituents, collectively referred to herein as "R^{BB}", include, but are not limited to, halogen, substituted or unsubstituted alkyl (*e.g.*, perhaloalkyl), substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-OR^{B3}$, $-N(R^{B4})_2$, $-SR^{B3}$, $-C(=O)R^{B3}$, $-C(=O)OR^{B3}$, $-C(=O)SR^{B3}$, $-C(=O)N(R^{B4})_2$, $-OC(=O)R^{B3}$, $-OC(=O)OR^{B3}$, $-OC(=O)SR^{B3}$, $-OC(=O)N(R^{B4})_2$, $-NR^{B4}C(=O)R^{B4}$, $-NR^{B4}C(=O)OR^{B3}$, $-NR^{B4}C(=O)SR^{B3}$, $-NR^{B4}C(=O)N(R^{B4})_2$, $-SC(=O)R^{B3}$, $-SC(=O)OR^{B3}$, $-SC(=O)SR^{B3}$, $-SC(=O)N(R^{B4})_2$, $-C(=NR^{B4})R^{B3}$, $-C(=NR^{B4})OR^{B3}$, $-C(=NR^{B4})SR^{B3}$, $-C(=NR^{B4})N(R^{B4})_2$, $-OC(=NR^{B4})R^{B3}$, $-OC(=NR^{B4})OR^{B3}$, $-OC(=NR^{B4})SR^{B3}$, $-OC(=NR^{B4})N(R^{B4})_2$, $-NR^{B4}C(=NR^{B4})R^{B2}$, $-NR^{B4}C(=NR^{B4})OR^{B3}$, $-NR^{B4}C(=NR^{B4})SR^{B3}$, $-NR^{B4}C(=NR^{B4})N(R^{B4})_2$, $-SC(=NR^{B4})R^{B3}$, $-SC(=NR^{B4})OR^{B3}$, $-SC(=NR^{B4})SR^{B3}$, $-SC(=NR^{B4})N(R^{B4})_2$, $-C(=S)R^{B3}$, $-C(=S)OR^{B3}$, $-C(=S)SR^{B3}$, $-C(=S)N(R^{B4})_2$, $-OC(=S)R^{B3}$, $-OC(=S)OR^{B3}$, $-OC(=S)SR^{B3}$, $-OC(=S)N(R^{B4})_2$, -

$NR^{B4}C(=S)R^{B4}$, $-NR^{B4}C(=S)OR^{B3}$, $-NR^{B4}C(=S)SR^{B3}$, $-NR^{B4}C(=S)N(R^{B4})_2$, $-SC(=S)R^{B3}$, $-SC(=S)OR^{B3}$, $-SC(=S)SR^{B3}$, $-SC(=S)N(R^{B4})_2$, $-S(=O)R^{B3}$, $-SO_2R^{B3}$, $-NR^{B4}SO_2R^{B3}$, $-SO_2N(R^{B4})_2$, $-N_3$, -CN, -SCN, and $-NO_2$, wherein each occurrence of R^{B3} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and each occurrence of R^{B4} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or a nitrogen protecting group, or two R^{B4} groups are joined to form an substituted or unsubstituted heterocyclic ring.

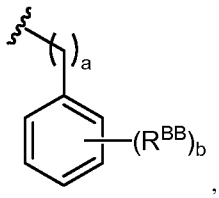
[00185] In certain embodiments, at least one R^{BB} substituent (*e.g.*, 1, 2, 3, or 4 R^{BB} substituents) is an electron-withdrawing group, *e.g.*, a substituent which pulls electron density away from the parent molecule (*e.g.*, a ring system) and/or stabilizes anions or electron rich structures. Exemplary electron-withdrawing substituents include, but are not limited to, halogen, perhaloalkyl, $-C(=O)R^{B3}$, $-C(=O)OR^{B3}$, $-C(=O)SR^{B3}$, $-C(=O)N(R^{B4})_2$, $-OC(=O)R^{B3}$, $-OC(=O)OR^{B3}$, $-OC(=O)SR^{B3}$, $-OC(=O)N(R^{B4})_2$, $-NR^{B4}C(=O)R^{B4}$, $-NR^{B4}C(=O)OR^{B3}$, $-NR^{B4}C(=O)SR^{B3}$, $-NR^{B4}C(=O)N(R^{B4})_2$, $-SC(=O)R^{B3}$, $-SC(=O)OR^{B3}$, $-SC(=O)SR^{B3}$, $-SC(=O)N(R^{B4})_2$, $-C(=NR^{B4})R^{B3}$, $-C(=NR^{B4})OR^{B3}$, $-C(=NR^{B4})SR^{B3}$, $-C(=NR^{B4})N(R^{B4})_2$, $-OC(=NR^{B4})R^{B4}$, $-OC(=NR^{B4})OR^{B3}$, $-OC(=NR^{B4})SR^{B3}$, $-OC(=NR^{B4})N(R^{B4})_2$, $-NR^{B4}C(=NR^{B4})R^{B3}$, $-NR^{B4}C(=NR^{B4})OR^{B3}$, $-NR^{B4}C(=NR^{B4})SR^{B3}$, $-NR^{B4}C(=NR^{B4})N(R^{B4})_2$, $-SC(=NR^{B4})R^{B3}$, $-SC(=NR^{B4})OR^{B3}$, $-SC(=NR^{B4})SR^{B3}$, $-SC(=NR^{B4})N(R^{B4})_2$, $-C(=S)R^{B3}$, $-C(=S)OR^{B3}$, $-C(=S)SR^{B3}$, $-C(=S)N(R^{B4})_2$, $-OC(=S)R^{B3}$, $-OC(=S)OR^{B3}$, $-OC(=S)SR^{B3}$, $-OC(=S)N(R^{B4})_2$, $-NR^{B4}C(=S)R^{B4}$, $-NR^{B4}C(=S)OR^{B3}$, $-NR^{B4}C(=S)SR^{B3}$, $-NR^{B4}C(=S)N(R^{B4})_2$, $-SC(=S)R^{B3}$, $-SC(=S)OR^{B3}$, $-SC(=S)SR^{B3}$, $-SC(=S)N(R^{B4})_2$, $-S(=O)R^{B3}$, $-SO_2R^{B3}$, $-NR^{B4}SO_2R^{B3}$, $-SO_2N(R^{B4})_2$, -CN, -SCN, and $-NO_2$, wherein R^{B3} and R^{B4} are as defined herein. In certain embodiments, at least one R^{BB} group is halogen, e.g., -F, -Br, -I, or –Cl.

[00186] However, in certain embodiments, at least one R^{BB} substituent (*e.g.*, 1, 2, 3, or 4 R^{BB} substituents) is an electron-donating group, *e.g.*, a substituent which donates electron density toward the parent molecule (*e.g.*, a ring system) and/or stabilizes cations or electron poor structures. Exemplary electron-donating substituents include, but are not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl,

substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-OR^{B3}$, $-N(R^{B4})_2$, $-SR^{B3}$.

[00187] Specific combinations of groups comprising R_2 are further contemplated.

[00188] In certain embodiments, R_2 is an optionally substituted arylalkyl group of the formula:



wherein:

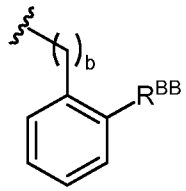
a is an integer between 1 and 6, inclusive;

b is 0 or an integer between 1 and 5, inclusive; and

each instance of R^{BB} is independently as defined herein.

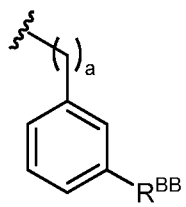
[00189] In some embodiments, a is 1. In some embodiments, a is 2. In some embodiments, a is 3. In some embodiments, a is 4. In some embodiments, a is 5. In some embodiments, a is 6. In certain embodiments, b is 0. In certain embodiments, b is 1. In certain embodiments, b is 2. In certain embodiments, b is 3. In certain embodiments, b is 4. In certain embodiments, b is 5. In certain embodiments, a is 1 and b is 0.

[00190] In certain embodiments, wherein b is 1, and R^{BB} is located in the *ortho* position relative to the point of attachment, R_2 is a monosubstituted arylalkyl group of the formula:



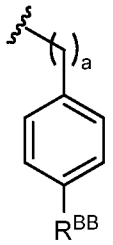
wherein a and R^{BB} are as defined herein.

[00191] In certain embodiments, wherein b is 1, and R^{BB} is located in the *meta* position relative to the point of attachment, R_2 is a monosubstituted arylalkyl group of the formula:



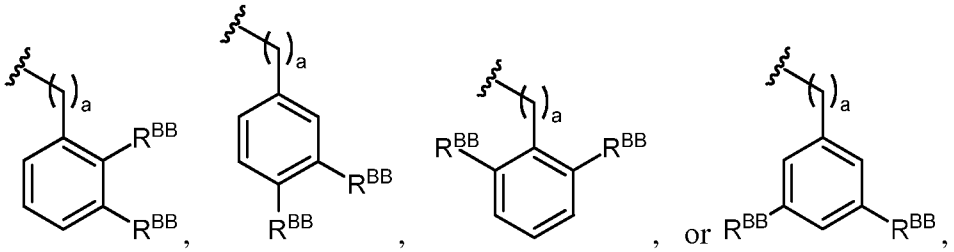
wherein a and R^{BB} are as defined herein.

[00192] In certain embodiments, wherein b is 1, and R^{BB} is located in the *para* position relative to the point of attachment, R_2 is a monosubstituted arylalkyl group of the formula:



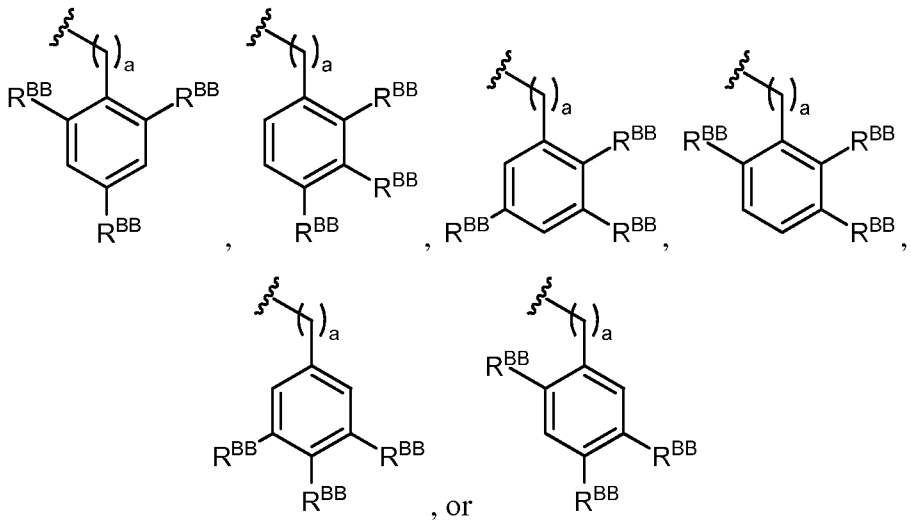
wherein a and R^{BB} are as defined herein.

[00193] In certain embodiments, wherein a is 2, R_2 is a disubstituted arylalkyl group of the formula:



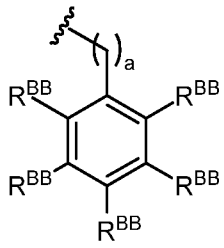
wherein a and R^{BB} are as defined herein.

[00194] In certain embodiments, wherein b is 3, R_2 is a trisubstituted arylalkyl group of the formula:



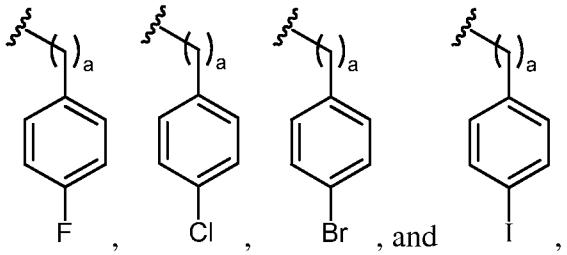
wherein a and R^{BB} are as defined herein.

[00195] In certain embodiments, wherein b is 4, R_2 is a tetrasubstituted arylalkyl group of the formula:



wherein a and R^{BB} are as defined herein.

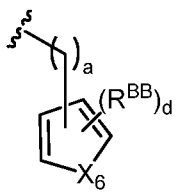
[00196] Exemplary substituted arylalkyl R_2 groups include, but are not limited to,



wherein a is as defined herein. In some embodiments, a is 1. In some embodiments, a is 2. In some embodiments, a is 3. In some embodiments, a is 4. In some embodiments, a is 5. In some embodiments, a is 6.

[00197] In certain embodiments, R_2 is alkyl substituted by an optionally substituted 5-membered heteroaryl group, *e.g.,* an optionally substituted pyrrolyl, furanyl, thiophenyl, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, triazolyl, oxadiazolyl, thiadiazolyl, or tetrazolyl group. In certain embodiments, R_2 is alkyl substituted by an optionally substituted pyrrolyl, optionally substituted furanyl, or optionally substituted thiophenyl. In certain embodiments, R_2 is alkyl substituted by an optionally substituted pyrrolyl (*e.g.,* 2-pyrrolyl, 3-pyrrolyl). In certain embodiments, R_2 is alkyl substituted by an optionally substituted furanyl (*e.g.,* 2-furanyl, 3-furanyl), *i.e.*, R_2 is a "furanylalkyl" group (*e.g.*, 2-furanylalkyl, 3-furanylalkyl). In certain embodiments, R_2 is alkyl substituted by an optionally substituted thiophenyl (*e.g.,* 2-thiophenyl, 3-thiophenyl).

[00198] In certain embodiments, wherein R_2 is alkyl substituted with an optionally substituted 5-membered heteroaryl ring, R_2 is a group of the formula:



wherein:

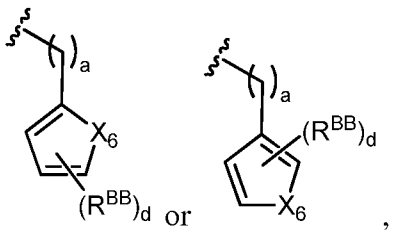
a is an integer between 1 and 6, inclusive;

d is 0, 1, or 2; and

X_6 is O, S, NH, or NR^{BB}; wherein R^{BB} is as defined above and herein.

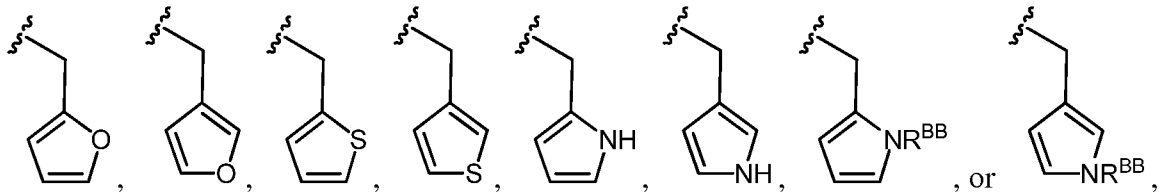
[00199] In some embodiments, a is 1. In some embodiments, a is 2. In some embodiments, a is 3. In some embodiments, a is 4. In some embodiments, a is 5. In some embodiments, a is 6. In certain embodiments, d is 0. In certain embodiments, d is 1. In certain embodiments, d is 2. In certain embodiments, X_6 is O. In certain embodiments, X_6 is S. In certain embodiments, X_6 is NH or NR^{BB}. In certain embodiments, X_6 is NH. In certain embodiments, X_6 is NR^{BB}.

[00200] In certain embodiments, wherein R_2 is alkyl substituted with an optionally substituted 5-membered heteroaryl ring, R_2 is a group of the formula:



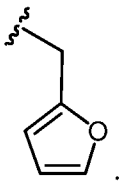
wherein a, d, X_6, and R^{BB} are as defined herein. In certain embodiments, X_6 is O. In certain embodiments, X_6 is S. In certain embodiments, X_6 is NH or NR^{BB}. In certain embodiments, d is 0. In certain embodiments, a is 1.

[00201] In certain embodiments, wherein R_2 is alkyl substituted with an optionally substituted 5-membered heteroaryl ring, R_2 is a group of the formula:



wherein R^{BB} is as defined herein.

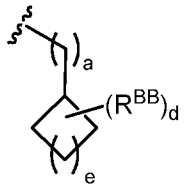
[00202] However, in some embodiments, R_2 is not:



[00203] In certain embodiments, R_2 is alkyl substituted by an optionally substituted C_{3-7}carbocyclyl group, *e.g.*, an optionally substituted cyclopropyl, cyclopropenyl, cyclobutyl, cyclobutenyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cyclohexadienyl, cycloheptyl, cycloheptenyl, cycloheptadienyl, or cycloheptatrienyl group. In certain embodiments, R_2 is alkyl substituted by an saturated carbocyclyl group, *e.g.*, an optionally substituted cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, or cycloheptyl group. In certain

embodiments, R_2 is alkyl substituted by an optionally substituted cyclopropyl group ("optionally substituted cyclopropylalkyl"). In certain embodiments, R_2 is alkyl substituted by an optionally substituted cyclobutyl group ("optionally substituted cyclobutylalkyl"). In certain embodiments, R_2 is alkyl substituted by an optionally substituted cyclopentyl group ("optionally substituted cyclopentylalkyl"). In certain embodiments, R_2 is alkyl substituted by an optionally substituted cyclohexyl group ("optionally substituted cyclohexylalkyl"). In certain embodiments, R_2 is alkyl substituted by an optionally substituted cycloheptyl group ("optionally substituted cycloheptylalkyl").

[00204] In certain embodiments, wherein R_2 is alkyl substituted with an optionally substituted carbocyclyl ring, R_2 is a group of the formula:



wherein:

a is an integer between 1 and 6, inclusive;

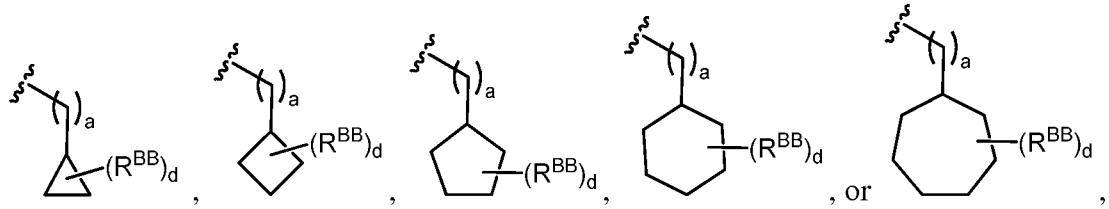
d is 0, 1, or 2;

e is 0 or an integer between 1 and 4, inclusive; and

R^{BB} is as defined above and herein.

[00205] In some embodiments, a is 1. In some embodiments, a is 2. In some embodiments, a is 3. In some embodiments, a is 4. In some embodiments, a is 5. In some embodiments, a is 6. In certain embodiments, d is 0. In certain embodiments, d is 1. In certain embodiments, d is 2. In certain embodiments, e is 0. In certain embodiments, e is 1. In certain embodiments, e is 2. In certain embodiments, e is 3. In certain embodiments, e is 4.

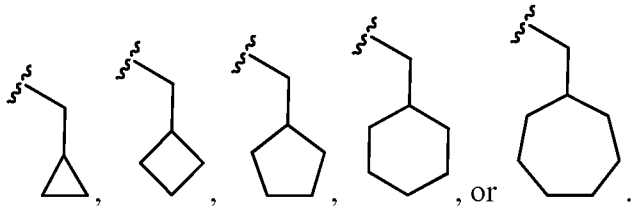
[00206] In certain embodiments, wherein R_2 is alkyl substituted with an optionally substituted carbocyclyl ring, R_2 is a group of the formula:



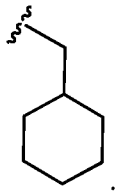
wherein a, d, and R^{BB} is as defined herein. In certain embodiments, a is 1. In certain embodiments, d is 0.

[00207] In certain embodiments, wherein R_2 is alkyl substituted with an optionally

substituted carbocyclyl ring, R_2 is a group of the formula:



[00208] However, in some embodiments, R_2 is not:



Embodiments of R_3

[00209] As generally defined above, each instance of R_3 is independently hydrogen; halogen; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; acyl; $-OR_C$; $-N(R_C)_2$; $-SR_C$; =O; -CN; $-NO_2$; -SCN; $-SOR_C$; or $-SO_2R_C$; wherein each occurrence of R_C is independently hydrogen; a protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; acyl; substituted or unsubstituted aryl; or substituted or unsubstituted heteroaryl.

[00210] In certain embodiments, R_3 is hydrogen. In certain embodiments, R_3 is halogen, *i.e.*, -F, -Cl, -Br, or –I. In certain embodiments, R_3 is $–OR_C$. In certain embodiments, R_3 is $-N(R_C)_2$. In certain embodiments, R_3 is $–SR_C$. In certain embodiments, R_3 is =O. In certain embodiments, R_3 is –CN. In certain embodiments, R_3 is $-NO_2$. In certain embodiments, R_3 is –SCN. In certain embodiments, R_3 is $–SOR_C$. In certain embodiments, R_3 is $-SO_2R_C$.

[00211] In certain embodiments, R_3 is acyl. For example, in certain embodiments, R_3 is acyl selected from the group consisting of $-C(=O)R^{C5}$, $-C(=O)OR^{C5}$, $-C(=O)SR^{C5}$, $-C(=O)N(R^{C6})_2$, $-C(=NR^{C6})R^{C5}$, $-C(=NR^{C6})OR^{C5}$, $-C(=NR^{C6})SR^{C5}$, $-C(=NR^{C6})N(R^{C6})_2$, $-C(=S)R^{C5}$, $-C(=S)OR^{C5}$, $-C(=S)SR^{C5}$, and $-C(=S)N(R^{C6})_2$, wherein each occurrence of R^{C5} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and each occurrence of R^{C6} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted

alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or a nitrogen protecting group, or two R^{C6} groups are joined to form an substituted or unsubstituted heterocyclic ring.

[00212] In certain embodiments, R_3 is substituted or unsubstituted aryl, *e.g.*, phenyl, napthyl. In certain embodiments, R_3 is substituted or unsubstituted phenyl. In certain embodiments, R_3 is substituted phenyl, *e.g.*, a monosubstituted, disubstituted, or trisubstituted phenyl.

[00213] In certain embodiments, R_3 is substituted or unsubstituted heteroaryl, *e.g.*, a substituted or unsubstituted 5- to 6-membered heteroaryl. In certain embodiments, R_2 is a substituted or unsubstituted 5-membered heteroaryl, *e.g.*, pyrrolyl, furanyl, thiophenyl, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, triazolyl, oxadiazolyl, thiadiazolyl, or tetrazolyl. In certain embodiments, R_3 is a substituted or unsubstituted 6-membered heteroaryl, *e.g.*, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, or tetrazinyl.

[00214] In certain embodiments, R_3 is substituted or unsubstituted aliphatic. In certain embodiments, R_3 is a substituted or unsubstituted aliphatic group comprising 1 to 10 carbon atoms ("C_{1-10}aliphatic"). In certain embodiments, R_3 is substituted or unsubstituted aliphatic group comprising 1 to 8 carbon atoms ("C_{1-8}aliphatic"). In certain embodiments, R_3 is a substituted or unsubstituted aliphatic group comprising 1 to 6 carbon atoms ("C_{1-6}aliphatic"). In certain embodiments, R_3 is a substituted or unsubstituted aliphatic group comprising 1 to 4 carbon atoms ("C_{1-4}aliphatic"). In certain embodiments, R_3 is a substituted or unsubstituted aliphatic group comprising 1 to 3 carbon atoms ("C_{1-3}aliphatic"). In certain embodiments, R_3 is a substituted or unsubstituted aliphatic group comprising 1 carbon atom ("C_1aliphatic"). In certain embodiments, R_3 is a substituted or unsubstituted aliphatic group comprising 2 carbon atoms ("C_2aliphatic"). In certain embodiments, R_3 is a substituted or unsubstituted aliphatic group comprising 3 carbon atoms ("C_3aliphatic"). In any of the above embodiments, R_3 is an acyclic aliphatic group. In any of the above embodiments, R_3 is an acyclic and substituted aliphatic group. In any of the above embodiments, the aliphatic group is an alkyl, alkenyl, or alkynyl group.

[00215] In certain embodiments, R_3 is substituted or unsubstituted alkyl. In certain embodiments, R_3 is unsubstituted alkyl. In certain embodiments, R_2 is substituted alkyl. In certain embodiments, R_3 is substituted C_{1-10}alkyl. In certain embodiments, R_3 is substituted C_{1-8}alkyl. In certain embodiments, R_3 is substituted C_{1-6}alkyl. In certain embodiments, R_3

is substituted C_{1-5}alkyl. In certain embodiments, R_3 is substituted C_{1-4}alkyl. In certain embodiments, R_3 is substituted C_{1-3}alkyl. In certain embodiments, R_3 is substituted C_{1-2}alkyl. In certain embodiments, R_3 is substituted C_{2-4}alkyl. In certain embodiments, R_2 is substituted C_1alkyl. In certain embodiments, R_3 is substituted C_2alkyl. In certain embodiments, R_3 is substituted C_3alkyl. In certain embodiments, R_3 is substituted C_4alkyl.

[00216] In certain embodiments, R_3 is substituted or unsubstituted alkenyl. In certain embodiments, R_3 is unsubstituted alkenyl. In certain embodiments, R_3 is substituted alkenyl. In certain embodiments, R_3 is substituted C_{2-10}alkenyl. In certain embodiments, R_3 is substituted C_{2-8}alkenyl. In certain embodiments, R_3 is substituted C_{2-6}alkenyl. In certain embodiments, R_3 is substituted C_{2-5}alkenyl. In certain embodiments, R_3 is substituted C_{2-4}alkenyl. In certain embodiments, R_3 is substituted C_{2-3}alkenyl. In certain embodiments, R_3 is substituted C_1alkenyl. In certain embodiments, R_3 is substituted C_2alkenyl. In certain embodiments, R_3 is substituted C_3alkenyl. In certain embodiments, R_3 is substituted C_4alkenyl.

[00217] In certain embodiments, R_3 is substituted or unsubstituted heteroaliphatic. In certain embodiments, R_3 is substituted or unsubstituted heteroaliphatic group comprising 1 to 10 carbon atoms and 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur ("C_{1-10}heteroaliphatic"). In certain embodiments, the heteroaliphatic group comprises 1 heteroatom selected from oxygen, nitrogen, and sulfur. In certain embodiments, the heteroaliphatic group comprises 2 heteroatoms independently selected from oxygen, nitrogen, and sulfur. In certain embodiments, the heteroaliphatic group comprises 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_3 is a substituted or unsubstituted heteroaliphatic group comprising 1 to 8 carbon atoms and 1-3 heteroatoms selected from oxygen, nitrogen, and sulfur ("C_{1-8}heteroaliphatic"). In certain embodiments, R_3 is a substituted or unsubstituted heteroaliphatic group comprising 1 to 6 carbon atoms and 1-2 heteroatoms selected from oxygen, nitrogen, and sulfur ("C_{1-6}heteroaliphatic"). In certain embodiments, R_3 is a substituted or unsubstituted heteroaliphatic group comprising 1 to 4 carbon atoms and 1-2 heteroatoms selected from oxygen, nitrogen, and sulfur ("C_{1-4}heteroaliphatic"). In certain embodiments, R_3 is a substituted or unsubstituted heteroaliphatic group comprising 1 to 3 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("C_{1-3}heteroaliphatic"). In certain embodiments, R_3 is a substituted or unsubstituted heteroaliphatic group comprising 1 carbon atom and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("C_1heteroaliphatic"). In certain embodiments, R_3 is a substituted or

unsubstituted heteroaliphatic group comprising 2 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("C_2heteroaliphatic"). In certain embodiments, R_3 is a substituted or unsubstituted heteroaliphatic group comprising 3 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("C_3heteroaliphatic"). In any of the above embodiments, R_3 is an acyclic heteroaliphatic group. In any of the above embodiments, R_3 is an acyclic and substituted heteroaliphatic group. In any of the above embodiments, the heteroaliphatic group is an heteroalkyl, heteroalkenyl, or heteroalkynyl group.

[00218] In certain embodiments, R_3 is substituted or unsubstituted heteroalkyl comprising 10 carbon atoms and 1-3 heteroatoms selected from oxygen, nitrogen, and sulfur ("substituted C_{1-10}heteroalkyl"). In certain embodiments, the heteroalkyl group comprises 1 heteroatom selected from oxygen, nitrogen, and sulfur. In certain embodiments, the heteroalkyl group comprises 2 heteroatoms independently selected from oxygen, nitrogen, and sulfur. In certain embodiments, the heteroalkyl group comprises 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_3 is a substituted heteroalkyl comprising 1-8 carbon atoms and 1-3 heteroatoms selected from oxygen, nitrogen, and sulfur ("substituted C_{1-8}heteroalkyl"). In certain embodiments, R_3 is a substituted heteroalkyl comprising 1-6 carbon atoms and 1-2 heteroatoms selected from oxygen, nitrogen, and sulfur ("substituted C_{1-6}heteroalkyl"). In certain embodiments, R_3 is a substituted heteroalkyl comprising 1-4 carbon atoms and 1-2 heteroatoms selected from oxygen, nitrogen, and sulfur ("substituted C_{1-4}heteroalkyl"). In certain embodiments, R_3 is a substituted heteroalkyl comprising 1-3 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("substituted C_{1-3}heteroalkyl"). In certain embodiments, R_3 is a substituted heteroalkyl comprising 1-2 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("substituted C_{1-2}heteroalkyl"). In certain embodiments, R_3 is a substituted heteroalkyl comprising 1 carbon atom and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("substituted C_1heteroalkyl"). In certain embodiments, R_3 is a substituted heteroalkyl comprising 2 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("substituted C_2heteroalkyl"). In certain embodiments, R_3 is a substituted heteroalkyl comprising 3 carbon atoms and 1 heteroatom selected from oxygen, nitrogen, and sulfur ("substituted C_3heteroalkyl"). In certain embodiments, R_3 is a substituted heteroalkyl comprising 4 carbon atoms and 1-2 heteroatoms selected from oxygen, nitrogen, and sulfur ("substituted C_4heteroalkyl").

[00219] As generally understood from the above, in certain embodiments, R_3 is a

group selected from aliphatic, heteroaliphatic, aryl, or heteroaryl which may be unsubstituted or substituted with one or more substituents. Exemplary R_3 group substituents, collectively referred to as "R^C", include, but are not limited to, halogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-OR^{C1}$, $-N(R^{C2})_2$, $-SR^{C1}$, $-C(=O)R^{C1}$, $-C(=O)OR^{C1}$, $-C(=O)SR^{C1}$, $-C(=O)N(R^{C2})_2$, $-OC(=O)R^{C1}$, $-OC(=O)OR^{C1}$, $-OC(=O)SR^{C1}$, $-OC(=O)N(R^{C2})_2$, $-NR^{C2}C(=O)R^{C2}$, $-NR^{C2}C(=O)OR^{C1}$, $-NR^{C2}C(=O)SR^{C1}$, $-NR^{C2}C(=O)N(R^{C2})_2$, $-SC(=O)R^{C1}$, $-SC(=O)OR^{C1}$, $-SC(=O)SR^{C1}$, $-SC(=O)N(R^{C2})_2$, $-C(=NR^{C2})R^{C1}$, $-C(=NR^{C2})OR^{C1}$, $-C(=NR^{C2})SR^{C1}$, $-C(=NR^{C2})N(R^{C2})_2$, $-OC(=NR^{C2})R^{C1}$, $-OC(=NR^{C2})OR^{C1}$, $-OC(=NR^{C2})SR^{C1}$, $-OC(=NR^{C2})N(R^{C2})_2$, $-NR^{C2}C(=NR^{C2})R^{C2}$, $-NR^{C2}C(=NR^{C2})OR^{C1}$, $-NR^{C2}C(=NR^{C2})SR^{C1}$, $-NR^{C2}C(=NR^{C2})N(R^{C2})_2$, $-SC(=NR^{C2})R^{C1}$, $-SC(=NR^{C2})OR^{C1}$, $-SC(=NR^{C2})SR^{C1}$, $-SC(=NR^{C2})N(R^{C2})_2$, $-C(=S)R^{C1}$, $-C(=S)OR^{C1}$, $-C(=S)SR^{C1}$, $-C(=S)N(R^{C2})_2$, $-OC(=S)R^{C1}$, $-OC(=S)OR^{C1}$, $-OC(=S)SR^{C1}$, $-OC(=S)N(R^{C2})_2$, $-NR^{C2}C(=S)R^{C2}$, $-NR^{C2}C(=S)OR^{C1}$, $-NR^{C2}C(=S)SR^{C1}$, $-NR^{C2}C(=S)N(R^{C2})_2$, $-SC(=S)R^{C1}$, $-SC(=S)OR^{C1}$, $-SC(=S)SR^{C1}$, $-SC(=S)N(R^{C2})_2$, $-S(=O)R^{C1}$, $-SO_2R^{C1}$, $-NR^{C2}SO_2R^{C1}$, $-SO_2N(R^{C2})_2$, $-N_3$, -CN, -SCN, and $-NO_2$, wherein each occurrence of R^{C1} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and each occurrence of R^{C2} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or a nitrogen protecting group, or two R^{C2} groups are joined to form an substituted or unsubstituted heterocyclic ring.

[00220] In certain embodiments, R_3 is aliphatic, as defined above and herein, substituted by one or more R^C substituents, as provided above, *e.g.,* 1, 2, 3, or 4 substituents. In certain embodiments, R_3 is alkyl, as defined above and herein, substituted by one or more R^C substituents, as provided above, *e.g.,* 1, 2, 3, or 4 substituents. In certain embodiments, R_3 is a C_{1-4}alkyl substituted by one or more R^C substituents, as provided above, *e.g.,* 1, 2, 3, or 4 substituents. In certain embodiments, R_3 is a C_{2-4}alkenyl substituted by one or more R^C substituents, as provided above, *e.g.,* 1, 2, 3, or 4 substituents.

[00221] For example, in certain embodiments, R_3 is aliphatic substituted by an

optionally substituted aryl or optionally substituted heteroaryl group, *e.g.*, an optionally substituted phenyl group or optionally substituted 5- to 6-membered heteroaryl group.

[00222] In certain embodiments, R_3 is aliphatic substituted by an optionally substituted aryl group ("optionally substituted arylaliphatic"). In certain embodiments, the aryl group is an optionally substituted phenyl ("optionally substituted phenylaliphatic"). In certain embodiments, the aryl group is unsubstituted. However, in certain embodiments, the aryl group is substituted. For example, in certain embodiments, the aryl group is a monosubstituted phenyl ring, *e.g.,* substituted at the *ortho*, *meta*, or *para* position of the phenyl ring relative to the point of attachment. In certain embodiments, the aryl group is a disubstituted phenyl ring, *e.g.,* substituted at the 1,2-, the 1,3-, the 1,4-, the 2,3-, the 3,4-, or the 2,4- positions of the phenyl ring relative to the point of attachment. In certain embodiments, the aryl group is a trisubstituted phenyl ring, *e.g.,* substituted at the 1,2,3-, the 1,2,4-, or the 2,3,4-, positions of the phenyl ring relative to the point of attachment. In certain embodiments, the aryl group is a tetrasubstituted phenyl ring. In any of the above embodiments, the aliphatic group is selected from alkyl, alkenyl, or alkynyl. In certain embodiments, the aliphatic group is alkyl. For example, in certain embodiments, R_3 is alkyl substituted by an optionally substituted aryl group ("optionally substituted arylalkyl"), *e.g.*, an optionally substituted phenyl group ("optionally substituted phenylalkyl"). In certain embodiments, R_3 is a C_{1-4}alkyl substituted by an optionally substituted aryl group, *e.g.*, an optionally substituted phenyl group. In certain embodiments, R_3 is a C_1alkyl substituted by an optionally substituted aryl group, *e.g.*, an optionally substituted phenyl group. In certain embodiments, the aliphatic group is alkenyl. For example, in certain embodiments, R_3 is a C_{2-4}alkenyl substituted by an optionally substituted aryl group ("optionally substituted arylalkenyl"), *e.g.*, an optionally substituted phenyl group ("optionally substituted phenylalkenyl"). In certain embodiments, R_3 is a C_2alkenyl substituted by an optionally substituted aryl group, *e.g.*, an optionally substituted phenyl group. In certain embodiments, R_3 is a C_3alkenyl substituted by an optionally substituted aryl group, *e.g.*, an optionally substituted phenyl group.

[00223] In certain embodiments, R_3 is aliphatic substituted by an optionally substituted 5- to 6-membered heteroaryl group, *e.g.,* a substituted or unsubstituted 5-membered heteroaryl, *e.g.*, pyrrolyl, furanyl, thiophenyl, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, triazolyl, oxadiazolyl, thiadiazolyl, or tetrazolyl, or a substituted or unsubstituted 6-membered heteroaryl, *e.g.*, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, or tetrazinyl. In certain embodiments, the heteroaryl group is a

substituted 5- to 6-membered heteroaryl ("substituted 5- to 6-membered heteroarylaliphatic"). In any of the above embodiments, the aliphatic group is selected from alkyl, alkenyl, or alkynyl. In certain embodiments, the aliphatic group is alkyl. For example, in certain embodiments, R_3 is alkyl substituted by a substituted by an optionally substituted heteroaryl group ("optionally substituted heteroarylalkyl"), *e.g.*, an optionally substituted 5- to 6-membered heteroaryl group ("optionally substituted 5- to 6-membered heteroarylalkyl"). In certain embodiments, R_3 is a C_{1-4}alkyl substituted by an optionally substituted heteroaryl group, *e.g.*, an optionally substituted 5- to 6-membered heteroaryl group. In certain embodiments, R_3 is a C_1alkyl substituted by an optionally substituted heteroaryl group, *e.g.*, an optionally substituted 5- to 6-membered heteroaryl group. In certain embodiments, R_3 is a C_{2-4}alkenyl substituted by an optionally substituted heteroaryl group, *e.g.*, an optionally substituted 5- to 6-membered heteroaryl group. In certain embodiments, R_3 is a C_2alkenyl substituted by an optionally substituted heteroaryl group, *e.g.*, an optionally substituted 5- to 6-membered heteroaryl group. In certain embodiments, R_3 is a C_3alkenyl substituted by an optionally substituted heteroaryl group, *e.g.*, an optionally substituted 5- to 6-membered heteroaryl group. In any of the above embodiments, the heteroaryl group is substituted. However, in any of the above embodiments, the heteroaryl group is unsubstituted.

[00224] In certain embodiments, R_3 is aliphatic substituted by a substituted or unsubstituted carbocyclyl ("optionally substituted carbocylcyl") or a substituted or unsubstituted heterocyclyl ("optionally substituted heterocyclyl"), *e.g.*, optionally substituted C_{3-8}carbocyclyl or optionally substituted 5- to 8-membered heterocyclyl.

[00225] For example, in certain embodiments, R_3 is aliphatic substituted by an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_3 is aliphatic substituted by an optionally substituted 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, *e.g.,* azocanyl, oxecanyl, or thiocanyl. In certain embodiments, R_3 is aliphatic substituted by an optionally substituted 7-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, *e.g.*, azepanyl, oxepanyl, or thiepanyl. In certain embodiments, R_3 is aliphatic substituted by an optionally substituted 6-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, *e.g.*, piperazinyl, morpholinyl, dithianyl, dioxanyl, triazinanyl, and 6,6-bicycles such as tetrahydroquinolinyl and tetrahydroisoquinolinyl. In certain embodiments, R_3 is aliphatic substituted by an optionally substituted 5-membered heterocyclyl comprising 1 to 3

heteroatoms selected from oxygen, nitrogen, and sulfur, *e.g.*, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothiophenyl, dihydrothiophenyl, pyrrolidinyl, dihydropyrrolyl, pyrrolyl–2,5–dione, dioxolanyl, oxasulfuranyl, disulfuranyl, oxazolidin-2-one, triazolinyl, oxadiazolinyl, thiadiazolinyl, and 5,6-bicycles such as indolinyl, isoindolinyl, dihydrobenzofuranyl, dihydrobenzothienyl, and benzoxazolinonyl. In any of the above embodiments, the heterocyclyl contains 1 heteroatom. In any of the above embodiments, the heterocyclyl contains 2 heteroatoms. In any of the above embodiments, the heterocyclyl contains 3 heteroatoms. In any of the above embodiments, the aliphatic group is selected from alkyl, alkenyl, or alkynyl. In certain embodiments, the aliphatic group is alkyl. For example, in certain embodiments, R_3 is alkyl substituted by a substituted by an optionally substituted heterocyclyl group ("optionally substituted heterocyclylalkyl"), *e.g.*, an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_3 is a C_{1-4}alkyl substituted by an optionally substituted heterocyclyl group, *e.g.*, an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_3 is a C_1alkyl substituted by an optionally substituted heterocyclyl group, *e.g.*, an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_3 is a C_{2-4}alkenyl substituted by an optionally substituted heterocyclyl group, *e.g.*, an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_3 is a C_2alkenyl substituted by an optionally substituted heterocyclyl group, *e.g.*, an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In certain embodiments, R_3 is a C_3alkenyl substituted by an optionally substituted heterocyclyl group, *e.g.*, an optionally substituted 5- to 8-membered heterocyclyl comprising 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. In any of the above embodiments, the heterocyclyl group is not substituted. However, in any of the above embodiments, the heterocyclyl group is substituted.

[00226] In certain embodiments, R_3 is aliphatic substituted by an optionally substituted C_{3-7}carbocyclyl. In certain embodiments, R_3 is aliphatic substituted by an optionally substituted C_{3-6}carbocyclyl. In certain embodiments, R_3 is aliphatic substituted by an optionally substituted C_{3-5}carbocyclyl. In certain embodiments, R_3 is aliphatic substituted by an optionally substituted C_{3-4}carbocyclyl. In certain embodiments, R_3 is aliphatic substituted by an optionally substituted C_{4-6}carbocyclyl. In certain embodiments, R_3 is aliphatic

substituted by an optionally substituted C_3carbocyclyl, *e.g.*, optionally substituted cyclopropyl or optionally substituted cyclopropenyl. In certain embodiments, R_3 is aliphatic substituted by an optionally substituted C_4carbocyclyl, *e.g.*, optionally substituted cyclobutyl or optionally substituted cyclobutenyl. In certain embodiments, R_3 is aliphatic substituted by an optionally substituted C_5carbocyclyl, *e.g.*, optionally substituted cyclopentyl or optionally substituted cyclopentenyl. In certain embodiments, R_3 is aliphatic substituted by an optionally substituted C_6carbocyclyl, *e.g.*, optionally substituted cyclohexyl, optionally substituted cyclohexenyl, or optionally substituted cyclohexadienyl. In certain embodiments, R_3 is aliphatic substituted by an optionally substituted C_7carbocyclyl, *e.g.*, optionally substituted cycloheptyl, optionally substituted cycloheptenyl, optionally substituted cycloheptadienyl, or optionally substituted cycloheptatrienyl. In any of the above embodiments, the aliphatic group is selected from alkyl, alkenyl, or alkynyl. In certain embodiments, the aliphatic group is alkyl. For example, in certain embodiments, R_3 is alkyl substituted by a substituted by an optionally substituted carbocyclyl group ("optionally substituted carbocyclylalkyl"), *e.g.*, an optionally substituted C_{3-7}carbocyclyl group. In certain embodiments, R_3 is a C_{1-4}alkyl substituted by an optionally substituted carbocyclyl group, *e.g.*, an optionally substituted C_{3-7}carbocyclyl. In certain embodiments, R_3 is a C_1alkyl substituted by an optionally substituted carbocyclyl group, *e.g.*, an optionally substituted C_{3-7}carbocyclyl. In certain embodiments, R_3 is a C_{2-4}alkenyl substituted by an optionally substituted carbocyclyl group, *e.g.*, an optionally substituted C_{3-7}carbocyclyl. In certain embodiments, R_3 is a C_2alkenyl substituted by an optionally substituted carbocyclyl group, *e.g.*, an optionally substituted C_{3-7}carbocyclyl. In certain embodiments, R_3 is a C_3alkenyl substituted by an optionally substituted carbocyclyl group, *e.g.*, an optionally substituted C_{3-7}carbocyclyl. In any of the above embodiments, the carbocyclyl group is not substituted. However, in any of the above embodiments, the carbocyclyl group is substituted.

[00227] As understood from the above, the carbocyclyl, heterocyclyl, aryl, and/or heteroaryl substituents present on the aliphatic group of R_3 may be unsubstituted or substituted with one or more substituents. Such substituents, collectively referred to herein as "R^{CC}", include, but are not limited to, halogen, substituted or unsubstituted alkyl (*e.g.*, perhaloalkyl), substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-OR^{C3}$, $-N(R^{C4})_2$, $-SR^{C3}$, $-C(=O)R^{C3}$, $-C(=O)OR^{C3}$, $-C(=O)SR^{C3}$, $-C(=O)N(R^{C4})_2$, $-OC(=O)R^{C3}$, $-OC(=O)OR^{C3}$, $-OC(=O)SR^{C3}$, $-OC(=O)N(R^{C4})_2$, $-NR^{C4}C(=O)R^{C4}$, $-NR^{C4}C(=O)OR^{C3}$, $-NR^{C4}C(=O)SR^{C3}$, -

$NR^{C4}C(=O)N(R^{C4})_2$, $-SC(=O)R^{C3}$, $-SC(=O)OR^{C3}$, $-SC(=O)SR^{C3}$, $-SC(=O)N(R^{C4})_2$, $-C(=NR^{C4})R^{C3}$, $-C(=NR^{C4})OR^{C3}$, $-C(=NR^{C4})SR^{C3}$, $-C(=NR^{C4})N(R^{C4})_2$, $-OC(=NR^{C4})R^{C3}$, $-OC(=NR^{C4})OR^{C3}$, $-OC(=NR^{C4})SR^{C3}$, $-OC(=NR^{C4})N(R^{C4})_2$, $-NR^{C4}C(=NR^{C4})R^{C2}$, $-NR^{C4}C(=NR^{C4})OR^{C3}$, $-NR^{C4}C(=NR^{C4})SR^{C3}$, $-NR^{C4}C(=NR^{C4})N(R^{C4})_2$, $-SC(=NR^{C4})R^{C3}$, $-SC(=NR^{C4})OR^{C3}$, $-SC(=NR^{C4})SR^{C3}$, $-SC(=NR^{C4})N(R^{C4})_2$, $-C(=S)R^{C3}$, $-C(=S)OR^{C3}$, $-C(=S)SR^{C3}$, $-C(=S)N(R^{C4})_2$, $-OC(=S)R^{C3}$, $-OC(=S)OR^{C3}$, $-OC(=S)SR^{C3}$, $-OC(=S)N(R^{C4})_2$, $-NR^{C4}C(=S)R^{C4}$, $-NR^{C4}C(=S)OR^{C3}$, $-NR^{C4}C(=S)SR^{C3}$, $-NR^{C4}C(=S)N(R^{C4})_2$, $-SC(=S)R^{C3}$, $-SC(=S)OR^{C3}$, $-SC(=S)SR^{C3}$, $-SC(=S)N(R^{C4})_2$, $-S(=O)R^{C3}$, $-SO_2R^{C3}$, $-NR^{C4}SO_2R^{C3}$, $-SO_2N(R^{C4})_2$, $-N_3$, -CN, -SCN, and $-NO_2$, wherein each occurrence of R^{C3} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and each occurrence of R^{C4} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or a nitrogen protecting group, or two R^{C4} groups are joined to form an substituted or unsubstituted heterocyclic ring.

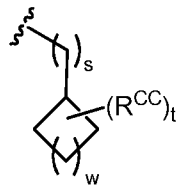
[00228] In certain embodiments, at least one R^{CC} substituent (*e.g.*, 1, 2, 3, or 4 R^{CC} substituents) is an electron-withdrawing group, *e.g.*, a substituent which pulls electron density away from the parent molecule (*e.g.*, a ring system) and/or stabilizes anions or electron rich structures. Exemplary electron-withdrawing substituents include, but are not limited to, halogen, perhaloalkyl, $-C(=O)R^{C3}$, $-C(=O)OR^{C3}$, $-C(=O)SR^{C3}$, $-C(=O)N(R^{C4})_2$, $-OC(=O)R^{C3}$, $-OC(=O)OR^{C3}$, $-OC(=O)SR^{C3}$, $-OC(=O)N(R^{C4})_2$, $-NR^{C4}C(=O)R^{C4}$, $-NR^{C4}C(=O)OR^{C3}$, $-NR^{C4}C(=O)SR^{C3}$, $-NR^{C4}C(=O)N(R^{C4})_2$, $-SC(=O)R^{C3}$, $-SC(=O)OR^{C3}$, $-SC(=O)SR^{C3}$, $-SC(=O)N(R^{C4})_2$, $-C(=NR^{C4})R^{C3}$, $-C(=NR^{C4})OR^{C3}$, $-C(=NR^{C4})SR^{C3}$, $-C(=NR^{C4})N(R^{C4})_2$, $-OC(=NR^{C4})R^{C3}$, $-OC(=NR^{C4})OR^{C3}$, $-OC(=NR^{C4})SR^{C3}$, $-OC(=NR^{C4})N(R^{C4})_2$, $-NR^{C4}C(=NR^{C4})R^{C4}$, $-NR^{C4}C(=NR^{C4})OR^{C3}$, $-NR^{C4}C(=NR^{C4})SR^{C3}$, $-NR^{C4}C(=NR^{C4})N(R^{C4})_2$, $-SC(=NR^{C4})R^{C3}$, $-SC(=NR^{C4})OR^{C3}$, $-SC(=NR^{C4})SR^{C3}$, $-SC(=NR^{C4})N(R^{C4})_2$, $-C(=S)R^{C3}$, $-C(=S)OR^{C3}$, $-C(=S)SR^{C3}$, $-C(=S)N(R^{C4})_2$, $-OC(=S)R^{C3}$, $-OC(=S)OR^{C3}$, $-OC(=S)SR^{C3}$, $-OC(=S)N(R^{C4})_2$, $-NR^{C4}C(=S)R^{C4}$, $-NR^{C4}C(=S)OR^{C3}$, $-NR^{C4}C(=S)SR^{C3}$, $-NR^{C4}C(=S)N(R^{C4})_2$, $-SC(=S)R^{C3}$, $-SC(=S)OR^{C3}$, $-SC(=S)SR^{C3}$, $-SC(=S)N(R^{C4})_2$, $-S(=O)R^{C3}$, $-SO_2R^{C3}$, $-NR^{C4}SO_2R^{C3}$, $-SO_2N(R^{C4})_2$, -CN, -SCN, and $-NO_2$, wherein R^{C3} and R^{C4} are as defined herein.

[00229] However, in certain embodiments, at least one R^{CC} substituent (*e.g.*, 1, 2, 3, or 4 R^{BB} substituents) is an electron-donating group, *e.g.*, a substituent which donates electron density toward the parent molecule (*e.g.,* a ring system) and/or stabilizes cations or electron poor structures. Exemplary electron-donating substituents include, but are not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-OR^{C3}$, $-N(R^{C4})_2$, $-SR^{C3}$, wherein R^{C3} and R^{C4} are as defined herein.

[00230] Specific combinations of groups comprising R_3 are further contemplated.

[00231] In certain embodiments, R_3 is alkyl substituted by an optionally substituted C_{3-7}carbocyclyl group, *e.g.*, an optionally substituted cyclopropyl, cyclopropenyl, cyclobutyl, cyclobutenyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cyclohexadienyl, cycloheptyl, cycloheptenyl, cycloheptadienyl, or cycloheptatrienyl group. In certain embodiments, R_3 is alkyl substituted by an saturated carbocyclyl group, *e.g.*, an optionally substituted cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, or cycloheptyl group. In certain embodiments, R_3 is alkyl substituted by an optionally substituted cyclopropyl group ("optionally substituted cyclopropylalkyl"). In certain embodiments, R_3 is alkyl substituted by an optionally substituted cyclobutyl group ("optionally substituted cyclobutylalkyl"). In certain embodiments, R_3 is alkyl substituted by an optionally substituted cyclopentyl group ("optionally substituted cyclopentylalkyl"). In certain embodiments, R_3 is alkyl substituted by an optionally substituted cyclohexyl group ("optionally substituted cyclohexylalkyl"). In certain embodiments, R_3 is alkyl substituted by an optionally substituted cycloheptyl group ("optionally substituted cycloheptylalkyl").

[00232] In certain embodiments, wherein R_3 is alkyl substituted with an optionally substituted carbocyclyl ring, R_3 is a group of the formula:



wherein:

s is an integer between 1 and 10, inclusive;

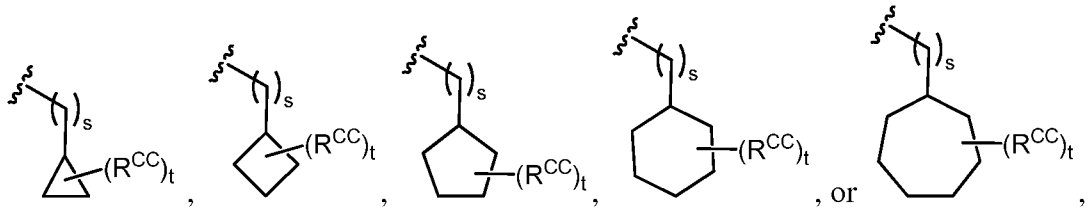
t is 0, 1, or 2;

w is 0 or an integer between 1 and 4, inclusive; and

each instance of R^{BB} is independently as defined above and herein.

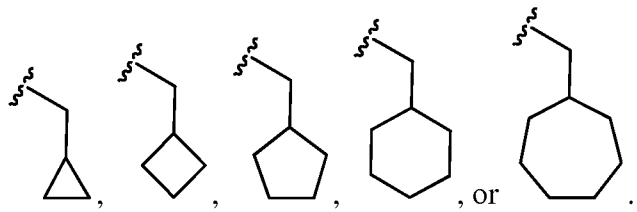
[00233] In some embodiments, s is 1. In some embodiments, s is 2. In some embodiments, s is 3. In some embodiments, s is 4. In some embodiments, s is 5. In some embodiments, s is 6. In some embodiments, s is 7. In some embodiments, s is 8. In some embodiments, s is 9. In some embodiments, s is 10. In some embodiments, s is an integer between 2 and 5, inclusive. In certain embodiments, t is 0. In certain embodiments, t is 1. In certain embodiments, t is 2. In certain embodiments, w is 0. In certain embodiments, w is 1. In certain embodiments, w is 2. In certain embodiments, w is 3. In certain embodiments, w is 4.

[00234] In certain embodiments, wherein R_3 is alkyl substituted with an optionally substituted carbocyclyl ring, R_3 is a group of the formula:

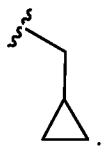


wherein s, t, and R^{CC} is as defined herein. In certain embodiments, s is 1. In certain embodiments, t is 0.

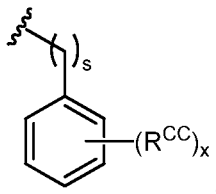
[00235] In certain embodiments, wherein R_3 is alkyl substituted with an optionally substituted carbocyclyl ring, R_3 is a group of the formula:



[00236] However, in some embodiments, R_3 is not:



[00237] In certain embodiments, R_3 is an optionally substituted arylalkyl group of the formula:



wherein:

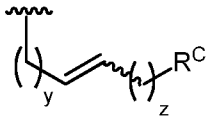
s is an integer between 1 and 10, inclusive;

x is 0 or an integer between 1 and 5, inclusive; and

each instance of R^{CC} is as defined herein.

[00238] In some embodiments, s is 1. In some embodiments, s is 2. In some embodiments, s is 3. In some embodiments, s is 4. In some embodiments, s is 5. In some embodiments, s is 6. In some embodiments, s is 7. In some embodiments, s is 8. In some embodiments, s is 9. In some embodiments, s is 10. In some embodiments, s is an integer between 2 and 5, inclusive. In certain embodiments, x is 0. In certain embodiments, x is 1. In certain embodiments, x is 2. In certain embodiments, x is 3. In certain embodiments, x is 4. In certain embodiments, x is 5. In certain embodiments, s is 1 and x is 0.

[00239] In certain embodiments, R_3 is an optionally substituted alkenyl group of the formula:



wherein:

y is 0 or an integer between 1 and 10, inclusive;

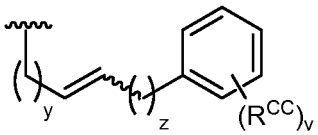
z is 0 or an integer between 1 and 4, inclusive;

provided that when z is > 0 and R^C is not hydrogen, the ∿∿∿ indicates that the adjacent C-C double bond is in a *cis* or *trans* configuration; and

R^C is hydrogen or as defined herein.

[00240] In certain embodiments, y is 0. In certain embodiments, y is 1. In certain embodiments, y is 2. In certain embodiments, y is 3. In certain embodiments, y is 4. In certain embodiments, y is 5. In certain embodiments, y is 6. In certain embodiments, y is 7. In certain embodiments, y is 8. In certain embodiments, y is 9. In certain embodiments, y is 10. In certain embodiments, z is 0. In certain embodiments, z is 1. In certain embodiments, z is 2. In certain embodiments, z is 3. In certain embodiments, z is 4. In certain embodiments, wherein z is > 0 and R^C is not hydrogen, the ∿∿∿ indicates that the adjacent C-C double bond is in a *cis* configuration. In certain embodiments, wherein z is > 0 and R^C is not hydrogen, the ∿∿∿ indicates that the adjacent C-C double bond is in a *trans* configuration. In certain embodiments, R^C is hydrogen. In certain embodiments, R^C is optionally substituted aryl.

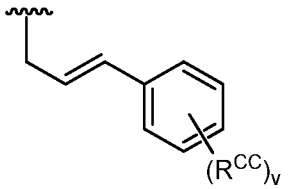
[00241] In certain embodiments of the above formula, wherein R^C is optionally substituted aryl, R_3 is an optionally substituted alkenylaryl group of the formula:



wherein y, z, and R^{CC} are as defined herein, and v is 0 or an integer between 1 and 5, inclusive.

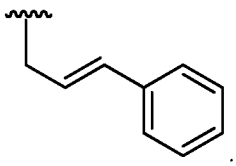
[00242] In certain embodiments, v is 0. In certain embodiments, v is 1. In certain embodiments, v is 2. In certain embodiments, v is 3. In certain embodiments, v is 4. In certain embodiments, v is 5.

[00243] In certain embodiments of the above formula, wherein R^C is optionally substituted aryl, R_3 is an optionally substituted alkenylaryl group of the formula:

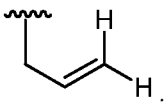


wherein y, z, v, and R^{CC} are as defined herein.

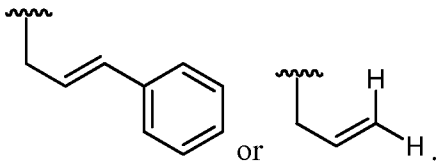
[00244] In certain embodiments, R_3 is



[00245] In certain embodiments, R_3 is



[00246] However, in certain embodiments, R_3 is not



Other Embodiments

[00247] As generally defined above, R_4 is substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; $-N(R_D)_2$; $-OR_D$; or $-SR_D$; wherein each occurrence of R_D is independently independently hydrogen; a protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; acyl; substituted or unsubstituted aryl;

or substituted or unsubstituted heteroaryl; or two R_D groups are joined to form a substituted or unsubstituted heterocyclic group; optionally wherein R^4 further comprises a label, resin, or therapeutic agent attached thereto.

[00248] In certain embodiments, R_4 is substituted or unsubstituted aliphatic.

[00249] In certain embodiments, R_4 is substituted or unsubstituted heteroaliphatic.

[00250] In certain embodiments, R_4 is substituted or unsubstituted aryl.

[00251] In certain embodiments, R_4 is substituted or unsubstituted heteroaryl.

[00252] In certain embodiments, R_4 is $-N(R_D)_2$. In certain embodiments, R_4 is $-NH(R_D)$. In certain embodiments, R_4 is $-NH_2$. In certain embodiments, R_D is hydrogen or substituted or unsubstituted alkyl, *e.g.*, C_{1-6} alkyl. In certain embodiments, R_D is hydrogen or substituted or unsubstituted C_{1-3} alkyl, *e.g.*, hydrogen, methyl, ethyl, n-propyl, or isopropyl.

[00253] In certain embodiments, R_4 is $-OR_D$. In certain embodiments, R_4 is -OH. In certain embodiments, R_D is hydrogen or substituted or unsubstituted alkyl, *e.g.*, C_{1-6} alkyl. In certain embodiments, R_D is hydrogen or substituted or unsubstituted C_{1-3} alkyl, *e.g.*, hydrogen, methyl, ethyl, n-propyl, or isopropyl.

[00254] In certain embodiments, R_4 is $-SR_D$. In certain embodiments, R_4 is -SH. In certain embodiments, R_D is hydrogen or substituted or unsubstituted alkyl, *e.g.*, C_{1-6} alkyl. In certain embodiments, R_D is hydrogen or substituted or unsubstituted C_{1-3} alkyl, *e.g.*, hydrogen, methyl, ethyl, n-propyl, or isopropyl.

[00255] It is understood from the above that in certain embodiments, R_4 is comprises a label, resin, or therapeutic agent attached thereto, *e.g.*, (i) the label, resin, or therapeutic agent is directly attached to the macrocycle *via* a bond; (ii) the label, resin, or therapeutic agent is attached to an R_4 group, *e.g.,* a substituted aliphatic, substituted heteroaliphatic, substituted aryl, or substituted heteroaryl wherein the substituent covalently or non-covalently attached thereto is a resin, a label, or a therapeutic agent; (iii) the label, resin, or therapeutic agent is attached an R^D group, wherein R^D is substituted aliphatic, substituted heteroaliphatic, substituted aryl, or substituted heteroaryl wherein the substituent covalently or non-covalently attached thereto is a resin, a label, or a therapeutic agent; or (iv) R^D is a label, resin, or therapeutic agent. In certain embodiments, at least one R^D is a label, resin, or therapeutic agent. In certain embodiments, the substituent is a resin. In certain embodiments, the substituent is a label. In certain embodiments, the substituent is a therapeutic agent. In certain embodiments, the therapeutic agent is a small molecule, *i.e.*, having a molecular weight under 800 g/mol, under 700 g/mol, under 600 g/mol, under 500 g/mol, under 400 g/mol, under 300 g/mol, under 200 g/mol, under 100 g/mol, under 50 g/mol, under 40 g/mol,

under 30 g/mol, under 20 g/mol, or under 15 g/mol, *e.g.*, between 15 g/mol and 800 g/mol. In certain embodiments, the therapeutic agent is an anti-cancer agent.

[00256] As generally defined above, each occurrence of R_E, R_F, R_G, R_H, and R_I is independently hydrogen; acyl; a nitrogen protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; substitute or unsubstituted hydroxyl; substituted or unsubstituted thiol; substituted or unsubstituted amino; or halogen; optionally wherein an R_1 group and R^F are joined to form a substituted or unsubstituted heterocyclic ring; an R_2 group and R^G are joined to form a substituted or unsubstituted heterocyclic ring; and/or an R_3 group and R^H are joined to form a substituted or unsubstituted heterocyclic ring.

[00257] In certain embodiments, each occurrence of R_E, R_F, R_G, R_H, and R_I is independently hydrogen, a nitrogen protecting group, or substituted or unsubstituted aliphatic. In certain embodiments, each occurrence of R_E, R_F, R_G, R_H, and R_I is independently hydrogen, a nitrogen protecting group, or substituted or unsubstituted alkyl. In certain embodiments, each occurrence of R_E, R_F, R_G, R_H, and R_I is independently hydrogen or alkyl (*e.g.*, $-CH_3$). In certain embodiments, each occurrence of R_E, R_F, R_G, R_H, and R_I is independently hydrogen.

[00258] In certain embodiments, an R_1 group and R^F are joined to form a substituted or unsubstituted heterocyclic ring; *e.g.,* an optionally substituted proline ring.

[00259] In certain embodiments, an R_2 group and R^G are joined to form a substituted or unsubstituted heterocyclic ring, *e.g.,* an optionally substituted proline ring.

[00260] In certain embodiments, an R_3 group and R^H are joined to form a substituted or unsubstituted heterocyclic ring; *e.g.,* an optionally substituted proline ring.

[00261] As generally defined above, n is 0 or an integer between 1-4, inclusive. In certain embodiments, n is an integer between 1-4, inclusive. In certain embodiments, n is an integer between 2-4, inclusive. In certain embodiments, n is an integer between 2-3, inclusive. In certain embodiments, n is an integer between 3-4, inclusive. In certain embodiments, n is 0. In certain embodiments, n is 1. In certain embodiments, n is 2. In certain embodiments, n is 3. In certain embodiments, n is 4.

[00262] As generally defined above, m is 0 or an integer between 1-4, inclusive. In certain embodiments, m is an integer between 1-4, inclusive. In certain embodiments, m is an integer between 2-4, inclusive. In certain embodiments, m is an integer between 2-3, inclusive. In certain embodiments, m is an integer between 3-4, inclusive. In certain

embodiments, m is 0. In certain embodiments, m is 1. In certain embodiments, m is 2. In certain embodiments, m is 3. In certain embodiments, m is 4.

[00263] In certain embodiments, n + m ≤ 4. In certain embodiments, n + m ≤ 3. In certain embodiments, n + m ≤ 2. In certain embodiments, n is an integer between 2-4, inclusive, and m is 0. In certain embodiments, n is 2, and m is 0. In certain embodiments, n is 3, and m is 0. In certain embodiments, n is 4, and m is 0.

[00264] As generally defined above, f is an integer between 1-3, inclusive. In certain embodiments, f is 1. In certain embodiments, f is 2. In certain embodiments, f is 3.

[00265] As generally defined above, g is an integer between 1-3, inclusive. In certain embodiments, g is 1. In certain embodiments, g is 2. In certain embodiments, g is 3.

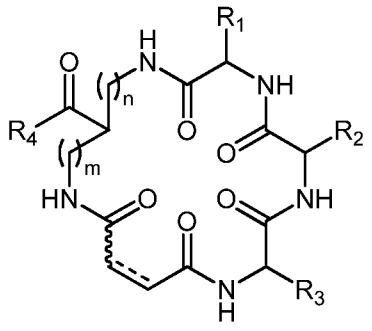
[00266] As generally defined above, h is an integer between 1-3, inclusive. In certain embodiments, h is 1. In certain embodiments, h is 2. In certain embodiments, h is 3.

[00267] As generally defined above, ------ represents a single or double C-C bond. In certain embodiments, ------ represents a single C-C bond. In certain embodiments, ------ represents a double C-C bond. Furthermore, as generally defined above, when ------ represents a double C-C bond, ∿∿∿ indicates that the adjacent C-C double bond in a *cis* or *trans* configuration. In some embodiments, ∿∿∿ represents an adjacent C-C double bond in the *cis* configuration. In other embodiments, ∿∿∿ represents an adjacent C-C double bond in the *trans* configuration.

Additional Embodiments of Formula (I)

[00268] Various embodiments of Formula (I) are futher contemplated herein.

[00269] In certain embodiments of Formula (I), when f is 1, g is 1, h is 1, R^E, R^F, R^G, R^H, and R^I, are hydrogen, the compound is of Formula (I-a):

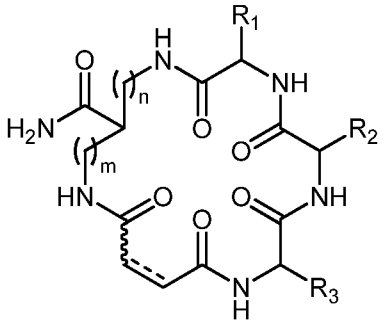


(I-a)

or a pharmaceutically acceptable salt thereof, wherein ------, ∿∿∿, R_1, R_2, R_3, n, and m are as defined herein.

[00270] In certain embodiments of Formula (I-a), when R_4 is $-NH_2$, the compound is

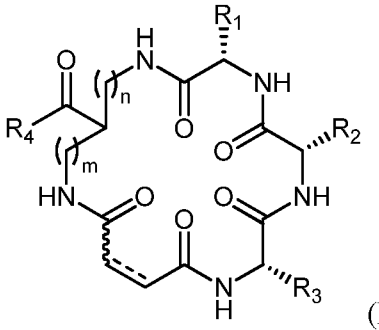
of Formula (I-b):



(I-b)

or a pharmaceutically acceptable salt thereof, wherein ======, ∿∿∿, R_1, R_2, R_3, n, and m are as defined herein.

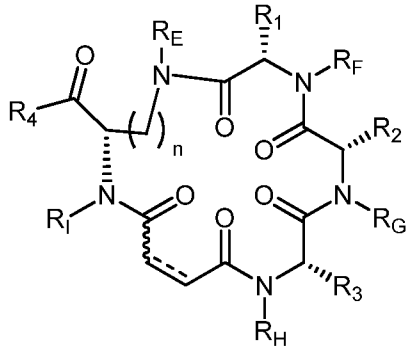
[00271] In certain embodiments of Formula (I-b), the compound is of Formula (I-c):



(I-c)

or a pharmaceutically acceptable salt thereof, wherein ======, ∿∿∿, R_1, R_2, R_3, R_4, n, and m are as defined herein.

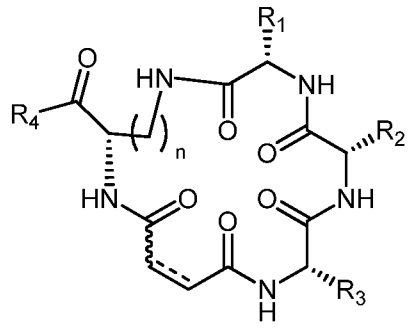
[00272] In certain embodiments of Formula (I), when f is 1, g is 1, h is 1, and m is 0, provided is a compound of Formula (II):



(II)

or a pharmaceutically acceptable salt thereof, wherein ======, ∿∿∿, R_1, R_2, R_3, R_4, R^E, R^F, R^G, R^H, R^I, n, and m are as defined herein.

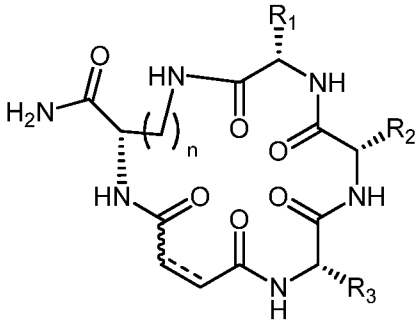
[00273] In certain embodiments of Formula (II), when R^E, R^F, R^G, R^H, and R^I, are hydrogen, the compound is of Formula (II-a):



(II-a)

or a pharmaceutically acceptable salt thereof, wherein ======, ∿∿∿, R_1, R_2, R_3, R_4, n, and m are as defined herein.

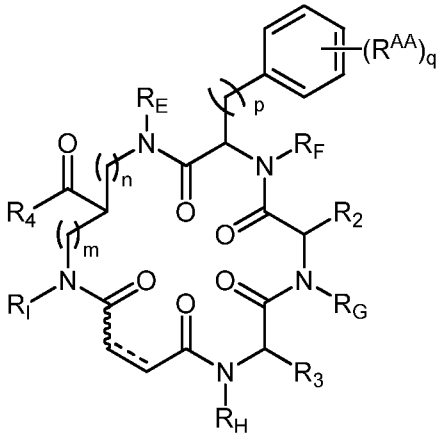
[00274] In certain embodiments of Formula (II-a), when R_4 is $-NH_2$, the compound is of Formula (II-b):



(II-b)

or a pharmaceutically acceptable salt thereof, wherein ======, ∿∿∿, R_1, R_2, R_3, n, and m are as defined herein.

[00275] In certain embodiments of Formula (I), when f is 1, g is 1, h is 1, and R_1 is an optionally substituted arylalkyl group, provided is a compound of Formula (III):

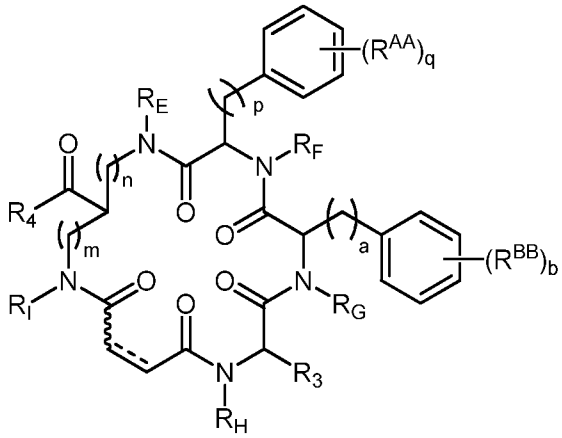


(III)

or a pharmaceutically acceptable salt thereof, wherein ======, ∿∿∿, R^{AA}, p, q, R_2, R_3, R^E, R^F, R^G, R^H, R^I, n, and m are as defined herein.

[00276] In certain embodiments of Formula (I), when f is 1, g is 1, h is 1, R_1 is an optionally substituted arylalkyl group and R_2 is an optionally substituted arylalkyl group,

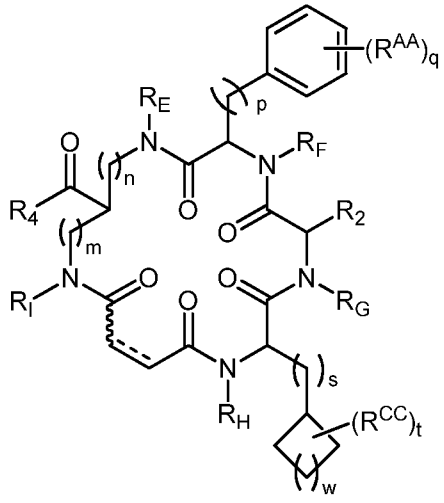
provided is a compound of Formula (IV):



(IV)

or a pharmaceutically acceptable salt thereof, wherein ======, ~~~~, R^{AA}, p, q, R^{BB}, a, b, R_3, R^E, R^F, R^G, R^H, R^I, n, and m are as defined herein.

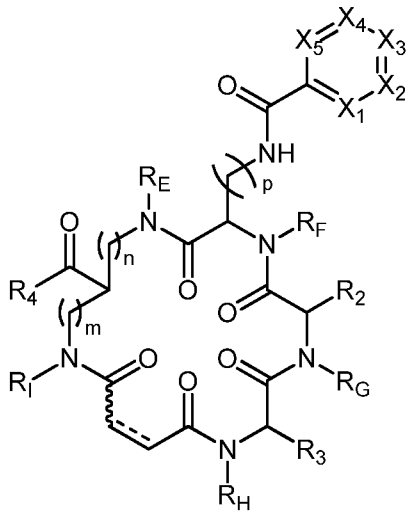
[00277] In certain embodiments of Formula (I), when f is 1, g is 1, h is 1, R_1 is an optionally substituted arylalkyl group, and R_3 is an optionally substituted carbocyclyl group, provided is a compound of Formula (V):



(V)

or a pharmaceutically acceptable salt thereof, wherein ======, ~~~~, R^{AA}, p, q, R_2, R^{CC}, t, w, R^E, R^F, R^G, R^H, R^I, n, and m are as defined herein.

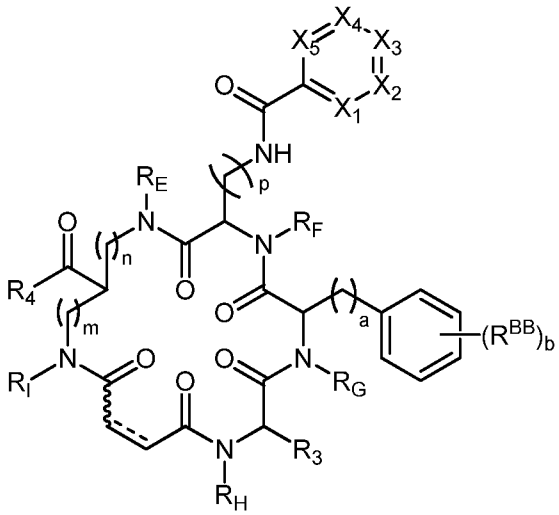
[00278] In certain embodiments of Formula (I), when f is 1, g is 1, h is 1, and R_1 is alkyl substituted by $-NR^{A2}C(=O)R^{A2}$, provided is a compound of Formula (VI):



(VI)

or a pharmaceutically acceptable salt thereof, wherein ======, ∿∿∿, X_1, X_2, X_3, X_4, X_5, p, R_2, R_3, R^E, R^F, R^G, R^H, R^I, n, and m are as defined herein.

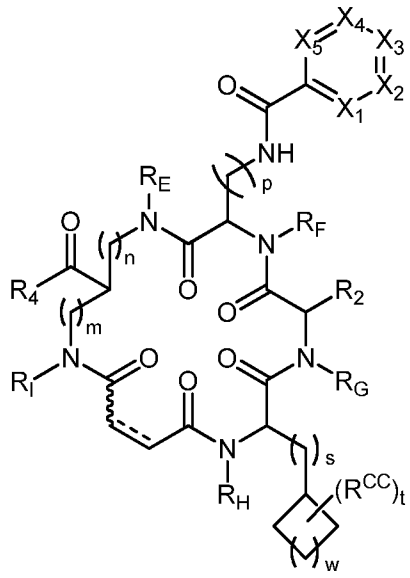
[00279] In certain embodiments of Formula (I), when f is 1, g is 1, h is 1, R_1 is alkyl substituted by $-NR^{A2}C(=O)R^{A2}$, and R_2 is an optionally substituted arylalkyl group, provided is a compound of Formula (VII):



(VII)

or a pharmaceutically acceptable salt thereof, wherein ======, ∿∿∿, X_1, X_2, X_3, X_4, X_5, p, R^{BB}, a, b, R_3, R^E, R^F, R^G, R^H, R^I, n, and m are as defined herein.

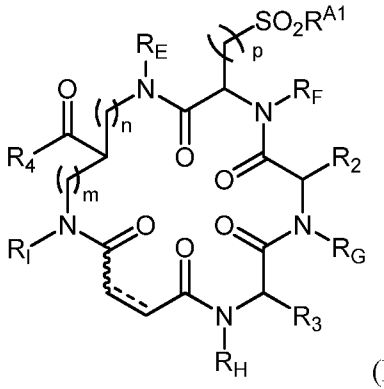
[00280] In certain embodiments of Formula (I), when f is 1, g is 1, h is 1, R_1 is alkyl substituted by $-NR^{A2}C(=O)R^{A2}$, and R_3 is an optionally substituted carbocyclyl group, provided is a compound of Formula (VIII):



(VIII)

or a pharmaceutically acceptable salt thereof, wherein ======, ∿∿∿, X_1, X_2, X_3, X_4, X_5, p, R_2, R^{CC}, s, t, w, R^E, R^F, R^G, R^H, R^I, n, and m are as defined herein.

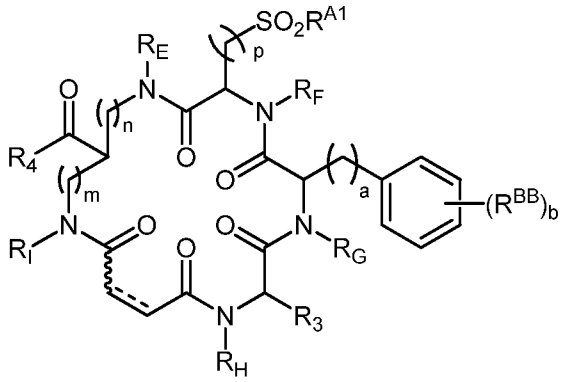
[00281] In certain embodiments of Formula (I), when f is 1, g is 1, h is 1, and R_1 is alkyl substituted with $-SO_2R^{A1}$, provided is a compound of Formula (IX):



(IX)

or a pharmaceutically acceptable salt thereof, wherein ======, ∿∿∿, R^{A1}, p, R_2, R_3, R^E, R^F, R^G, R^H, R^I, n, and m are as defined herein.

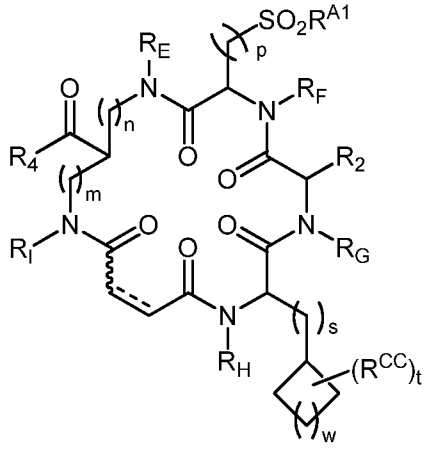
[00282] In certain embodiments of Formula (I), when f is 1, g is 1, h is 1, R_1 is alkyl substituted with $-SO_2R^{A1}$ and R_2 is an optionally substituted arylalkyl group, provided is a compound of Formula (X):



(X)

or a pharmaceutically acceptable salt thereof, wherein ======, ~~~~, R^{A1}, p, R_2, R_3, R^E, R^F, R^G, R^H, R^I, n, and m are as defined herein.

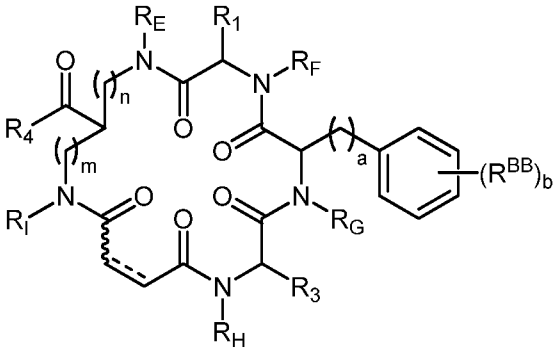
[00283] In certain embodiments of Formula (I), when f is 1, g is 1, h is 1, R_1 is alkyl substituted with $-SO_2R^{A1,}$ and R_3 is an optionally substituted carbocyclyl group, provided is a compound of Formula (XI):



(XI)

or a pharmaceutically acceptable salt thereof, wherein ======, ~~~~, R^{A1}, p, R_2, R_3, R^E, R^F, R^G, R^H, R^I, n, and m are as defined herein.

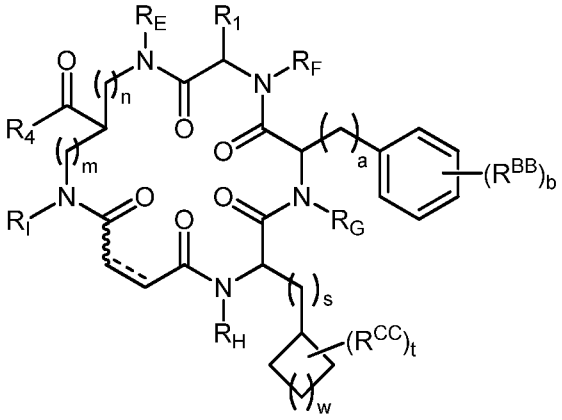
[00284] In certain embodiments of Formula (I), when f is 1, g is 1, h is 1, and R_2 is an optionally substituted arylalkyl group, provided is a compound of Formula (XII):



(XII)

or a pharmaceutically acceptable salt thereof, wherein ======, ~~~~, R_1, R^{BB}, a, b, R_3, R_4, R^E, R^F, R^G, R^H, R^I, n, and m are as defined herein.

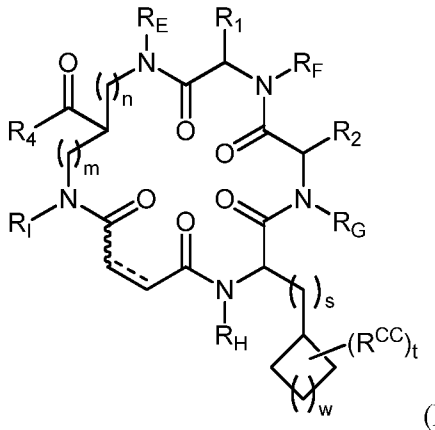
[00285] In certain embodiments of Formula (I), when f is 1, g is 1, h is 1, R_2 is an optionally substituted arylalkyl group, and R_3 is an optionally substituted carbocyclyl group, provided is a compound of Formula (XIII):



(XIII)

or a pharmaceutically acceptable salt thereof, wherein ======, ∿∿∿, R_1, R^{BB}, a, b, R^{CC}, s, w, R_4, R^E, R^F, R^G, R^H, R^I, n, and m are as defined herein.

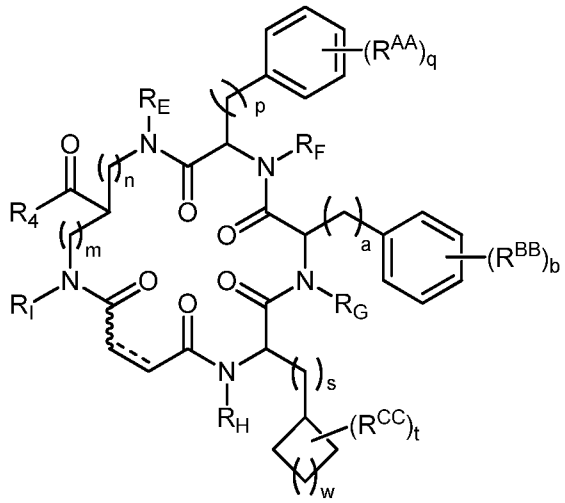
[00286] In certain embodiments of Formula (I), when f is 1, g is 1, h is 1, and R_3 is an optionally substituted carbocyclyl group, provided is a compound of Formula (XIV):



(XIV)

or a pharmaceutically acceptable salt thereof, wherein ======, ∿∿∿, R_1, R^{BB}, a, b, R^{CC}, s, w, R_4, R^E, R^F, R^G, R^H, R^I, n, and m are as defined herein.

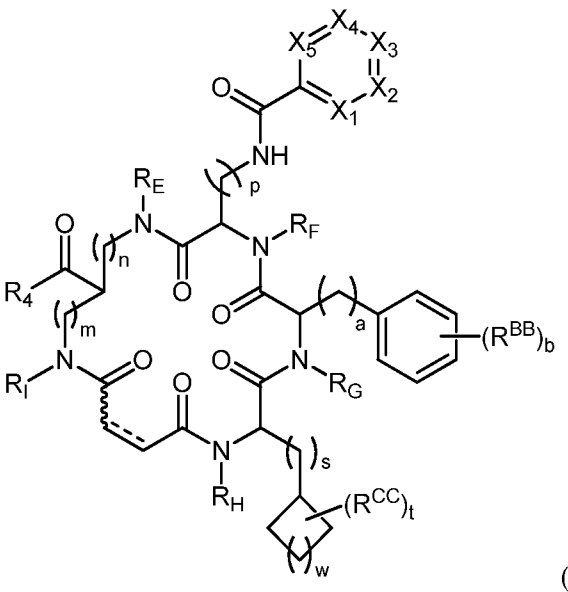
[00287] In certain embodiments of Formula (I), when f is 1, g is 1, h is 1, R_1 is an optionally substituted arylalkyl group, R_2 is an optionally substituted arylalkyl group, and R_3 is an optionally substituted carbocyclyl group, provided is a compound of Formula (XV):



(XV)

or a pharmaceutically acceptable salt thereof, wherein ======, ~~~~, R^{AA}, p, q, R^{BB}, a, b, R^{CC}, s, t, w, R^{E}, R^{F}, R^{G}, R^{H}, R^{I}, n, and m are as defined herein.

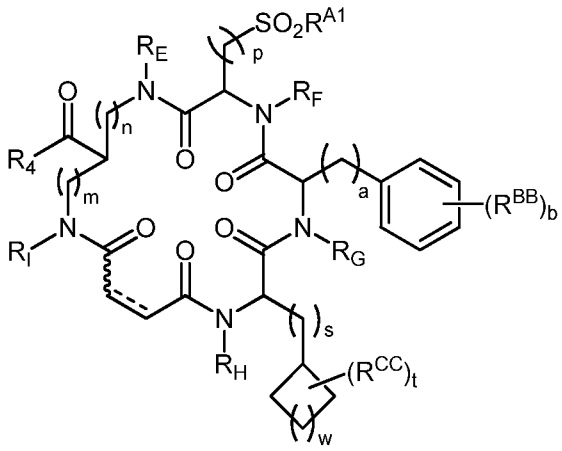
[00288] In certain embodiments of Formula (I), when f is 1, g is 1, h is 1, R_1 is alkyl substituted by $-NR^{A2}C(=O)R^{A2}$, R_2 is an optionally substituted arylalkyl group, and R_3 is an optionally substituted carbocyclyl group, provided is a compound of Formula (XVI):



(XVI)

or a pharmaceutically acceptable salt thereof, wherein ======, ~~~~, X_1, X_2, X_3, X_4, X_5, p, R^{BB}, a, b, R^{CC}, s, t, w, R^{E}, R^{F}, R^{G}, R^{H}, R^{I}, n, and m are as defined herein.

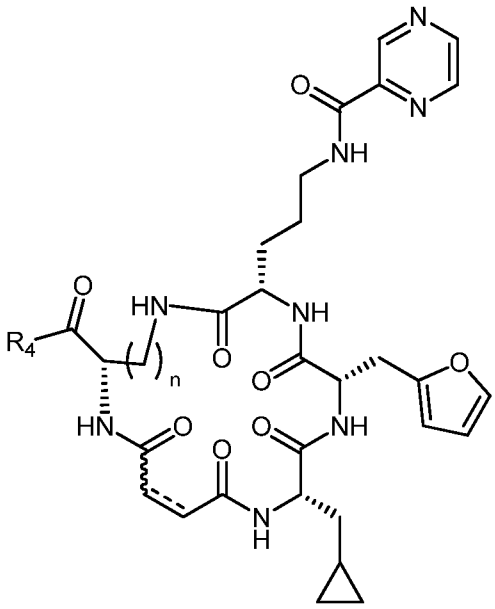
[00289] In certain embodiments of Formula (I), when f is 1, g is 1, h is 1, R_1 is alkyl substituted with $-SO_2R^{A1}$, R_2 is an optionally substituted arylalkyl group, and R_3 is an optionally substituted carbocyclyl group, provided is a compound of Formula (XVI):



(XVI)

or a pharmaceutically acceptable salt thereof, wherein ======, ~~~~, R^{A1}, p, q, R^{BB}, a, b, R^{CC}, s, t, w, R^{E}, R^{F}, R^{G}, R^{H}, R^{I}, n, and m are as defined herein.

[00290] In certain embodiments, the compound is selected from the group consisting of:



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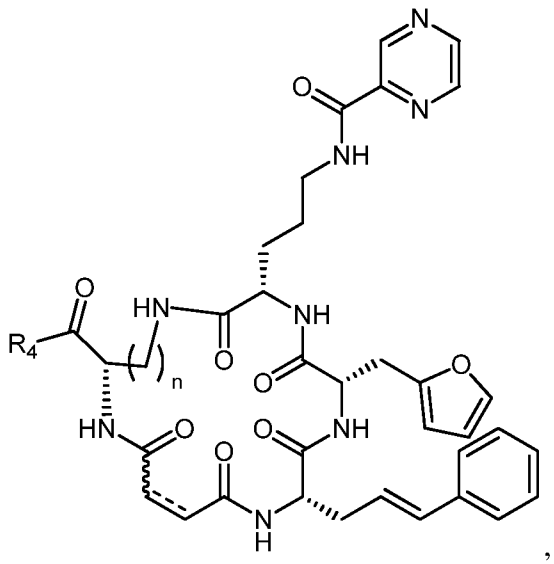

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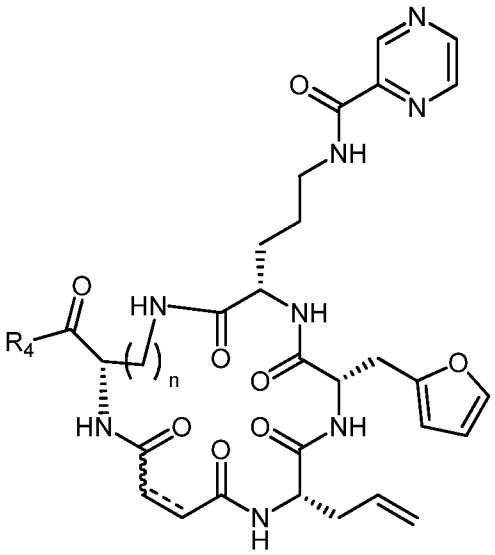
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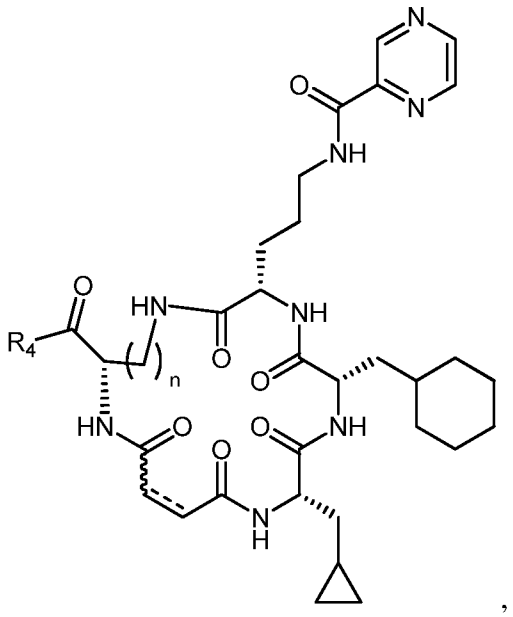
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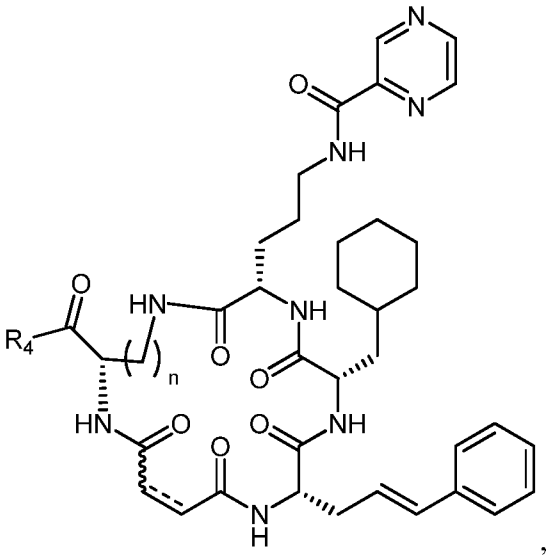
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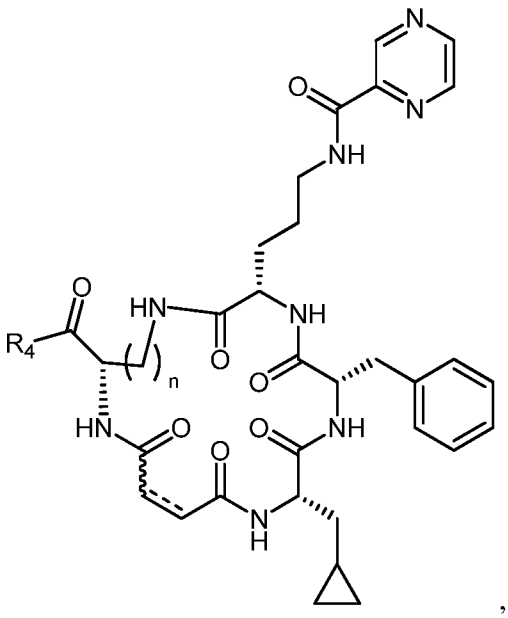
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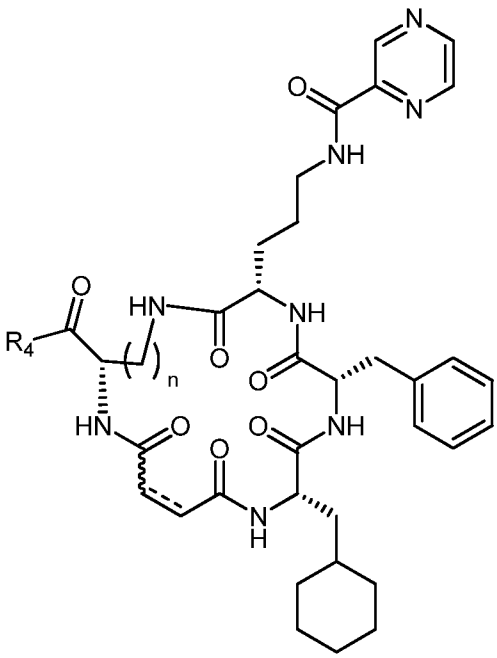

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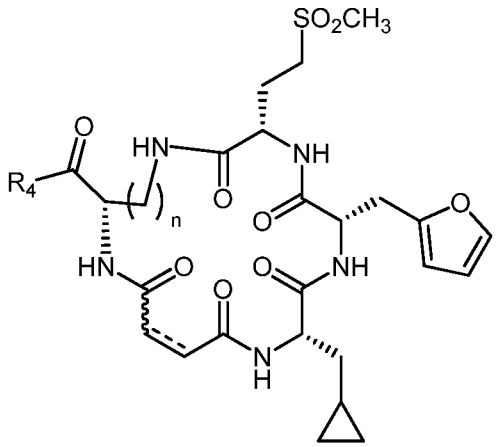

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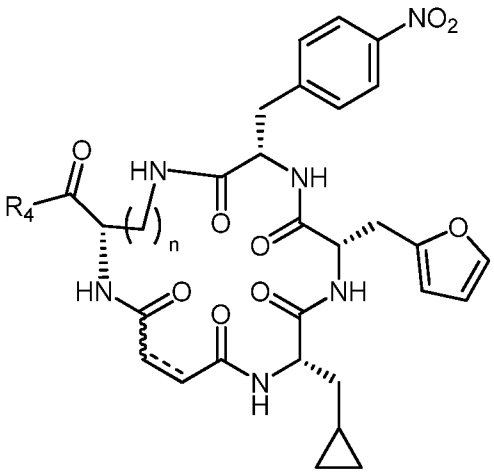

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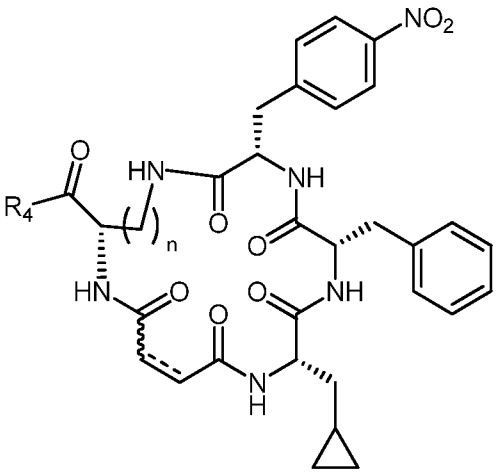

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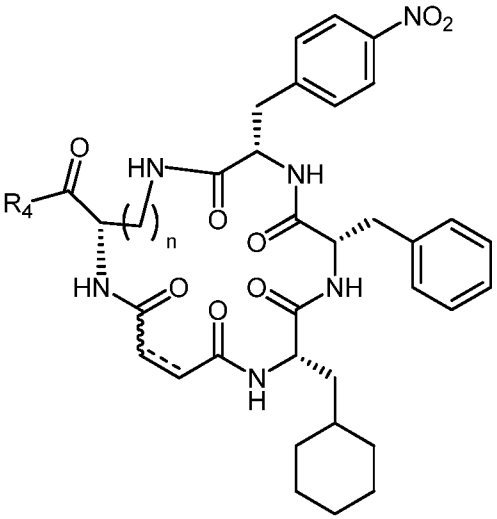

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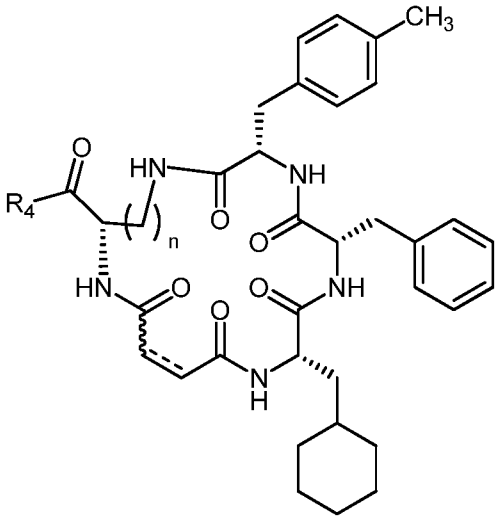

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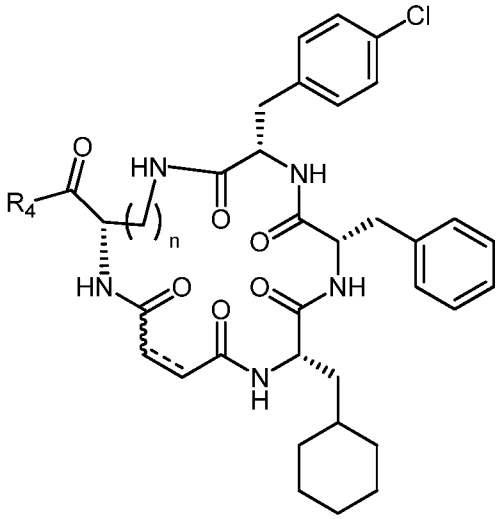

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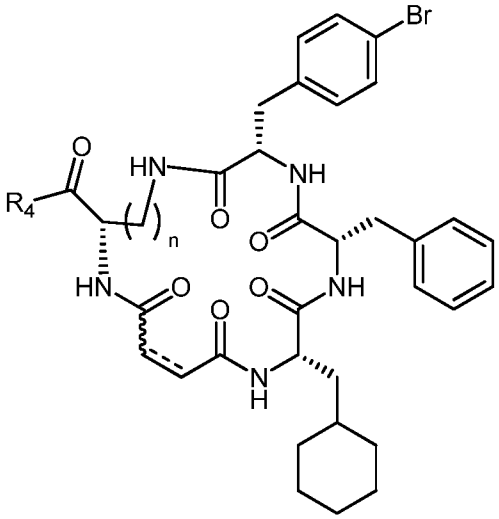

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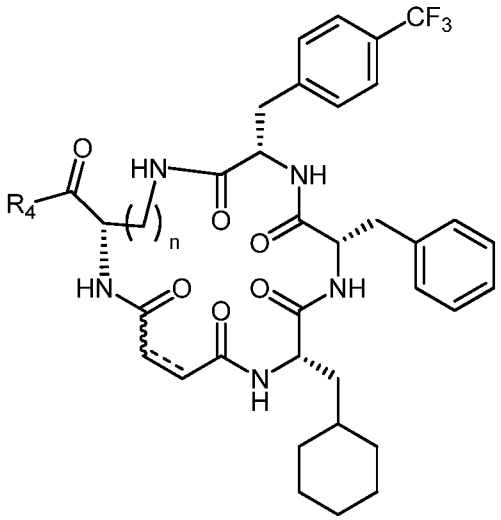

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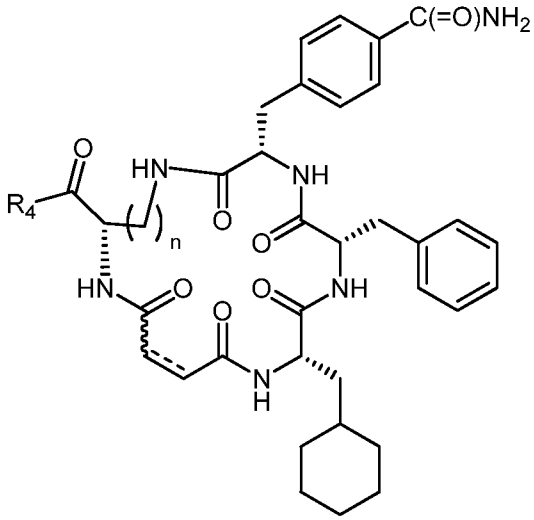

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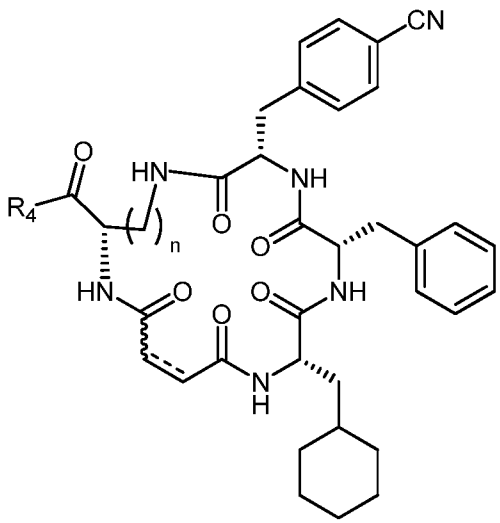

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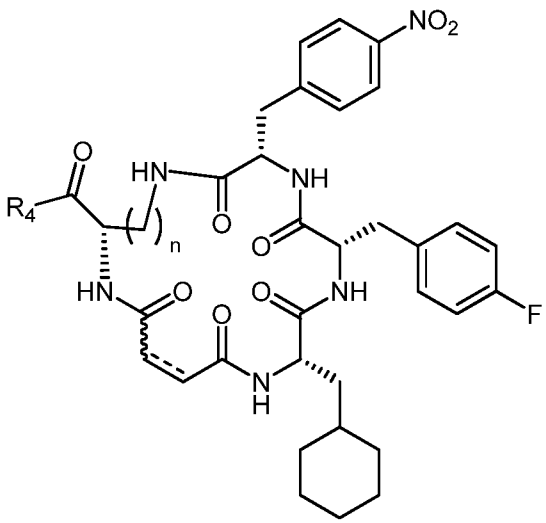


,

and pharmaceutically acceptable salts thereof,

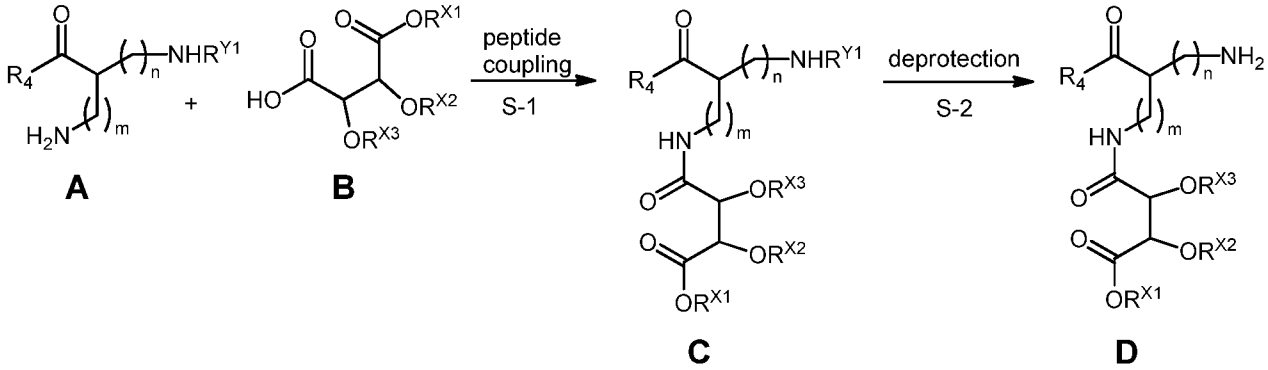
wherein ======, ∿∿∿, R^4, and n are as defined herein. In certain embodiments, n is 2. In certain embodiments, n is 3. In certain embodiments, R_4 is $-NH_2$. In certain embodiments, R_4 is $-NHR^D$, wherein R^D is a resin covalently attached thereto.

Methods of Preparation

[00291] The present invention further provides methods for preparing macrocyclic compounds of the present invention, *e.g.*, following the syntheses depicted in below Schemes 1- 6 and Figure 7.

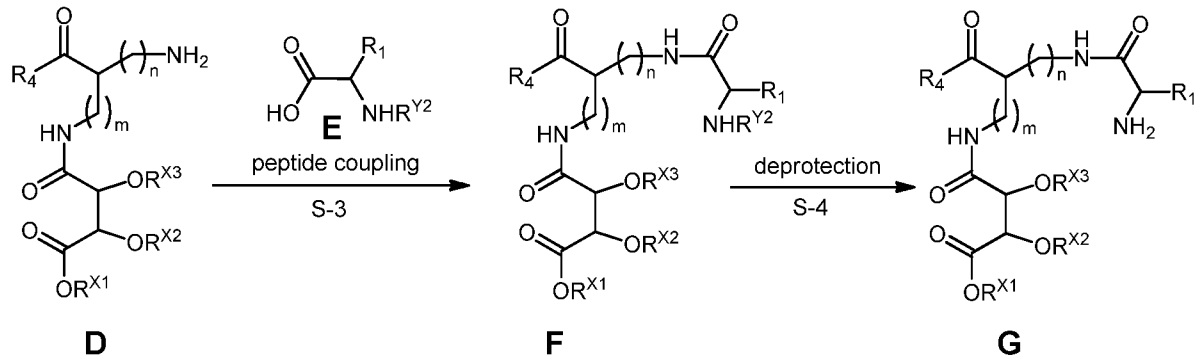
[00292] Scheme 1 depicts the first two steps in the synthesis of a compound of Formula (I). Step 1 (S-1) comprises providing a compound of formula (A), wherein R^4 is as defined herein and R^{Y1} is a nitrogen protecting group, providing a compound of formula (B), wherein R^{X1} is a carboxylic acid protecting group, and R^{X2} and R^{X3} are each independently oxygen protecting groups or are cyclized to form a 1,2-diol protecting group (*e.g.*, a dioxolanyl group), and coupling the compound of formula (A) and the compound of formula (B) under peptide coupling conditions to provide the coupled product (C). Step 2 (S-2) comprises deprotecting the coupled product (C) to provide a compound of formula (D).

Scheme 1.



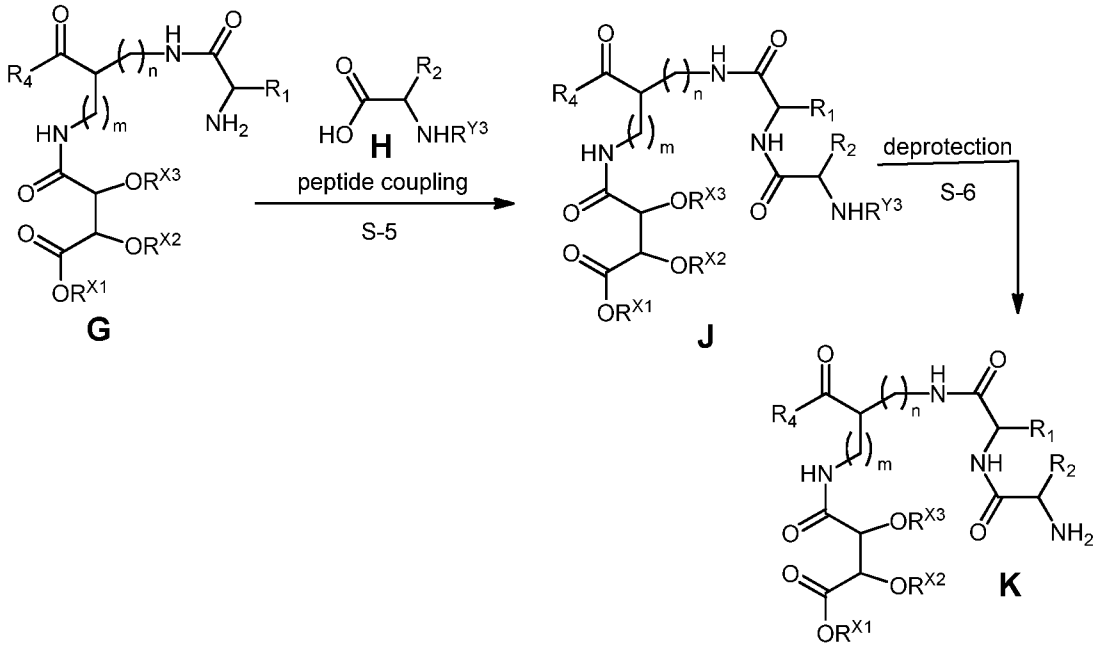
[00293] Step 3 (S-3), depicted in Scheme 2, comprises coupling the compound of formula (D) with a compound of formula (E), wherein R^{Y2} is a nitrogen protecting group, under peptide coupling conditions to provide the coupled product (F). Step 4 (S-4), also depicted in Scheme 2, comprises deprotecting the coupled product (F) to provide a compound of formula (G).

Scheme 2.



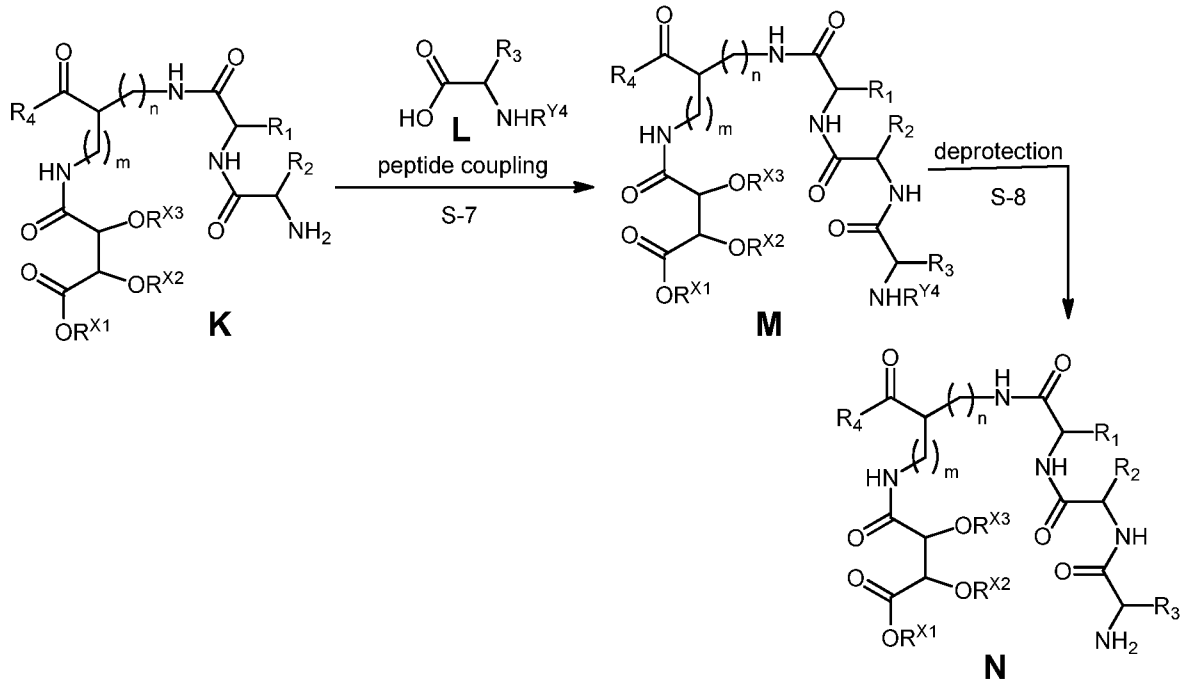
[00294] Step 5 (S-5), depicted in Scheme 3, comprises coupling the compound of formula (G) with a compound of formula (H), wherein R^{Y3} is a nitrogen protecting group, under peptide coupling conditions to provide the coupled product (J). Step 6 (S-6), also depicted in Scheme 3, comprises deprotecting the coupled product (J) to provide a compound of formula (K).

Scheme 3.



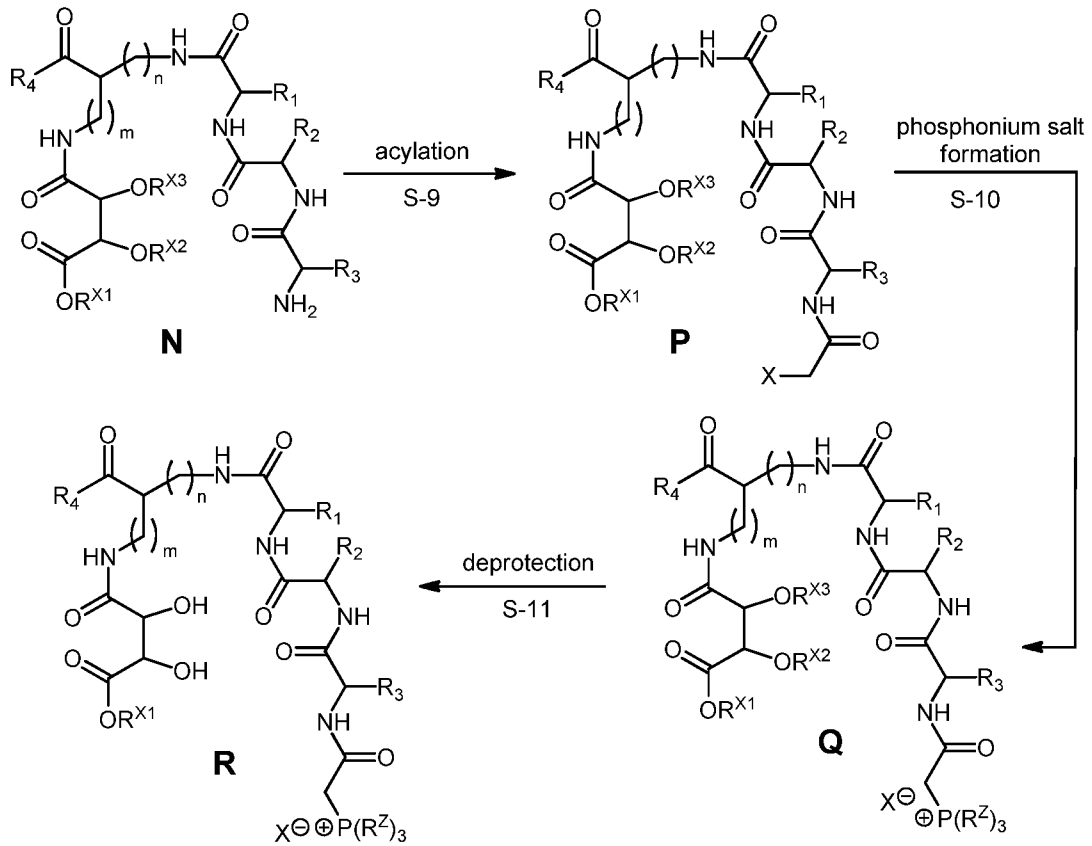
[00295] Step 7 (S-7), depicted in Scheme 4, comprises coupling the compound of formula (K) with a compound of formula (L), wherein R^{Y4} is a nitrogen protecting group, under peptide coupling conditions to provide the coupled product (M). Step 8 (S-8), also depicted in Scheme 4, comprises deprotecting the coupled product (M) to provide a compound of formula (N).

Scheme 4.



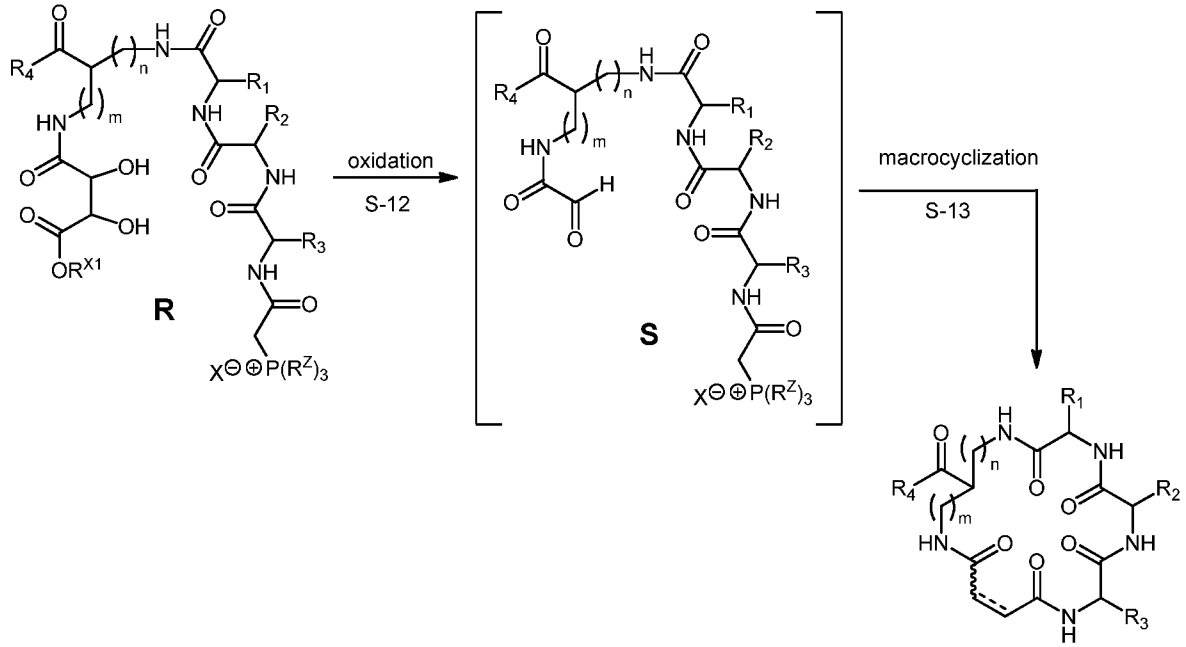
[00296] Step 9 (S-9), depicted in Scheme 5, comprises acylating the compound of formula (N) to provide the acylated product (P), wherein X is –Br, -Cl, or -I. An exemplary acylating reagent is an amine reactive ester of the formula Y-C(=O)CH_2X, wherein Y is a N-hydroxysuccinimide (NHS) or sulfo-NHS. Step 10 (S-10) comprises contacting the acylated product (P) with a phosphine of formula $P(R^Z)_3$, wherein each R^Z is independently optionally substituted aryl or optionally substituted heteroaryl, to provide a phosphonium salt of the formula (Q). Step 11 (S-11) comprises deprotecting the 1,2-diol to provide a compound of formula (R).

Scheme 5.



[00297] Step 12 (S-12), depicted in Scheme 6, comprises cleaving the 1,2-diol under oxidative conditions to provide the aldehyde intermediate (S) *in situ*. Exposure of the aldehyde intermediate (S) under basic conditions (*e.g.*, pH >8) generates a phosphonium ylide, and the subsequent intramolecular Wittig reaction provides an exemplary macrocyclic compound of the present invention.

Scheme 6.



[00298] As is understood from the above, the synthesis utilizes peptide coupling methods. Such methods are known in the art, see generally, *March's Advanced Organic Chemistry: Reactions*, Mechanisms, and Structure, M.B. Smith and J. March, 5th Edition, John Wiley & Sons, 2001, and *Comprehensive Organic Transformations*, R.C. Larock, 2nd Edition, John Wiley & Sons, 1999.

[00299] The peptide coupling reaction requires a peptide coupling reagent. Exemplary peptide coupling reagents include, but are not limited to, benzotriazol–1–yloxy–tris(dimethylamino)–phosphonium hexafluorophosphate (BOP), benzotriazole–1–yl–oxy–tris–pyrrolidino–phosphonium hexafluorophosphate (PyBOP), bromo–tris–pyrrolidino phosphonium hexafluorophosphate (PyBroP), 1–ethyl–3–(3–dimethyllaminopropyl) carbodiimide (EDC), N,N'–carbonyldiimidazole (CDI), 3–(diethoxyphosphoryloxy)–1,2,3–benzotriazin–4(3H)–one (DEPBT), 1–hydroxy–7–azabenzotriazole (HOAt), 1–hydroxy–7–benzotriazole (HOBt), 2–(7–aza–1H–benzotriazole–1–yl)–1,1,3,3–tetramethyluronium hexafluorophosphate (HATU), 2–(6–chloro–1H–benzotriazole–1–yl)–1,1,3,3–tetramethylaminium hexafluorophosphate (HCTU), 2–(1H–benzotriazole–1–yl)–1,1,3,3–tetramethyluronium hexafluorophosphate (HBTU), O–(7–azabenzotriazole–1–yl)–N,N,N',N'–tetramethyluronium tetrafluoroborate (TATU), 2–(1H–benzotriazole–1–yl)–1,1,3,3–tetramethyluronium tetrafluoroborate (TBTU), N,N,N',N'–tetramethyl–O–(3,4–dihydro–4–oxo–1,2,3–benzotriazin–3–yl)uranium tetrafluoroborate (TDBTU), and O–(N–succinimidyl)–

1,1,3,3–tetramethyl uranium tetrafluoroborate (TSTU)).

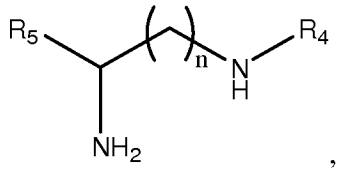
[00300] In certain embodiments, the peptide coupling further comprises a base, *e.g.*, potassium carbonate, potassium hydroxide, sodium hydroxide, tetrabutylammonium hydroxide, benzyltrimethylammonium hydroxide, triethylbenzylammonium hydroxide, 1,1,3,3–tetramethylguanidine, 1,8–diazabicyclo[5.4.0]undec–7–ene (DBU), N–methylmorpholine, diisopropylethylamine (DIPEA), tetramethylethylenediamine (TMEDA), pyridine (Py), 1,4–diazabicyclo[2.2.2]octane (DABCO), N,N–dimethylamino pyridine (DMAP), or triethylamine (NEt_3).

[00301] In certain embodiments, the peptide coupling is solid phase peptide coupling. For example, in some embodiments, the macrocyclic compound may be synthesized using solid-phase peptide synthesis. In this particular instance, in certain embodiments, R^4 is –NHR^D, wherein R^D is a resin, *e.g*, a Rink resin. An overview of exemplary solid phase methods can be found, for example, in Chan, WC, White, PD, *Fmoc Solid Phase Peptide Synthesis: A Practical Approach (Practical Approach Series)*, Oxford University Press, USA; 1 edition (March 2, 2000), ISBN-10: 0199637245; incorporated herein in its entirety for disclosure of Fmoc and solid phase Fmoc synthetic methods and related protocols). In certain embodiments, the method comprises generating the phosphonium salt (Q) on the resin, and then cleaving the compound from the resin prior to Step 12 (S-12). In certain embodiments, the phosphonium salt (Q) is cleaved from the resin by treatment with an acid (*e.g.,* trifluoroacetic acid, TFA). In certain embodiments, the acidic conditions also cleave the 1,2-diol protecting group to provide a compound of formula (R). In certain embodiments, prior to the oxidative and cyclization steps (Steps 12 and 13), the method further comprises purifying the compound of formula (R). Methods for isolating and/or purifying synthesized peptides are well known to those of skill in the art and include, but are not limited to high performance liquid chromatography (HPLC), conventional column chromatography, or recrystallization.

[00302] However, in certain embodiments, the peptide coupling is solution phase peptide coupling, and the intermediates are not attached to a resin.

[00303] In another aspect, provided is a method of a macrocyclic compound, the method comprising:

(a) providing a differentially protected diamino acid macrocyclization precursor of the formula:



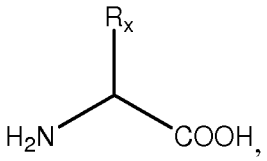
wherein:

n is 0 or is an integer between 0 and 10, inclusive;

R_4 is an amino protecting group;

R_5 is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; acyl; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; $-OR_D$; =O; $-C(=O)R_D$; $-CO_2R_D$; -CN; -SCN; $-SR_D$; $-SOR_D$; $-SO_2R_D$; $-NO_2$; $-N(R_D)_2$; $-NHC(O)R_D$; or $-C(R_D)_3$; wherein each occurrence of R_D is independently hydrogen, a protecting group, aliphatic, heteroaliphatic, acyl; aryl; heteroaryl; alkoxy; aryloxy; alkylthio; arylthio; amino, alkylamino, dialkylamino, heteroaryloxy; or heteroarylthio; and optionally, wherein the macrocyclization precursor is coupled to a solid support via R_5;

(b) contacting the macrocyclization precursor provided under (a) with a building block of the formula:



under conditions suitable for the formation of a peptide bond between the carboxyl group of the building block provided under (b) with the unprotected amino group of the macrocyclization precursor, wherein R_x, is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; acyl; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; $-OR_E$; =O; $-C(=O)R_E$; $-CO_2R_E$; -CN; -SCN; $-SR_E$; $-SOR_E$; $-SO_2R_E$; $-NO_2$; $-N(R_E)_2$; $-NHC(O)R_E$; or $-C(R_E)_3$; wherein each occurrence of R_E is independently hydrogen, a protecting group, aliphatic, heteroaliphatic, acyl; aryl; heteroaryl; alkoxy; aryloxy; alkylthio; arylthio; amino, alkylamino, dialkylamino, heteroaryloxy; or heteroarylthio;

(c) performing 1-5 additional steps of contacting the reaction product generated under (b) with an additional building block of the formula provided in (b), wherein R_x is defined for each building block separately and individually as under (b);

(d) optionally, cleaving the macrocyclization precursor from the solid support and/or purifying the macrocyclization precursor; and

(e) effecting cyclization of the macrocyclization precursor.

[00304] In certain embodiments, n is an integer between 1 and 4, inclusive.

[00305] In certain embodiments, R_4 is an amino protecting group. In certain embodiments, R_4 is isopropylidene-protected tartrate monomethyl ester.

[00306] In certain embodiments, the macrocyclization precursor is coupled to an amide resin. In certain embodiments, the building blocks are added to the macrocyclization precursor by Fmoc synthesis. In certain embodiments, the method comprises generating a phosphonium salt of the macrocyclization precursor on resin.

[00307] In certain embodiments, the macrocyclization precursor is cleaved from the solid support by treatment with a strong acid. In certain embodiments, the cleavage reaction generates a carboxamide at the C-terminus and deprotects a tartrate diol group.

[00308] In certain embodiments, the macrocyclization precursor is purified before cyclization. In certain embodiments, the method further comprising a step of oxidatively cleaving the diol group, thus generating an aldehyde group. In certain embodiments, the cyclization is a Wittig cyclization. In certain embodiments, the Wittig cyclization is effected by raising the pH to generate a phosphonium ylide.

[00309] In some embodiments, a compound of the invention may be synthesized according to the synthesic scheme shown in **Figure 7**. It will be understood by those of skill in the art that the synthesis scheme depicted is exemplary and can be used, or, where necessary, adapted with no more than routine experimentation, for the synthesis of any compound described herein by the use of the appropriate building blocks, for example, the building blocks described in **Figure 2b**, reagents, protecting groups, and reaction conditions.

[00310] In some embodiments, a macrocyclic compound provided herein may be synthesized using Fmoc solid-phase peptide synthesis technology. Fmoc synthesic methods are known in the art. An overview of exemplary suitable methods can be found, for example, in Chan, WC, White, PD, *Fmoc Solid Phase Peptide Synthesis: A Practical Approach (Practical Approach Series)*, Oxford University Press, USA; 1 edition (March 2, 2000), ISBN-10: 0199637245; incorporated herein in its entirety for disclosure of Fmoc and solid phase Fmoc synthesic methods and related protocols). The synthesis of inventive macrocycles may, in some embodiments, be performed on a multi-milligram scale or a multi-gram scale. In some embodiments, a differentially protected diamino acid building block, for example, a building block described in **Figure 2b** under D1-D8 may be employed as the

starting material for macrocycle synthesis. In some embodiments, an isopropylidene-protected tartrate monomethyl ester may be reacted with the amine of the first building block. An additional building block may be reacted with the first building block by a round of Fmoc synthesis. In some embodiments, multiple rounds of Fmoc synthesis may be performed to synthesize multi-block peptides. For example, in some embodiments, three rounds of Fmoc synthesis are employed to generate a tripeptide. In some embodiments, a phosphonium salt of the synthesized peptide is generated for macrocyclization. In some embodiments, the synthesized peptide, or macrocyclization precursor is cleaved from a solid support, *e.g.* a resin, before cyclization. In some embodiments, cleavage of the macrocyclization precursor is achieved by treatment with a strong acid, *e.g.*, TFAD.. In some embodiments, a carboxamide is generated at the C-terminus of the synthesized peptide, for example, during the cleavage reaction. In some embodiments, a tartrate diol is revealed by removal of a protecting group. In some embodiments, the macrocyclization precursor is isolated and/or purified before cyclization is effected. Methods for isolating and/or purifying synthesized peptides are well known to those of skill in the art and include, but are not limited to high performance liquid chromatography (HPLC), conventional column chromatography, or recrystallization. In some embodiments, the macrocyclization precursor is characterized before cyclization, for example, by mass spectroscopy (MS) and/or NMR. In some embodiments, the deprotected diol is cleaved oxidatively to yield an aldehyde. In some embodiments, the cyclization reaction is a Wittig cyclization. In some embodiments, cyclization is effected by raising the pH of a solution comprising the macrocyclization precursor to generate a phosphonium ylide. Other cyclization reactions known in the art may also be used to yield the macrocycle.

[00311] While the compounds provided herein can be synthesized by the exemplary synthesic methods described herein, it will be understood by those of skill in the art that other synthetic methods may be used to synthesize the described compounds and the scope of the invention is not limited in this aspect.

Uses

[00312] The compounds of the invention, for example, the macrocyclic kinase inhibitors provided herein, are useful for modulating the activity of a kinase, for example, Src kinase. In some embodiments, modulating the activity of a kinase with a compound described herein involves contacting the kinase with an effective amount of the compound. In certain embodiments, the kinase is contacted *in vitro*. In some embodiments, an isolated or

purified kinase is contacted. In some embodiments, the kinase is contacted *in vivo*, *e.g.*, in a cell, tissue, or subject. In some embodiments, the cell or tissue is contacted *in vitro*, for example, in a cell or tissue culture environment. In some embodiments, the kinase is contacted *in vivo*, for example in a subject. In some embodiments, the kinase is contacted in vivo with the compound, e.g., a macrocycle described herein, or a pharmaceutically acceptable salt, solvate, or derivative thereof. In some embodiments, the subject has or is diagnosed with a disease or disorder associated with aberrant kinase activity. In some embodiments, a compound as provided herein is administered to a subject to inhibit the activity of a kinase in the subject, for example, in a cell or tissue of the subject. In some embodiments, a kinase-modulatory compound as provided herein is administered to a subject based on the subject, or a cell or tissue of the subject, exhibiting aberrant kinase activity. Methods to determine kinase activity in a subject are known to those of skill in the art. For example, such methods may, in some embodiments, include obtaining a biological sample from a subject, for example, a cell, tissue, or body fluid sample, and performing a suitable kinase assay on the sample. Suitable kinase assays are well known to those in the art. For an overview on some exemplary and non-limiting kinase assays, see Sefton and Hunter, *Protein Phosphorylation (Selected Methods in Enzymology)* Academic Press; 1st edition (May 1, 1998), ISBN: 0126344906; Reith, *Protein Kinase Protocols (Methods in Molecular Biology)*, Humana Press; 1st edition (January 15, 2001) ISBN: 0896037002; and Hardie, *Protein Phosphorylation: A Practical Approach (Practical Approach Series)*, Oxford University Press, USA; 2 edition (December 15, 1999), ISBN: 0199637288; all incorporated by reference in their entirety herein for disclosure of protein kinase assays). In some embodiments, the method includes determining an aberrant level of kinase activity, for example, Src kinase activity, in the subject. In some embodiments, the method includes determining an aberrant level of kinase activity in a cell or tissue of the subject. In some embodiments, the method includes obtaining a biological sample, for example, a tumor biopsy, from a subject, determining the activity level of a kinase, for example, Src kinase, in the biological sample (*e.g.*, by performance of a suitable kinase assay (*e.g.* a Src kinase assay)), and comparing the kinase activity measured in the sample to a reference or control activity, wherein, if the activity level measured in the sample from the subject is higher or lower than the reference or control activity, then the biological sample is determined to exhibit an aberrant kinase activity, or if the kinase activity level in the biological sample from the subject is similar or equal to the reference or control level, then the biological sample is determined to exhibit normal kinase activity. In some embodiments, "lower" refers to a level

of kinase activity that is at least 10%, at least 20%, at least 30%, at least 40%, at least 50% at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% lower than a reference or control level, for example, an average level observed in healthy individuals. In some embodiments, "higher" refers to a level of kinase activity that is at least 25%, at least 50%, at least 75%, at least 100%, at least 200% at least 250%, at least 300%, at least 400%, or at least 500% higher than a reference or control level, for example, an average level observed in healthy individuals.In some embodiments, "lower" or "higher" refers to a statistically significant elevation or decrease in the level of activity. In some embodiments, the reference or control level is a level measured in a cell or tissue of the same type that is not affected by disease. For example, if the tissue from the subject is colonic tissue, then the reference or control level may, in some embodiments, be the level of activity of the respective kinase measured in healthy colonic tissue, *e.g.* in colonic tissue from a healthy subject or from healthy subjects or in colonic tissue that is not affected by disease from the same subject. For another example, if the tissue obtained from the subject is a tumor biopsy, the reference or control level may be the average level observed in healthy cells, or in tumors of the same tissue or organ, or in tumors of the same tissue of origin. In some embodiments, for example, where a change in kinase activity is related to a mutation in the kinase-encoding gene, the reference or control level may be a level measured in a cell, tissue, or biological sample known to have or express a non-mutated kinase gene. Accordingly, a higher level of, *e.g.*, Src kinase activity in a colon tumor cell or tissue may, in some embodiments, be a level that is statistically significantly higher than a reference or control level of Src kinase activity, for example, an average level measured in colon tumor biopsies, or a level measured in colon tumors known to express a non-mutated Src protein.

[00313] Certain compounds of the invention are useful for modulating the activity of a mutant kinase, for example, a kinase comprising a getkeeper mutation, such as a Src kinase comprising a T338I or T341I mutation. In some embodiments, modulating the activity of a mutant kinase with a compound described herein involves contacting the kinase with an effective amount of the compound. In certain embodiments, the mutant kinase is contacted *in vitro*. In some embodiments, an isolated or purified mutant kinase is contacted. In some embodiments, the mutant kinase is contacted *in vivo*, *e.g.*, in a cell, tissue, or subject. In some embodiments, the cell or tissue is contacted *in vitro*, for example, in a cell or tissue culture environment. In some embodiments, the mutant kinase is contacted *in vivo*, for example in a subject. In some embodiments, the subject has or is diagnosed with a disease or disorder associated with aberrant kinase activity. In some embodiments, the subject has been

determined to express a mutant kinase. In some embodiments, the mutant kinase is resistant to an ATP-competitive kinase inhibitor. In some embodiments, a compound as provided herein is administered to a subject to inhibit the activity of a mutant kinase in the subject, for example, in a cell or tissue of the subject. In some embodiments, a mutant-kinase-modulatory compound as provided herein is administered to a subject based on the subject, or a cell or tissue of the subject, exhibiting aberrant kinase activity and/or being determined to express a mutant or ATP-competitive kinase inhibitor-resistant kinase. Methods to determine kinase activity and kinase mutations in a subject are known to those of skill in the art. For example, such methods may, in some embodiments, include obtaining a biological sample from a subject, for example, a cell, tissue, or body fluid sample, and performing a suitable kinase assay on the sample. Kinase mutations can be detected by sequencing the kinase-encoding genomic region or analyzing the kinase amino acid sequence, using methods well known to those of skill in the art. In some embodiments, the method includes determining an aberrant level of kinase activity, for example, Src kinase activity, in the subject, e.g., as described elsewhere herein. In some embodiments, the method includes detecting a gatekeeper mutation in a kinase in the subject, for example, in a kinase exhibting aberrant kinase activity in a cell or tissue of the subject. In some embodiments, the method includes obtaining a biological sample, for example, a tumor biopsy, from a subject, determining the activity level of a kinase, for example, Src kinase, in the biological sample (*e.g.*, by performance of a suitable kinase assay (*e.g.* a Src kinase assay)), comparing the kinase activity measured in the sample to a reference or control activity, wherein, if the activity level measured in the sample from the subject is higher or lower than the reference or control activity, then the biological sample is determined to exhibit an aberrant kinase activity, or if the kinase activity level in the biological sample from the subject is similar or equal to the reference or control level, then the biological sample is determined to exhibit normal kinase activity. In some embodiments, "lower" refers to a level of kinase activity that is at least 10%, at least 20%, at least 30%, at least 40%, at least 50% at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% lower than a reference or control level, for example, an average level observed in healthy individuals. In some embodiments, "higher" refers to a level of kinase activity that is at least 25%, at least 50%, at least 75%, at least 100%, at least 200% at least 250%, at least 300%, at least 400%, or at least 500% higher than a reference or control level, for example, an average level observed in healthy individuals. In some embodiments, "lower" or "higher" refers to a statistically significant elevation or decrease in the level of activity. In some embodiments, the reference

or control level is a level measured in a cell or tissue of the same type that is not affected by disease. In some embodiments, for example, where a change in kinase activity is related to a mutation in the kinase-encoding gene, the reference or control level may be a level measured in a cell, tissue, or biological sample known to have or express a non-mutated kinase gene. Accordingly, a higher level of, *e.g.*, Src kinase activity in a colon tumor cell or tissue may, in some embodiments, be a level that is statistically significantly higher than a reference or control level of Src kinase activity, for example, an average level measured in colon tumor biopsies, or a level measured in colon tumors known to express a non-mutated Src protein. In some embodiments, the method further comprises detecting a mutation, for example, a gatekeeper mutation, in a kinase expressed in the subject, e.g., a kinase determined to exhibit an aberrant activity level in the subject. In some embodiments, the method comprises administering to a cell, tissue, or subject determined to express a kinase comprising a gatekeeper mutation, for example, a src kinase with a T338I or T341I mutation, or a kinase having a homologous gatekeeper mutation, a compound provided herein that inhibits the mutated kinase. In some embodiments, a method is provided that includes administering a kinase inhibitor that inhibits the activity of a kinase comprising a gatekeeper mutation, for example, of a src kinase comprising a T338I or T341I mutation to a subject, cell, or tissue expressing such a mutated kinase. For example, in some embodiments, the cell, tissue, or subject expresses a src kinase with a T338I or T341I mutation, and the method includes administering to the cell, tissue, or subject macrocycle A10-Phe-C5-D6, [4-Me-Phe]-Phe-C5-D6, [4-Cl-Phe]-Phe-C5-D6, [4-Br-Phe]-Phe-C5-D6, [4-CF_3-Phe]-Phe-C5-D6, [4-$CONH_2$-Phe]-Phe-C5-D6, [4-CN-Phe]-Phe-C5-D6, A10-Phe-Cha-D6, [4-Me-Phe]-Phe-Cha-D6, [4-Cl-Phe]-Phe-Cha-D6, [4-Br-Phe]-Phe-Cha-D6, [4-CF_3-Phe]-Phe-Cha-D6, [4-$CONH_2$-Phe]-Phe-Cha-D6, [4-CN-Phe]-Phe-Cha-D6, A10-[4-F-Phe]-Cha-D6, [4-Me-Phe]-[4-F-Phe]-Cha-D6, [4-Cl-Phe]-[4-F-Phe]-Cha-D6, [4-Br-Phe]-[4-F-Phe]-Cha-D6, [4-CF_3-Phe]-[4-F-Phe]-Cha-D6, [4-$CONH_2$-Phe]-[4-F-Phe]-Cha-D6, or [4-CN-Phe]-[4-F-Phe]-Cha-D6, where Phe is phenylalanine, Cha is cyclohexylalanine, 4-Me-Phe is 4-methyl-phenylalanine, 4-F-Phe is 4-fluoro-phenylalanine, etc. (A-B-C-D nomenclature as illustrated in e.g., **Figures 5 and 19**). In some embodiments, the macrocycle is in trans-configuration.

[00314] In some embodiments, a macrocycle inhibitors provided herein is administered to a target kinase, cell, tissue, or subject in an amount effective to achieve a reduction of the activity level of the target kinase, for example, src kinase, of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99%.

[00315] In some embodiments, methods of treating a disease using a compound described herein are provided. In some embodiments, the disease is associated with, caused by, and/or characterized by aberrant kinase activity. In some embodiments, a method provided involves the administration of a therapeutically effective amount of an inventive compound to a subject (including, but not limited to, a human or other animal) in need thereof. In some embodiments, an effective amount is an amount sufficient to decrease the level of kinase activity by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99%, in a cell or tissue of the subject. In some embodiments, an effective amount is an amount sufficient to increase the level of kinase activity by at least 25%, at least 50%, at least 75%, at least 100%, at least 200%, at least 250%, at least 300%, at least 400%, or at least 500%.

[00316] Compounds and compositions described herein are generally useful for the modulation of kinase activity, for example, for the inhibition of the activity of a kinase, such as Src kinase or a mutant thereof, *e.g.*, a constitutively active src mutant or a src mutant carrying a gatekeeper mutation.

[00317] The compounds and pharmaceutical compositions provided herein may be used in treating or preventing any disease or condition characterized by increased activity of a kinase, including, but not limited to, proliferative diseases (*e.g.*, cancer, benign neoplasms, diabetic retinopathy), neurodegenerative diseases, autoimmune diseases (*e.g.*, rheumatoid arthritis, lupus, multiple sclerosis), metabolic syndrome, diabetes mellitus, and inflammatory diseases.

[00318] The inventive compounds and pharmaceutical compositions may be administered to animals, preferably mammals (*e.g.*, domesticated animals, cats, dogs, mice, rats), and more preferably humans. Any suitable method of administration may be used to deliver the inventive compound or pharmaceutical composition to the animal. In certain embodiments, the compound or pharmaceutical composition is administered orally. In other embodiments, the compound or pharmaceutical composition is administered parenterally.

[00319] In certain embodiments, the inventive compounds are useful in treating a proliferative disease. In some embodiments, methods for treating cancer are provided. In some embodiments, methods for treating tumorigenesis are provided. Examples of cancers that may be treated with a compound provided by some aspects of the invention include, but are not limited to, tumors of the breast; biliary tract; bladder; bone; brain, including glioblastomas and medulloblastomas; central and peripheral nervous system; cervix; colon;

connective tissue; endocrine glands (*e.g.*, thyroid and adrenal cortex); esophagus; endometrium; germ cells; gastrointestinal tract; head and neck; kidney; liver; lung; larynx and hypopharynx; mesothelioma; muscle; ovary, including those arising from epithelial cells, stromal cells, germ cells and mesenchymal cells; pancreas; prostate; rectum; renal, including adenocarcinoma and Wilm's tumor; small intestine; soft tissue; testis, including germinal tumors such as seminoma, non-seminoma (teratomas, choriocarcinomas), stromal tumors, and germ cell tumors; thyroid, including thyroid adenocarcinoma and medullar carcinoma; stomach; skin, including melanoma, Kaposi's sarcoma, basocellular cancer, and squamous cell cancer; ureter; vagina; and vulva; retinoblastoma; leukemia and lymphoma, namely non-Hodgkin's disease, lymphocytic lymphomas, chronic and acute myeloid leukemia (CML/AML), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), Hodgkin's disease, multiple myeloma, and T-cell lymphoma; myelodysplastic syndrome; plasma cell neoplasia; paraneoplastic syndromes; intraepithelial neoplasms including Bowen's disease and Paget's disease; neuroblastomas; oral cancer including squamous cell carcinoma; sarcomas including leiomyosarcoma, rhabdomyosarcoma, liposarcoma, fibrosarcoma, and osteosarcoma; cancers of unknown primary site; and AIDS-related malignancies. Other cancers that may be treated by administering a compound provided herein, for example, cancers that exhibit increased kinase activity (*e.g.*, Src kinase activity) or expression, will be known to one of ordinary skill in the art or can be identified with no ore than routine experimentation.

[00320] In certain embodiments, the invention provides methods for treating or lessening the severity of a proliferative disease including, but not limited to, smooth muscle cell proliferation including vascular proliferative disorders, atherosclerosis, and restenosis. In certain embodiments, the invention provides methods for treating or lessening the severity of endometriosis, uterine fibroids, endometrial hyperplasia, or benign prostate hyperplasia.

[00321] In certain embodiments, the invention provides methods for treating or lessening the severity of neurodegenerative disorders and/or tauopathies including, but not limited to, Alzheimer's disease, progressive supranuclear palsy, corticobasal degeneration, frontotemporal lobar degeneration, Pick's disease, Parkinson's disease, Lewy body disease, or amyotropic lateral sclerosis (ALS).

[00322] In some embodiments, the invention provides methods for treating or lessening the severity of autoimmune diseases including, but not limited to, inflammatory bowel disease, arthritis, systemic lupus erythematosus, rheumatoid arthritis, psoriatic arthritis, osteoarthritis, Still's disease, juvenile arthritis, diabetes, myasthenia gravis, Hashimoto's

thyroiditis, Ord's thyroiditis, Graves' disease, Sjogren's syndrome, multiple sclerosis, Guillain-Barre syndrome, acute disseminated encephalomyelitis, Addison's disease, opsoclonus-myoclonus syndrome, ankylosing spondylosis, antiphospholipid antibody syndrome, aplastic anemia, autoimmune hepatitis, celiac disease, Goodpasture's syndrome, idiopathic thrombocytopenic purpura, optic neuritis, scleroderma, primary biliary cirrhosis, Reiter's syndrome, Takayasu's arteritis, temporal arteritis, warm autoimmune hemolytic anemia, Wegener's granulomatosis, psoriasis, alopecia universalis, Behcet's disease, chronic fatigue, dysautonomia, endometriosis, interstitial cystitis, neuromyotonia, scleroderma, or vulvodynia.

[00323] In some embodiments, the invention provides a method for treating or lessening the severity of one or more diseases and conditions, wherein the disease or condition is selected from immune-related conditions or diseases, which include, but are not limited to, graft versus host disease, transplantation, transfusion, anaphylaxis, allergies (*e.g.*, allergies to plant pollens, latex, drugs, foods, insect poisons, animal hair, animal dander, dust mites, or cockroach calyx), type I hypersensitivity, allergic conjunctivitis, allergic rhinitis, and atopic dermatitis.

[00324] In some embodiments, the present invention provides a method for treating or lessening the severity of an inflammatory disease including, but not limited to, asthma, appendicitis, Blau syndrome, blepharitis, bronchiolitis, bronchitis, bursitis, cervicitis, cholangitis, cholecystitis, chronic obstructive pulmonary disease (COPD), chronic recurrent multifocal osteomyelitis (CRMO), colitis, conjunctivitis, cryopyrin associated periodic syndrome (CAPS), cystitis, dacryoadenitis, dermatitis, dermatomyositis, dry eye syndrome, encephalitis, endocarditis, endometritis, enteritis, enterocolitis, epicondylitis, epididymitis, familial cold-induced autoinflammatory syndrome, familial Mediterranean fever (FMF), fasciitis, fibrositis, gastritis, gastroenteritis, hepatitis, hidradenitis suppurativa, laryngitis, mastitis, meningitis, mevalonate kinase deficiency (MKD), Muckle-Well syndrome, myelitis myocarditis, myositis, nephritis, oophoritis, orchitis, osteitis, inflammatory osteolysis, otitis, pancreatitis, parotitis, pericarditis, peritonitis, pharyngitis, pleuritis, phlebitis, pneumonitis, pneumonia, proctitis, prostatitis, pulmonary fibrosis, pyelonephritis, pyoderma gangrenosum and acne syndrome (PAPA), pyogenic sterile arthritis, rhinitis, salpingitis, sinusitis, stomatitis, synovitis, systemic juvenile rheumatoid arthritis, tendonitis, TNF receptor associated periodic syndrome (TRAPS), tonsillitis, undifferentiated spondyloarthropathy, undifferentiated arthropathy, uveitis, vaginitis, vasculitis, vulvitis, chronic inflammation

resulting from chronic viral or bacteria infections, or psoriasis (*e.g.*, plaque psoriasis, pustular psoriasis, erythrodermic psoriasis, guttate psoriasis or inverse psoriasis).

[00325] In some embodiments, methods are provided for treating or lessening the severity of arthropathies and osteopathological diseases including, but not limited to, rheumatoid arthritis, osteoarthrtis, gout, polyarthritis, and psoriatic arthritis.

[00326] In some embodiments, methods are provided for treating or lessening the severity of acute and chronic inflammatory diseases including, but not limited to, ulcerative colitis, inflammatory bowel disease, Crohn's disease, dry eye syndrome, allergic rhinitis, allergic dermatitis, cystic fibrosis, chronic obstructive bronchitis, and asthma.

[00327] In some embodiments, a method is provided for the treatment of mammals, including humans, which are suffering from one of the above-mentioned conditions, illnesses, disorders, or diseases. In some embodiments, the method comprises that a therapeutically effective amount of one or more of the compounds according to this invention or a composition thereof is administered to the subject in need of such treatment.

[00328] In some embodiments, a method is provided for inhibiting or activating a kinase in a cell or tissue using a compound of the invention. In some embodiments, inhibition or activation of a kinase in a cell or a tissue is achieved by contacting the cell or tissue with a compound of the invention. In some embodiments, the activity of a specific kinase, for example, Src kinase, is measured prior to contacting the cell or tissue with a compound of the invention. In some embodiments, a cell or tissue is contacted with a compound provided herein, based on the cell or tissue exhibiting abnormal kinase activity. For example, in some embodiments, a cell or tissue is contacted with a compound inhibiting Src kinase activity, as provided herein, based on the cell or tissue exhibiting increased or constitutive Src kinase activity. In some embodiments, the cell is a tumor cell. In some embodiments, the cell is a malignant cell. In some embodiments, the tissue is tumor tissue. In some embodiments, the cell or tissue are in culture. In some embodiments, the cell or the tissue are comprised within a subject. In some embodiments, the contacting is performed *in vitro*. In some embodiments, the contacting is performed *in vivo*.

[00329] In some embodiments, a method is provided comprising administering to a subject having a tumor an effective amount of a compound modulating a kinase as provided herein based on the tumor exhibiting aberrant kinase activity. In some embodiments, a method is provided comprising administering to a subject having a tumor exhibiting increased kinase activity, for example, increased Src kinase activity, a compound inhibiting a

kinase as provided herein, for example, an Src-inhibitory compound, based on the tumor exhibiting aberrant kinase activity.

[00330] In some embodiments a compound modulating the activity of a kinase is a compound selectively binding the kinase. Accordingly, in some embodiments a selective kinase-inhibitory compound is provided. In some embodiments, a selective Src kinase inhibitor is provided. In some embodiments, a compound selectively binding a kinase binds the kinase with high affinity, for example, with a dissociation constant (K_D) of less than 10^{-7}, less than 10^{-8}, less than 10^{-9}, less than 10^{-10}, less than 10^{-11}, less than 10^{-12}, or less than 10^{-13}, but does not bind with comparable affinity to other kinases. In some embodiments, a compound selectively binding a kinase binds the kinase with a dissociation constant (K_D) of less than 10^{-7}, less than 10^{-8}, less than 10^{-9}, less than 10^{-10}, less than 10^{-11}, less than 10^{-12}, or less than 10^{-13}, but binds other proteins with a KD of more than 10^{-7}, more than 10^{-6}, more than 10^{-5}, or more than 10^{-4}. Similarly, a compound selectively inhibiting a kinase inhibits the activity of the kinase, but does not significantly inhibit other kinases. In some embodiments, a compound provided herein that selectively inhibits a kinase, decreases the activity of the kinase by at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, at ;east about 98%, or at least about 99%, but does not decrease the activity of a different kinase by more than about 40%, more than about 30%, more than about 25%, more than about 20%, more than about 10%, or more than about 5%.

[00331] Some aspects of this invention relate to the use of the inventive compounds for the production of pharmaceutical compositions for the treatment and/or prophylaxis and/or amelioration of a disease or disorder associated with aberrant kinase activity, for example, any of the diseases, disorders, illnesses, and/or conditions as mentioned herein.

[00332] Some aspects of this invention relate to the use of the inventive compounds for the production of pharmaceutical compositions that modulate kinase activity, for example, compositions that inhibit Src kinase activity, in a subject.

[00333] Some aspects of this invention relate to the use of the inventive compounds for the production of pharmaceutical compositions which can be used for treating, preventing, or ameliorating diseases responsive to modulating kinase activity, for example, diseases responsive to the inhibition of Src kinase activity, *e.g.*, cancers, and particularly cancers exhibiting increased or constitutive Src kinase activity.

[00334] The amount of a compound provided herein that is required for effective treatment of a disease will vary from subject to subject, depending on the species, age, and

general condition of the subject, the particular compound, its mode of administration, its mode of activity, and the like. The compounds of the invention are preferably formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be determinedby the attending physician based on sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific protein employed; the specific composition employed; the age, body weight, general health, sex, and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

[00335] Furthermore, after formulation with an appropriate pharmaceutically acceptable carrier in a desired dosage, the pharmaceutical compositions of this invention can be administered to humans and other animals orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, or drops), bucally, as an oral or nasal spray, or the like. In certain embodiments, the compounds of the invention may be administered orally or parenterally at dosage levels sufficient to deliver from about 0.001 mg/kg to about 100 mg/kg, from about 0.01 mg/kg to about 50 mg/kg, from about 0.1 mg/kg to about 40 mg/kg, from about 0.5 mg/kg to about 30 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, and from about 1 mg/kg to about 25 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic effect. The desired dosage may be delivered three times a day, two times a day, once a day, every other day, every third day, every week, every two weeks, every three weeks, or every four weeks. In certain embodiments, the desired dosage may be delivered using multiple administrations (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations).

[00336] Liquid dosage forms for oral and parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and

sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents. In certain embodiments for parenteral administration, the compounds of the invention are mixed with solubilizing agents such polyethoxylated castor oil, alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and combinations thereof.

[00337] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

[00338] The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[00339] In order to prolong the effect of a drug, it is often desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as poly(lactide-co-glycolide). Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

[00340] Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the compounds of this invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

[00341] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

[00342] Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

[00343] The active compounds can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active protein may be admixed with at least one inert diluent

such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, *e.g.*, tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

[00344] Formulations suitable for topical administration include liquid or semi-liquid preparations such as liniments, lotions, gels, applicants, oil-in-water or water-in-oil emulsions such as creams, ointments, or pastes; or solutions or suspensions such as drops. Formulations for topical administration to the skin surface can be prepared by dispersing the drug with a dermatologically acceptable carrier such as a lotion, cream, ointment, or soap. Useful carriers are capable of forming a film or layer over the skin to localize application and inhibit removal. For topical administration to internal tissue surfaces, the agent can be dispersed in a liquid tissue adhesive or other substance known to enhance adsorption to a tissue surface. For example, hydroxypropylcellulose or fibrinogen/thrombin solutions can be used to advantage. Alternatively, tissue-coating solutions such as pectin-containing formulations can be used. Ophthalmic formulation, ear drops, and eye drops are also contemplated as being within the scope of this invention. Additionally, the present invention contemplates the use of transdermal patches, which have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms can be made by dissolving or dispensing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

[00345] Additionally, the carrier for a topical formulation can be in the form of a hydroalcoholic system (*e.g.*, liquids and gels), an anhydrous oil or silicone based system, or an emulsion system, including, but not limited to, oil-in-water, water-in-oil, water-in-oil-in-water, and oil-in-water-in-silicone emulsions. The emulsions can cover a broad range of consistencies including thin lotions (which can also be suitable for spray or aerosol delivery), creamy lotions, light creams, heavy creams, and the like. The emulsions can also include microemulsion systems. Other suitable topical carriers include anhydrous solids and semisolids (such as gels and sticks); and aqueous based mousse systems.

[00346] It will also be appreciated that the compounds and pharmaceutical compositions of the present invention can be employed in combination therapies, that is, the compounds and pharmaceutical compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, an inventive compound may be administered concurrently with another anticancer agent), or they may achieve different effects (*e.g.*, control of any adverse effects).

[00347] In still another aspect, the present invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention, and in certain embodiments, includes an additional approved therapeutic agent for use as a combination therapy. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceutical products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

EXAMPLES

[00348] The function and advantage of these and other embodiments of the present invention will be more fully understood from the examples below. The following examples are intended to illustrate the benefits of the present invention, but do not exemplify the full scope of the invention.

Example 1: Identification of macrocycles having kinase-inhibiting properties

[00349] DNA-linked small molecules with protein binding affinity can be enriched from complex mixtures containing predominantly non-binding DNA-linked small molecules,[20,29] and that a library of 13,824 DNA templates can be translated into a corresponding library of synthetic macrocycles through three DNA-templated synthesis reactions.[24] In this example, these capabilities are integrated into a broad effort to discover and synthetic small-molecule macrocycles that inhibit or activate protein targets of interest are characterized.

Validating the In vitro Selection of DNA-Linked Small Molecules that Bind Proteins Fused to GST

[00350] Prior to undertaking library selections, we first validated a general and efficient *in vitro* selection strategy that would support selections against many different protein targets. We sought to improve our earlier *in vitro* selection efforts that used proteins covalently bound to beads[29] by implementing a strategy that would not perturb the covalent structure of the target protein upon immobilization. Glutathione-*S*-transferase (GST) fusions of many biomedically relevant proteins are readily available, typically retain native function, and can be immobilized in a defined way using glutathione-linked resin. Furthermore, library members that bind targets presented in this manner can be specifically eluted under mild conditions with free glutathione. This elution strategy should minimize the enrichment of library members that bind molecules other than the target of interest, such as resins or resin-bound linkers.

[00351] We tested the ability of immobilized GST-fusion proteins to enrich known small-molecule ligands covalently linked to DNA oligonucleotides in several mock selections using GST-tagged MDM2, FKBP, Bcl-xL, cRaf-1 and HSP90 as protein targets. In brief, a known small-molecule ligand linked to DNA (the positive control) was combined with a 100- to 10,000-fold molar excess of a non- binding DNA sequence (the negative control). The negative and positive control sequences were of identical length and shared common PCR primer-binding sequences. The mixture was incubated with the corresponding protein target immobilized on glutathione-linked beads. Following several successive washes with buffer, molecules that remained bound to the resin were eluted with free glutathione and subjected to PCR amplification or carried through to another round of selection. The ratio of positive control DNA to non-binding DNA was measured using a restriction endonuclease[29] or by qPCR.[43-45] After only one round of selection, we observed substantial (~10- to 1000-fold) enrichment of the positive control-encoding DNA sequence against all five GST-linked protein targets (**Figure 1** and **Table 1**). These results confirmed our ability to enrich DNA-linked small–molecule protein ligands using immobilized GST-tagged proteins.

Protein	Ligand	interaction affinity (not DNA-linked)	Enrichment Factor	Analysis
MDM2 (1-118, N-terminal	Novartis Peptide	5 nM[1]	2,000	Taqman qPCR
MDM2 (1-118, N-terminal	control peptide	1.5 μM[2]	40	Taqman qPCR
FKBP (N-terminal GST- fused)	Holt ligand	8 nM[3]	1,100	restriction digestion
BcLxL (1-209,Δ45-84, N-terminal GST- fused)	Bak peptide	0.34 μM[4]	200	restriction digestion
Human Hsp90 (1-235, N-terminal GST-fused)	Geldanamycin	23 nM (as FITC conjugate)[5]	40	Taqman qPCR
cRaf-1 (51-200, N-terminal	Ras peptide	~100 μM[6]	30	Taqman qPCR

Table 1. Single round enrichment factors for positive-control DNA-small molecule conjugates after affinity selection using GST-linked MDM2, FKBP, Bcl-xL, cRaf-1, and HSP90.

References Cited in Table 1

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6. Barnard, D., Sun, H., Baker, L. & Marshall, M.S. *In vitro* inhibition of Ras-Raf association by short peptides. *Biochem. Biophys. Res. Commun.* **247**, 176-180 (1998).

[00352] All references cited herein, including any references cited below, are included herein in their entirety by reference.

In vitro Selection of 13,824 DNA-Templated Small-Molecule Macrocycles

[00353] The DNA-templated small-molecule macrocycle library used in this *in vitro* selection effort was generated in three DNA-templated reactions followed by a non-templated Wittig macrocyclization reaction to generate the final product (**Figure 2a**).[20,24] Each DNA-templated library synthesis step relied on a set of 12 DNA-linked building blocks comprising both natural and non-natural amino acids (**Figure 2b**).[24] These 36 building blocks together with eight variable diamino- acid scaffolds (**Figure 2b**) result in a theoretical diversity of 13,824 DNA-templated macrocycles. The fidelity of DNA-templated macrocycle library synthesis was extensively characterized by high- resolution LC-MS analysis and by gel electrophoresis as previously described.[24]

[00354] We chose a diverse set of biomedically relevant protein targets including protein kinases, protein phosphatases, small GTPases, PDZ domains, SH2 domains, nuclear receptors, and anti- apoptotic proteins for *in vitro* selection (**Table 2**).

Kinases	Phosphatases	PDZ domains	SH2 domains
Akt3	DEP1	Dvl1-3	Abl1
AMPK ERBB4	MEG2	Erbin G1syntrophin Magi1	Abl2
MK2	PRL2	MUPP1	P85αN P85αC P55γC
p38α	**GTPases**	PAR6B PSD95	
MKK6	Cdc42	RGS3	
Pim1	H-Ras-V12	SAP97	**other proteins**
Src	RhoA	Semcap3	Bcl-xL
VEGFR2		Shank3	BIR3 (XIAP) PPARδ

Table 2. Protein targets for *in vitro* selection. All proteins were obtained or purified as GST-tagged fusion proteins.

[00355] Multiple representatives were chosen from each protein class so that we could compare selection results within protein families. In total, we performed a single round of *in vitro* selection against 36 protein targets. Additionally, we also performed a selection for binding to GST (with no fused target protein) immobilized on glutathione-linked resin. Although successive rounds of selection (with or without DNA amplification and translation between rounds) can multiply net enrichment factors,[29] we hypothesized that recent advances in ultra high- throughput DNA sequencing would enable even modest enrichment factors to be robustly detected, and the findings in this work are the result of a single round of selection on each protein target.

[00356] Following *in vitro* selection, DNA templates linked to target-binding macrocycles were amplified in PCR reactions using primers containing "barcode" sequences specific to each selection (**Figure 3**). We also amplified DNA templates from eight aliquots of the unselected library to determine the initial abundance of each library member. A set of 12 five-base barcodes used in pairwise combinations provided sufficient encoding complexity for one round of selection against 36 protein targets, a control selection against immobilized GST, and eight pre-selection library samples (**Table 3**). These barcodes were designed to differ by at least three bases from one another so that common DNA sequencing errors would not preclude barcode assignment. After affinity selection and PCR amplification, barcoded samples were combined in equimolar amounts and submitted for ultra high-throughput DNA sequencing[46,47] as a single sample.

5'-ACTGA-3'	5'-GTATC-3'
5'-AGCTG-3'	5'-GAGCT-3'
5'-ATACG-3'	5'-GTCAT-3'
5'-TACGC-3'	5'-CGTAT-3'
5'-TCATG-3'	5'-CATCG-3'
5'-TCGAT-3'	5'-CTGTA-3'

Table 3. Five-base PCR barcodes used to identify each selection during Solexa sequencing. Barcodes were appended to the 5'-terminus of the DNA-templated library-specific primers and used in pairwise combinations to provide sufficient encoding complexity.

Ultra High-Throughput DNA Sequencing and Analysis of Selection Results

[00357] To rapidly identify the DNA-linked macrocycles that survived target affinity selection, we used Solexa (Illumina) DNA sequencing technology.[48] Because standard Solexa sequencing read lengths at the time of this experiment were shorter than the length of our barcoded library templates (84 base pairs), the paired-end sequencing method, which provides both forward and reverse sequencing reads for each template, was used. We created a PERL computer program to analyze the large amount of data emerging from high-throughput sequencing. First, forward and reverse sequence reads were merged to give complete template sequences (**Figure 4a**). Each complete DNA sequence was converted into a combination of a barcode (selection identity) and three codons (building block identities). Template sequences were then binned by barcode to deconvolute the results of each selection. The abundance fraction of each library member was determined within each selection by counting the number of corresponding DNA sequence occurrences and dividing by the total number of interpretable sequence reads for that selection. Finally, enrichment factors for each library member were computed by dividing post-selection abundance fraction by pre-selection abundance fraction. In total, we received 25.6 million paired forward and reverse sequence reads from two Solexa sequencing runs. Of these, all four codons (which require five out of six correct bases per codon for the building blocks, and three out of three correct bases per codon for the scaffold[24]) and the selection barcode (which requires four out of five correct bases) could be conclusively assigned for 12.4 million sequences. As a result, we obtained ~200,000 sequence reads for each selection, and 1.7 million sequence reads of the pre-selection library.

[00358] Although 100% of the 13,824 possible library DNA sequences were observed at least once, due to the varying efficiencies of library member synthesis,[24] library members in the pre-selection pool were not expected to be present in equal abundance. Since underrepresented library members are more prone to inaccurate enrichment factors arising from limited sampling, we evaluated selection results using scatter plots of enrichment factor versus sequence counts in order to better identify unusually high enrichment factors that may arise from macrocycle-target binding (**Figure 4b**). Indeed, in most selections we observed that those sequences with the fewest sequence counts exhibited both the largest and the smallest enrichment factors (**Figure 4b**), presumably due to statistical noise among underrepresented library members. The large number of library members enabled a general

relationship between sequence abundance and typical enrichment levels to emerge for each selection. Enrichment factors were therefore deemed of interest not only on the basis of their absolute value, but also only if they fell above the typical enrichment factor range observed for sequences of that abundance.

Protein Kinase Selection Results

[00359] Because most of the significantly enriched DNA sequences arose from selections against protein kinases, we focused our characterization efforts on this class of targets. After one round of selection against Src kinase, macrocycle A11-B1-C5-D7 was enriched 62-fold, representing the highest degree of enrichment for any library member in any of the selections described above. We also identified macrocycles A11-B1-C10-D7, A11-B1-C3-D7, A10-B1-C5-D6, A10-B1-C5-D7, A11-B1- C5-D5, A9-B1-C5-D7, A11-B8-C5-D7, and A11-B8-C10-D7 (**Figure 4b** and **Figure 5**) as enriched between 2- to 7-fold in the Src selection. The significant enrichment factors, strong degree of structural similarity among these molecules, and lack of enrichment of these macrocycles in the other eight kinase selections performed (**Table 4**), collectively suggested that this class of molecules may correspond to authentic Src ligands that are selective for binding to Src over other protein kinases.

	Src	Akt3	AMPK	ERBB4	MK2	p38α	MKK6	Pim1	VEGFR2
1	62	2.2	0.69	0.63	2.8	1.1	2.7	2.4	2.4
2	7.1	1.2	0.86	0.82	1.8	0.84	1.1	0.91	0.95
3	4.8	0.97	0.94	0.91	1.2	1.0	0.81	0.93	1.4
4	4.1	0.81	0.8	1.1	0.97	0.91	1.1	0.89	0.65
5	4.0	1.5	1.4	1.7	2.0	1.7	2.2	1.3	1.6
6	3.6	1.3	1.0	1.0	0.65	1.1	1.4	1.1	0.66
7	3.5	1.5	1.1	1.2	1.5	0.99	1.0	0.39	1.4
8	2.6	1.5	1.2	1.2	2.0	1.6	1.3	2.2	1.5
9	2.6	0.98	0.88	1.3	2.6	1.4	2.4	1.9	1.6

Table 4. Enrichment factors of positives from the Src selection in all protein kinase selections performed. Macrocycle numbering is from **Figure 5**. Enrichment factors > 10 are colored red; those > 4 are colored orange; those > 2 are colored yellow.

[00360] We also identified two additional families of macrocycles enriched in selections against several other kinase targets. Macrocycles A10-Y-C11-D5 (where Y = B1 or B8) were enriched against Akt3, MAPKAPK2, Pim1, and VEGFR2 (**Figure 6**). Macrocycles A12-B8-C10-X (where X = D1-D8) exhibited unusually high enrichment

factors in the MAPKAPK2 and Pim1 selections (**Figure 6**). While we were encouraged by the ability of these molecules to bind similar targets, the high degree of hydrophobicity of the building blocks in these macrocycles raised the possibility that they may bind their targets in a non-classical mode.[49,50] Moreover, the enrichment of macrocycles of structure A12- B8-C10-X appears to be insensitive to changes in ring size (caused by substitution at the "D" scaffold position) that we anticipated would have large effects on macrocycle conformation, suggesting that these macrocycles were interacting with their targets in a non-classical binding mode.[24] The possibility that these compounds represent non-specific ligands or "promiscuous aggregators"[49,50] was examined as described below.

Solid-Phase Macrocycle Synthesis

[00361] To test if the enriched DNA sequences emerging from selection correspond to macrocycles with target-binding or target-inhibiting activities, the corresponding macrocycles were synthesized on multi-milligram scale using Fmoc solid-phase peptide synthesis (**Figure 7**).[20] In brief, NovaPEG Rink amide resin (Merck) was coupled to a differentially protected diamino acid building block. An isopropylidene-protected tartrate monomethyl ester was reacted with one amine, and a tripeptide was synthesized on the other amine using three cycles of Fmoc peptide synthesis. After generating the phosphonium salt on resin, we cleaved the macrocyclization precursor from resin using strong acid. This cleavage reaction simultaneously generated a carboxamide at the C-terminus and also revealed the tartrate diol. After HPLC purification and LC/MS characterization of the linear product, we oxidiatively cleaved the diol to reveal an aldehyde and effected Wittig cyclization by raising the pH of the solution to generate a phosphonium ylide. Macrocycle syntheses typically yielded two HPLC- separable isomers that were characterized as the desired *cis* and *trans* macrocycles using NMR spectroscopy and high-resolution mass spectrometry (**Table 5**). In total, 27 macrocycles corresponding to enriched DNA sequences were synthesized on 1-3 mg scale using this route. Overall yields for each 14-step synthesis (13 solid-phase steps and one solution-phase step) ranged from 1-12%.

Macrocycle	Expected $(M+H)^+$	Observed $(M+H)^+$
cis-A11-B1-C5-D7	666.2994	666.2995
trans-A11-B1-C5-D7	666.2994	666.2986
cis-A11-B1-C10-D7	728.3151	728.3120
Trans-A11-B1-C10-D7	728.3151	728.3118
cis-A11-B1-C3-D7	652.2838	652.2811
trans-A11-B1-C3-D7	652.2838	652.2809
cis-A10-B1-C5-D6	652.2726	652.2714
trans-A10-B1-C5-D6	652.2726	652.2703
cis-A10-B1-C5-D7	638.2569	638.2558
trans-A10-B1-C5-D7	638.2569	638.2549
Cis-A9-B1-C5-D7	609.2337	609.2321
trans-A9-B1-C5-D7	609.2337	609.2330
cis-A11-B1-C5-D5	694.3307	694.3289
trans-A11-B1-C5-D5	694.3307	694.3298
cis-A11-B8-C5-D7	682.3671	682.3647
trans-A11-B8-C5-D7	682.3671	682.3654
cis-A11-B8-C10-D7	744.3828	744.3802
Trans-A11-B8-C10-D7	744.3828	744.3804
cis-Ala-B1-C5-D7	517.2405	517.2384
cis-A11-Ala-C5-D7	600.2889	600.2847
cis-A11-B1-Ala-D7	626.2681	626.2674
trans-Phe-B1-C5-D6	607.2874	607.2851
linear-A11-B1-C5-D7	670.3307	670.3296
linear-A10-B1-C5-D6	656.3039	656.3015
cis-A10-B1-C11-D5	883.4560	883.4545
cis-A10-B8-C11-D5	899.5237	899.5232
cis-A12-B8-C10-D3	906.4760	906.4734
cis-A12-B8-C10-D4	892.4604	892.4568
cis-A12-B8-C10-D8	892.4604	892.4589

Table 5. High-resolution ESI mass spectrometry data for macrocycles.

Macrocycles Selected for Binding to Src Inhibit Src Kinase Activity

[00362] Nine macrocycles enriched in the Src selection (**Figure 5**) and synthesized as described above were assayed *in vitro* for the ability to inhibit Src kinase activity. Kinase activity was assayed in the presence of varying concentrations of macrocycle using the FRET-based Z'-LYTE assay (Invitrogen). Although our selections did not explicitly select for target inhibition, all but one of the nine macrocycles tested inhibited Src kinase activity (**Table 6** and **Figure 8**). All of the assayed macrocycles with Src selection enrichment factors ≥ 4-fold exhibited IC_{50} values in the range of ~500nM to 10 μM. The most potent molecules, macrocycles *trans*-A10-B1-C5-D6 and *cis*-A11-B1-C5-D7, which share the furylalanine (B1) and cyclopropylalanine (C5) building blocks, but otherwise display significant structural diversity, inhibited Src with IC_{50} values of 680 nM and 960 nM respectively (**Table 6** and **Figure 8**). We consistently observed large differences in potency between macrocycle *cis* and *trans* stereoisomers (**Table 6**), highlighting the importance of macrocycle stereochemistry and conformation for activity. For example, the *cis* and *trans* stereoisomers of macrocycles A11-B1-C5- D7 and A10-B1-C5-D6 exhibited a ~10-fold difference in potency (**Table 6**). Likewise, variants of highly-enriched macrocycles such as A11-B1-C5-D7 (62-fold enrichment) that differ by the addition or loss of a single methylene group within the macrocycle were not significantly enriched, further suggesting that even closely related library members can access significantly different three-dimensional structures.

Macrocycle	Src IC_{50} (μM)
cis-A11-B1-C5-D7	0.96
trans-A11-B1-C5-D7	12
cis-A11-B1-C10-D7	7.0
trans-A11-B1-C10-D7	21
cis-A11-B1-C3-D7	10
trans-A11-B1-C3-D7	48
cis-A10-B1-C5-D6	7.4
trans-A10-B1-C5-D6	0.68
cis-A10-B1-C5-D7	8.9
trans-A10-B1-C5-D7	1.5
cis-A9-B1-C5-D7	>100
trans-A9-B1-C5-D7	>100
cis-A11-B1-C5-D5	85
trans-A11-B1-C5-D5	>100
cis-A11-B8-C5-D7	36
trans-A11-B8-C5-D7	>100
cis-A11-B8-C10-D7	25

Macrocycle	Src IC_{50} (μM)
trans-A11-B8-C10-D7	>100

Table 6. Macrocycles corresponding to Src selection survivors inhibit Src kinase. Src kinase activity was assayed in the presence of increasing concentrations of macrocycle using the Z'-LYTE assay (Invitrogen).

Macrocycles Selected for Binding to Akt3, MAPKAPK2, Pim1, and VEGFR2 Modulate Kinase Activity

[00363] Macrocycles A10-B1-C11-D5 and A10-B8-C11-D5 were enriched 2- to 6-fold upon selection for binding Akt3, MAPKAPK2, Pim1, and VEGFR2 (**Figure 6**). These two macrocycles are structurally similar, sharing nitrophenylalanine (A10) and cyclohexylstatine (C11) building blocks as well as the same lysine scaffold (D5), and differ only at the B building block with either furylalanine (B1) or cyclohexylalanine (B8) (**Figure 6**). For assay purposes, the macrocycles were synthesized with C-terminal PEG-diamine linkers to aid solubility, and then tested for the ability to inhibit Akt3, MAPKAPK2, Pim1 and VEGFR2. The more hydrophobic of the two compounds, macrocycle *cis*-A10-B8-C11-D5, inhibited Akt3, MAPKAPK2, Pim1, and p38α-MAPKAPK2 with IC_{50} values of 8.7 μM, 6.8 μM, 7.5 μM, and 3.1 μM respectively (**Table 7** and **Figure 9**). Macrocycle *cis*-A10-B1-C11-D5 did not inhibit any of these kinases when assayed with isolated kinase enzymes; however we observed inhibition of the p38α-MAPKAPK2 kinase cascade with an IC_{50} value of 6.4 μM (**Table 7** and **Figure 9**), suggesting that the molecule may interact with either or both kinases but only in a conformation that is adopted upon formation of the p38α-MAPKAPK2 complex. Interestingly, we observed strong, dose-dependent *activation* of VEGFR2 in the presence of *cis*-A10-B1-C11-D5 (**Figure 10**) with enzyme activity increasing 70% upon treatment with 10 μM of this macrocycle and 300% upon treatment with 100 μM compound, suggesting that this macrocycle may bind to VEGFR2 in a manner that enhances kinase activity.

Macrocycle	Kinase	IC_{50} (μM)
cis-A10-B1-C11-D5	P38α-MK2	6.5
cis-A10-B1-C11-D5	Pim1	21
cis-A10-B8-C11-D5	Akt3	8.4
cis-A10-B8-C11-D5	MK2	6.1
cis-A10-B8-C11-D5	P38α-MK2	3.4
cis-A10-B8-C11-D5	Pim1	7.3
cis-A12-B8-C10-D3	P38α-MK2	12
cis-A12-B8-C10-D3	Pim1	25
cis-A12-B8-C10-D4	P38α-MK2	20

Macrocycle	Kinase	IC_{50} (μM)
cis-A12-B8-C10-D4	Pim1	100
cis-A12-B8-C10-D8	MK2	47
cis-A12-B8-C10-D8	P38α-MK2	14
cis-A12-B8-C10-D8	Pim1	17

Table 7. Enriched macrocycles inhibit Pim1, MK2, Akt3, and p38a-MK2. Kinase activity was assayed in the presence of increasing concentrations of macrocycles using Invitrogen's Z-LYTE assay.

[00364] We also synthesized and assayed three macrocycles from the A12-B8-C10-X series that were enriched 2- to 10-fold after *in vitro* selection against MAPKAPK2 and Pim1 (**Figure 6**). These macrocycles possess three hydrophobic building blocks, benzoyl-*D*-phenylalanine (A12), cyclohexylalanine (B8), and styrylalanine (C10), with variable scaffold diamino acid building blocks. Macrocycles A12-B8-C10-D3, enriched 3-fold against MAPKAPK2 and 3.5-fold against Pim1; A12- B8-C10-D4, enriched 3-fold against MAPKAPK2 and 10-fold against Pim1; and A12-B8-C10-D8, enriched 2.5-fold against MAPKAPK2 and 6-fold against Pim1 (**Figure 6**), were synthesized and assayed for inhibition of MAPKAPK2, Pim1, and the p38α-MAPKAPK2 cascade. We observed modest inhibition of these kinase targets, with the most potent molecules being *cis*-A12-B8-C10-D3 against the p38α-MAPKAPK2 cascade (IC_{50} = 11 μM) and *cis*-A12-B8-C10-D8 against Pim1 (IC_{50} = 19 μM) (**Figure 11** and **Table 7**).

[00365] Taken together, these results indicate that DNA-templated macrocycles that are enriched from *in vitro* selections for kinase affinity frequently possess the ability to inhibit protein kinases with reasonable potency (low- to mid-micromolar IC_{50} values) when synthesized and assayed in a non-DNA-linked form. Among the nine macrocycles chosen based on the magnitude of their enrichment during Src selection, or based on their structurally similarity to the highly enriched A11-B1-C5-D7, we characterized only one enriched macrocycle (A9-B1-C5-D7) that did not inhibit Src, representing a false positive rate of 11%. While we characterized only five molecules from selections against Akt3, MAPKAPK2, Pim1, and VEGFR2, due to enrichment of the same molecule against multiple targets, we validated 12 distinct protein-macrocycle interactions. One molecule, macrocycle A10-B9-C11-D5 (B9 = citrulline), similar to the validated A10-Y-C11-D5 family of hits, was observed enriched above background in the Pim1 (3.5-fold enrichment) and VEGFR2 (2.4-fold enrichment) selections, but did not inhibit either kinase when assayed in its non-DNA-linked form. In total, we observed a similarly low false positive

rate of 14% (2/14) from the Akt3, MAPKAPK2, Pim1, and VEGFR2 selections.

Kinase Inhibition Selectivity of Enriched Src-Inhibiting Macrocycles

[00366] We chose to characterize in greater depth macrocycles *cis*-A11-B1-C5-D7 and *trans*-A10-B1- C5-D6 (**Figure 5** and **Table 6**) since they represent potent and structurally diverse inhibitors that are both predicted based on *in vitro* selection results (**Table 4**) to be selective for Src kinase. We first characterized their mode of inhibition. Although the selection used to identify the inhibitors is agnostic with respect to target binding site, we determined that *cis*-A11-B1-C5-D7 and *trans*-A10-B1-C5-D6 are ATP-competitive inhibitors (**Figure 12** and **Figure 13**). We next determined the selectivity of these molecules against a representative set of human kinases. Although kinase selectivity can be modest for ATP-competitive inhibitors since the ATP-binding site is highly conserved among protein kinases,[51-54] our selection data (**Table 4**) suggested that the Src-inhibiting macrocycles may exhibit unusual selectivity.

[00367] Macrocycle *cis*-A11-B1-C5-D7 was assayed at 50 μM against a representative panel of 58 commercially available human protein kinases including all nine mammalian Src family kinases (**Table 8**). For those kinases that displayed significant inhibition (> 40%) at 50 μM *cis*-A11-B1-C5-D7, we re-assayed at 5 μM compound concentration to estimate rough IC_{50} values and performed 10-point IC_{50} titrations for the most potently inhibited kinases (**Table 8**). Using this data, we calculated a selectivity score[55] for the macrocycle based on the number of non-target proteins exhibiting IC_{50} values within 10-fold of the 960 nM IC_{50} of *cis*-A11-B1-C5-D7 for Src. Macrocycle *cis*-A11-B1-C5-D7 displayed a selectivity score of 0.05, indicating that only 5% of the kinases assayed were inhibited with IC_{50} values within 10-fold of the IC_{50} for Src. When judged by similar criteria, *cis*-A11-B1-C5-D7 is significantly more selective than the promiscuous ATP-competitive inhibitor staurosporine (selectivity score = 0.50), and slightly more selective than two kinase inhibitor drugs: sorafenib (selectivity score = 0.10) and dasatanib (selectivity score = 0.10).[55] The most potently inhibited proteins other than Src were closely related kinases such as Fgr (a Src family kinase, IC_{50} = 4.0 μM) and RET (a tyrosine kinase, IC_{50} = 5.7 μM), as well as Aurora A, a serine/threonine kinase which shares close structural homology to Src-family kinases (IC_{50} = 1.7 μM)[56].

kinase	% inhibition (50 μM)	% inhibition (5 μM)	IC_{50}
ABL1	7		>50 μM
ACVR1B (ALK4)	5		>50 μM
AKT1 (PKB alpha)	89		19.4 μM
AMPK A1/B1/G1	57	7	40 μM
AURKA	105		1.7 μM
BLK	42	13	>50 μM
BTK	59	20	31 μM
CDK1/cyclin B	9		>50 μM
CHEK1 (CHK1)	18		>50 μM
CSNK1G2 (CK1 gamma 2)	2		>50 μM
CSNK2A1 (CK2 alpha 1)	4		>50 μM
DYRK3	44	8	>50 μM
EGFR (ErbB1)	-1		>50 μM
EPHA2	0		>50 μM
ERBB2 (HER2)	-1		>50 μM
FGFR1	2		>50 μM
FGR	96		4.0 μM
FLT3	53	16	41 μM
FRAP1 (mTOR)	0		>50 μM
FRK (PTK5)	71	23	19 μM
FYN	39	8	>50 μM
GSK3B (GSK3 beta)	93		26.8 μM
HCK	16		>50 μM
IGF1R	11		>50 μM
IKBKB (IKK beta)	78		43.9 μM
INSR	4		>50 μM
IRAK4	-5		>50 μM
JAK3	58	11	37 μM
KDR (VEGFR2)	96		15.9 μM
KIT	-6		>50 μM
LCK	70	25	18 μM
LYN A	67	21	23 μM
LYN B	75	14	21 μM
MAP2K1 (MEK1)	14		>50 μM
MAP4K4 (HGK)	40	-13	>50 μM
MAPK1 (ERK2)	18		>50 μM
MAPK14 (p38 alpha)	102		10.0 μM
MAPK8 (JNK1)	1		>50 μM
MAPKAPK2	63	14	30 μM
MARK2	15		>50 μM
MET (cMet)	85	11	>50 μM
NEK1	-17		>50 μM
NTRK1 (TRKA)	4		>50 μM
PAK4	26		>50 μM
PDGFRB (PDGFR beta)	6		>50 μM
PHKG2	4		>50 μM
PIM1	3		>50 μM
PLK1	2		>50 μM
PRKACA (PKA)	27		>50 μM
PRKCB1 (PKC beta I)	4		>50 μM
RET	82		5.2 μM
ROCK1	11		>50 μM
RPS6KA3 (RSK2)	97		10.6 μM
RPS6KB1 (p70S6K)	4		>50 μM
SRC	100	92	960 nM
SYK	16		>50 μM
TEK (Tie2)	6		>50 μM
YES1	56	36	23 μM

Table 8. Inhibition activity of *cis*-A11-B1-C5-D7 against a panel of human kinase enzymes. > 80% inhibition is highlighted in red, < 40% inhibition is highlighted in blue. IC_{50} values falling within 10-fold of the Src IC_{50} (960 nM) are highlighted in orange. Assay data for Src kinase is from **Figure 8** and **Table 6**. All non-Src assay points were measured by the Invitrogen Select Screen Profiling Service. IC_{50} values < 18 μM were calculated from 10-

point titrations. All other IC_{50} values were estimated from % inhibition at 5 and 50 μM.

[00368] In addition to good overall selectivity, *cis*-A11-B1-C5-D7 exhibited exceptional selectivity among Src-like protein kinases that have traditionally been difficult to distinguish by small-molecule inhibition (**Figure 14**). For example, this macrocycle exhibits > 50-fold selectivity for Src over Abl kinase, > 50-fold selectivity for Src over the Src-family kinases Hck and Fyn, and > 10-fold selectivity for Src over all remaining Src-family kinases with the exception of Fgr (**Figure 14** and **Table 8**). Similarly, among the closely related Aurora kinases we observed > 50-fold selectivity for Aurora A over Aurora B (**Table 9** and **Table 8**).

[00369] We also investigated the selectivity of the nitrophenylalanine (A10)-containing macrocycle *trans*-A10-B1-C5-D6. While it shares two out of four building blocks in common with *cis*-A11-B1- C5-D7 and also inhibits Src in an ATP-competitive manner, building block composition in this macrocycle is strikingly different at the A position and the *trans* stereochemistry of the olefin contrasts with the olefin geometry of the active isomer of A11-B1-C5-D7. The differences between these two molecules were further revealed upon assays against a kinase panel, in which *trans*-A10-B1-C5-D6 exhibited even greater selectivity for Src than macrocycle *cis*-A11-B1-C5-D7 (**Figure 14**). Macrocycle *trans*-A10-B1-C5-D6 did not significantly inhibit Aurora A, RET, or any of the other Src-family kinases (the primary kinases beyond Src that are inhibited by *cis*-A11-B1-C5-D7) at the concentration tested (5 μM) (**Figure 14**). Indeed, of all 44 human kinases against which this macrocycle was assayed, Src was the only kinase observed to be significantly inhibited by this macrocycle (**Figure 14** and **Table 10**). This level of Src selectivity is unprecedented among currently available Src-selective kinase inhibitors such as PP1 and PP2,[57] which are significantly more potent against Src-family members Fyn, Hck and Lyn than against Src itself. Taken together, these results establish that macrocycles emerging from the *in vitro* selection of a DNA-templated small-molecule library include kinase inhibitors that exhibit unusual selectivity.

KINASE	% inhibition
ABL1	18
AKT1 (PKB alpha)	-7
AKT3 (PKB gamma)	11
AMPK A1/B1/G1	-1
AURKA (Aurora A)	10
AURKB (Aurora B)	0
AURKC (Aurora C)	2
BLK	10
BTK	4
CHEK1 (CHK1)	-15
DYRK3	4
ERBB4 (HER4)	4
FGR	13
FLT3	7
FRK (PTK5)	15
FYN	-1
GSK3B (GSK3 beta)	-6
HCK	9
IGF1R	9
IKBKB (IKK beta)	-1
JAK3	-7
KDR (VEGFR2)	7
LCK	17
LYN A	9
LYN B	7
MAP2K1 (MEK1)	-6
MAP2K6 (MKK6)	-1
MAP4K4 (HGK)	-1
MAPK1 (ERK2)	-3
MAPK14 (p38 alpha)	32
MAPK14 (p38 alpha) Direct	-9
MAPKAPK2	-3
MARK2	1

MET (cMet)	**-8**
PAK4	**-2**
PIM1	**-3**
PRKACA (PKA)	**-1**
RET	**6**
ROCK1	**12**
RPS6KA3 (RSK2)	**-4**
SRC	**86**
SYK	**-1**
YES1	**19**

Table 10. Inhibition activity of *trans*-A10-B1-C5-D6 at 5 μM against a set of human kinase enzymes. Assay data for Src kinase is from **Figure 8** and **Table 6**. All non-Src assay points were measured by the Invitrogen Select Screen Profiling Service.

SAR Analysis of Src-Inhibiting Macrocycles

[00370] We analyzed the set of macrocycles enriched in the Src selection to develop basic structure-activity relationships for Src inhibition. Our selection data shows a strong preference for a small set of building blocks at each non-scaffold position (**Figure 5**), suggesting that all three macrocyclic building blocks are important for binding. To test this hypothesis, we synthesized three derivatives of macrocycle *cis*-A11-B1-C5-D7 by systematically replacing each of the non-scaffold building blocks (positions A, B, and C) with alanine, in analogy to alanine-scanning mutagenesis of proteins. We assayed the resulting three single-alanine mutants for Src inhibition and observed that none of the three mutant macrocycles at 5 μM significantly inhibit Src or the Src-family kinase Fgr (**Figure 15a**), indicating that all three building blocks contribute to the kinase inhibition activity of *cis*-A11-B1-C5-D7.

[00371] To evaluate the importance of the macrocyclic nature of these molecules for kinase inhibition, we synthesized two diacetylated linear peptides as linear versions of macrocycles A11-B1-C5-D7 and A10-B1-C5-D6 (**Figure 15b**) and assayed their abilities to inhibit Src. These two compounds are identical to their corresponding macrocycles with the exception that the olefin group resulting from Wittig macrocyclization is replaced by two methyl groups in the linear peptides. Both linear peptides were virtually inactive against Src kinase, confirming the necessity of a cyclic scaffold to pre-organize these sets of building blocks into a three-dimensional conformation capable of binding to and inhibiting

the kinase.

[00372] Olefin stereochemistry plays a role in the ability of these compounds to inhibit Src kinase activity. Macrocycles possessing the ornithine(pyrazinylcarbonyl) building block (A11) and diaminobutyric acid scaffold (D7) are more potent Src inhibitors in their *cis*-isomeric form (**Table 6**). These macrocycles favor furylalanine (B1) over cyclohexylalanine (B8) at the B position, as evidenced by the weakened potency of macrocycles *cis*-A11-B8-C5-D7 and *cis*-A11-B8-C10-D7 compared to *cis*-A11-B1-C5-D7 and *cis*-A11-B1-C10-D7 (**Table 6**), respectively. All of these compounds exhibit hydrophobic building blocks at the C position, especially cyclopropylalanine (C5) (**Figure 5** and **Table 6**).

[00373] In contrast, macrocycles with nitrophenylalanine (A10) at the A position are more active Src inhibitors in their *trans*-isomeric form (**Table 6**). The ornithine (D6) scaffold also supports more potent Src inhibition than the diaminobutyric acid (D7) scaffold that differs by the loss of a single methylene group (**Table 6**). We assayed the importance of the nitrophenylalanine (A10) building block for Src inhibition by replacing it with phenylalanine in macrocycle *trans*-A10-B1-C5-D6 to create macrocycle *trans*-Phe-B1-C5-D6. This substitution abolished Src inhibition (**Figure 15c**), indicating the importance of the nitro group among A10 macrocycle subfamily kinase inhibitors. Due to the altered olefin stereochemistry of the nitrophenylalanine (A10)-containing macrocycles, the difference in macrocycle ring size, and the structural differences between the nitrophenylalanine (A10) and ornithine(pyrazinylcarbonyl) (A11) building blocks, we believe that these two classes of macrocycles interact in distinct ways with the Src active site. Indeed, modeling the three-dimensional conformations adopted by *cis*-A11-B1-C5-D7 and *trans*-A10-B1-C5-D6 using an MM2 energy- minimization algorithm (Chem3D, CambridgeSoft) predicts large differences in both preferred backbone conformation and preferred side-chain orientation for these two macrocycles (**Figure 16**). This hypothesis is further supported by the substantial differences in kinase selectivity (**Figure 14**) exhibited by *cis*-A11-B1-C5-D7 and *trans*-A10-B1-C5-D6.

Src-Inhibiting Macrocycles Do Not Resemble Promiscuous Aggregators

[00374] High-throughput screening can result in the discovery of non-canonical ligands known as promiscuous aggregators that form colloidal aggregates in aqueous solution and inhibit enzymes by a non-specific sequestration mechanism rather than through a classical one-to-one binding mode.[49] A common property of promiscuous aggregators is a

steep dose-response curve that can result from the enzyme concentration in the kinase assay exceeding the K_i of the aggregated species, which is typically present at significantly lower concentrations than that of the small molecule itself.[49] We suspected that A10-Y-C11-D5 and A12-B8-C10-X macrocycles (**Figure 6** and **Table 7**) might be promiscuous aggregators due to their high hydrophobicity, broad specificity, and steep dose-response curves (**Figures 9 and 11**).

[00375] To test this hypothesis, we assayed macrocycle *cis*-A10-B8-C11-D5 against Akt3, MAPKAPK2, and Pim1 and assayed macrocycle *cis*-A12-B8-C10-D3 against Pim1 while varying the concentration of kinase in the reactions. If aggregate formation results in stoichiometric titration of the kinase, the observed IC_{50} value should vary linearly with enzyme concentration.[49]

[00376] Despite their steep dose-response curves, inhibition of MAPKAPK2 and Pim1 did not vary with kinase concentration (**Figure 17**), suggesting a different mechanism of inhibition. For example, steep dose-response curves can also be caused by multi-site binding or by a phase transition in the inhibitor that is coupled with inhibition.[49] In contrast, we observed that inhibition of Akt3 by *cis*-A10-B8-C11-D5 varied linearly with the kinase concentration used in the assay (**Figure 17**), indicating that this macrocycle may be inhibiting Akt3 through a promiscuous aggregator mechanism.

[00377] In contrast, the family of Src-inhibiting macrocycles discovered in this work do not exhibit steep dose-response curves (**Figure 8**), bind in an ATP-competitive manner (**Figure 12** and **Figure 13**), and do not vary significantly in observed inhibitory potency as a function of enzyme concentration (**Figure 17**). These results collectively indicate that the Src-inhibiting macrocycles described above are not promiscuous aggregators.

Discussion

[00378] A 13,824-membered DNA-templated macrocycle library was subjected to 36 protein affinity-based selections in a parallel manner. PCR-installed DNA barcodes enabled all of the selections to be sequenced as one sample and deconvoluted for analysis by binning sequence data by barcode. The ability of current DNA sequencing technology to detect even modestly enriched library members is demonstrated here, obviating the need to perform multiple rounds of selection for the library members characterized in this work. The low false positive rate among the macrocycles enriched during selections studied here suggests that the evaluation of small molecule-protein interactions based on *in vitro* selection and modern DNA sequencing methods is robust.

[00379] After one round of *in vitro* binding selection, high-throughput sequencing enabled the identification of three families of enriched macrocycles in the protein kinase selections, despite enrichment factors that were as low as ~2- to 3-fold. All three families of macrocycles enriched after selection were shown to possess the ability to inhibit their target kinase Macrocycles enriched in the Src selection possessed classical single-site dose response curves with IC_{50} values as low as 680 nM. The potency of these compounds is promising given that they emerged from the broad selection of an untargeted 13,824-membered library.

[00380] Consistent with the highly target-specific *in vitro* selection results, assays of Src-enriched macrocycles against a broad set of human kinases revealed that these molecules indeed possess very good overall selectivity among a panel of human kinases. The specificity of these macrocycles is especially intriguing since these macrocycles are ATP-competitive inhibitors and the ATP-binding site is highly conserved among protein kinases.[51-53] Furthermore, in addition to possessing good overall selectivity, the molecules were exceptionally selective among kinases that have been traditionally very difficult to distinguish by small-molecule inhibitors. For example, Src and Abl are two closely related non-receptor tyrosine kinases that have exhibited very similar small-molecule binding specificities.[58-61]

[00381] Towards the goal of distinguishing Src and Abl using a small molecule, Maly and co-workers[62] recently generated bivalent kinase inhibitors that rely on peptidic recognition outside of the ATP- binding cleft to generate binding selectivity. Macrocycle A11-B1-C5-D7 displays > 50-fold selectivity for Src over Abl kinase while binding in an ATP-competitive manner. We also observed > 100-fold selectivity for Aurora A among the closely-related Aurora family of serine/threonine protein kinases.[63] Most reported small molecule Aurora A inhibitors also inhibit Aurora B and/or Aurora C with comparable affinity[64-67] although examples of Aurora A selective inhibitors have begun to emerge.[68]

[00382] Macrocycle *trans*-A10-B1-C5-D6 exhibited even better selectivity for Src, with no significant off-target inhibition in the panel of 44 human kinases against which this compound was assayed. This panel included all nine highly-related Src-family kinases. The generation of small molecules that are selective among the Src-family kinases has posed a significant challenge to academic and pharmaceutical drug-discovery efforts, and the new class of macrocyclic kinase inhibitors described here represent promising starting points for future probe or therapeutic development efforts.

[00383] The observed selectivity may arise at least in part from the design of the library to contain rigid macrocyclic structures with a high ratio of atoms derived from varying building blocks to atoms shared in common by all library members. Kinase selectivity may also result from the larger size of these molecules compared with traditional ATP-competitive kinase inhibitors; the presence of additional groups may support interactions both inside and outside of the ATP-binding site. The macrocyclic kinase inhibitors discovered in this work share very few chemical features with classical ATP-competitive kinase inhibitors, which are typically planar heterocycles based on adenine. In contrast, our peptidic macrocycles contain several stereocenters and can adopt distinct three- dimensional conformations required for selective target binding. These findings also demonstrate that changes in building block composition that do not abrogate Src inhibition can modulate the selectivity of these macrocycles among human kinases.

Materials and Methods

General Methods

[00384] Macrocycles were purified by reverse-phase HPLC using a C18 stationary phase and an acetonitrile/0.1% trifluoroacetic acid gradient. All ^{1}H NMR spectra were recorded on a Varian INOVA 600 (600 MHz) instrument (**Figure 18**).

In vitro Selections with Known Protein-Binding Ligands

[00385] A stock solution of GST-labeled protein target was diluted in protein-binding buffer (50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 10 mM β-mercaptoethanol) to a final concentration of 1 μM. The protein target (200 pmol) was incubated with 2 μL MagneGST (Promega) glutathione-linked magnetic particles for one hour at 4 °C with gentle shaking. The beads were washed three times with 100 μL TBST buffer supplemented with 1 M NaCl, followed by two washes with 300 μL protein-binding buffer. The beads were then diluted in 20 μL selection buffer (TBST, 3 mg/mL yeast tRNA, 1 mM DTT) and 10 μL of resulting protein bead solution was combined with 1 pmol of a non-binding DNA sequence and 1/10, 1/100, 1/1000, or 1/10,000 pmol of the known protein-binding ligand conjugated to DNA. After removing unbound molecules, the beads were washed three times with 200 μL selection buffer, and bound molecules were eluted in 20 μL of 0.1 mM glutathione in 50 mM Tris pH 8.0 buffer for 15 minutes. Of this eluant, 5 μL was subjected to PCR amplification.

Positive Control Selection Analysis

[00386] Positive-control selections were analyzed using either a *Taqman* qPCR assay or restriction- endonuclease digestion. *Taqman* qPCR was performed according to the manufacturer's protocols. For restriction-endonuclease analysis, 20 μL of PCR reaction were combined with 5 units of *Ear*I restriction endonuclease (NEB), and incubated for two hours at 37 °C. Digested PCR amplicons were separated by agarose gel electrophoresis and stained with EtBr. The ratio of digested to undigested DNA was determined by ethidium bromide staining and densitometry using UV light.

In vitro Selections with the DNA-Templated Macrocycle Library

[00387] *In vitro* selections of the 13,824-membered library were conducted exactly as described above for the positive control selections. For each selection, 5 pmol of library were used. PCR amplification of selection survivors was performed with 5'-barcoded forward and reverse primers. Barcoded PCR amplicons were quantitated using Picogreen dsDNA quantitation reagent (Invitrogen), mixed in equimolar amounts, and submitted for high-throughput DNA sequencing.

Kinase Assays

[00388] Src IC_{50} determinations and single-point inhibition measurements were performed using Invitrogen's Z'-LYTE Tyr 02 assay kit according to the manufacturer's protocols. All kinase assays were performed at ATP concentrations near $K_{M,ATP}$ values, except where noted otherwise. Active human Src kinase was obtained from Millipore. For kinome profiling and IC_{50} determinations against non-Src kinases, compounds were submitted to Invitrogen's SelectScreen Kinase Profiling Service.

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[00389] All publications, patents and sequence database entries mentioned herein, including those items listed below, are hereby incorporated by reference in their entirety for disclosure of the teachings relevant to this invention, as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of the present specification and a document incorporated by reference including conflicting disclosure, the present specification shall control.

Example 2: Development of macrocyclic Src inhibitors

[00390] Since protein kinases play a central role in cell signaling, the discovery and development of protein kinase inhibitors have become the focus of intensive chemical and biological research over the past two decades (Cohen 2002). Currently, all FDA-approved small-molecule inhibitors of protein kinases target the binding site for ATP, the common substrate in the enzymatic reaction of all 518 kinases in the human kinome (Manning, Whyte et al. 2002). The high sequence conservation within the kinase domain and in particular around the ATP binding pocket makes it challenging to develop specific inhibitors of protein kinases. However, when such specificity can be achieved, kinase inhibitors can become effective drugs. The small-molecule inhibitor IMATINIB™ (GLEEVEC™, Novartis) is selective for c-Abl, PDFGR and c-Kit kinases, which makes imatinib a powerful therapeutic for the treatment of chronic myelogenic leukemia (CML), hypereosinophile syndrome (HES)

and gastrointestinal stromal tumors (GIST), respectively (Capdeville, Buchdunger et al. 2002; Cools, Stover et al. 2003; Bikker, Brooijmans et al. 2009). The importance of high specificity is further illustrated by the second-generation drugs approved for the treatment of CML. Dasatinib (Sprycel, Bristol-Meyers Squibb) inhibits a broader spectrum of protein kinases than imatinib, including the Src-family tyrosine kinases (SFKs), and consequently dasatinib has more side effects and increased toxicity (Lombardo, Lee et al. 2004; Bikker, Brooijmans et al. 2009). Since the introduction of imatinib, another ten small-molecule kinase inhibitors have been approved for clinic use and 80 are in clinical trials (Bikker, Brooijmans et al. 2009; Zhang, Yang et al. 2009; Krishnamurty and Maly 2010). The application of these inhibitors ranges from the treatment of chronic myeloid leukemia and renal cell carcinoma to non-small-cell lung cancers (Bikker, Brooijmans et al. 2009; Zhang, Yang et al. 2009).

[00391] The catalytic domains of protein kinases can adopt multiple conformations as illustrated by the model tyrosine kinases Src and Abl. The active conformation of the Src and Abl kinase domains is characterized by two hallmarks: (i) a salt bridge between a glutamate in helix αC (Glu310, chicken c-Src numbering) and the catalytic lysine in the amino terminal N-lobe of the kinase (Lys295) and (ii) an aspartate side chain (Asp404) facing into the active site of the kinase where it coordinates Mg^{2+}/ATP during the catalytic cycle (Azam, Seeliger et al. 2008). Disruption of either structural hallmark of the active conformation renders the kinases inactive. The outward rotation of helix αC disrupts this first hallmark by breaking the Lys295-Glu310 salt bridge. This conformation was first observed in the structures of Src and Cdk2 and is referred to as the "Src/Cdk-like" or the "helix αC-out" inactive conformation. The second hallmark of the active conformation can be disrupted by a crankshaft-like 180° rotation of the Asp-Phe-Gly (DFG) motif at the beginning of the activation loop. This rotation forces Asp404 out of the active side and Phe405 into the active site, thereby preventing the coordination of Mg^{2+}/ATP for catalysis. The movement of Phe405 opens up the specificity pocket - a hydrophobic pocket with low sequence homology among kinases. This conformation is termed the "c-Kit/Abl-like" or the "DFG-Asp-out" inactive conformation as it was described first in the complex of c-Abl and c-Kit with imatinib (Schindler, Bornmann et al. 2000; Mol, Dougan et al. 2004; Levinson, Kuchment et al. 2006). While it is not clear whether all kinases can access these three distinct conformations, Src and Abl kinase (Sicheri, Moarefi et al. 1997; Xu, Harrison et al. 1997; Azam, Seeliger et al. 2008) have been observed in all three conformations indicating that these conformations are relevant for the function of these kinases.

[00392] ATP competitive kinase inhibitors can be classified into two groups: (i) type I inhibitors (such as PP1) that bind deeply into the binding pocket of adenine and mimic the hydrogen bonding pattern of the base (Zhang, Yang et al. 2009) and (ii) type II inhibitors such as imatinib, nilotinib and the DSA series of compounds (Seeliger, Ranjitkar et al. 2009) that bind to the specificity pocket of Src and Abl kinase that only opens upon rotation of the Asp-Phe-Gly motif.

[00393] Non-ATP competitive inhibitors have the potential for excellent specificity. Type III kinase inhibitors bind to allosteric sites, which exist in a number of kinases. The small-molecule inhibitors GNF-2 (targeting Abl kinase) and CI-1040 (targeting MEK kinases) have been shown to be potent and highly specific (Ohren, Chen et al. 2004; Adrian, Ding et al. 2006; Zhang, Yang et al. 2009). One of the main challenges in finding type III inhibitors is the identification of allosteric sites. Recently we used unusually long computer simulations that may help to predict such allosteric binding sites for Src kinase (Shan, Kim et al. 2011).

[00394] Finally, substrate-peptide-competitive inhibitors can potentially be highly specific because of the sequence variation in the substrate-peptide binding sites of protein kinases. For example, tyrphostins potently and specifically inhibit EGFR activity by targeting the substrate binding site (Gazit, Yaish et al. 1989; Levitzki 1999).

[00395] The identification of macrocyclic kinase inhibitors that bind to Src kinase and potently inhibit the enzyme activity from a library of macrocyclic compounds is described elsewhere herein. Interestingly, two of these macrocyclic compounds, cis-A11-B1-C5-D7 and trans-A10-B1-C5-D6 (referred to also herein as **MC1** and **MC4**, respectively) displayed a remarkable level of specificity, inhibiting Src kinase but not Abl kinase or the highly-related Src-family kinases Hck (68% identical to Src kinase domain in sequence), Blk, Frk, Fyn, and Lyn (see **Figure 14**).

[00396] Macrocycle derivatives of **MC1** and **MC4** with potencies as high as Kd = 4 nM were developed by systematic modulation of individual macrocycle building blocks. Some of the most potent of these macrocycle derivatives inhibit Src kinase activity in mammalian cells. The mechanism of inhibition of the macrocycle kinase inhibitors was investigated, and it was determined that the src kinase inhibiting macrocycles compete with both ATP and substrate peptide for binding to and inhibition of Src kinase domain.

[00397] Further, two amino acid substitutions between Src and Hck were identified that explain the specificity of the macrocycles described herein for Src over Hck. Finally, the ability of macrocycles to inhibit the Thr338Ile Src gatekeeper mutation was characterized,

and it was discovered that **MC4**-based but not **MC1**-based macrocycles are insensitive to this mutation and inhibit Thr338Ile Src kinase with similar potency as wild type Src kinase. Taken together, these findings establish that macrocycles can serve as potent Src kinase inhibitors that demonstrate uncommon kinase selectivity, insensitivity to common drug-resistant forms of tyrosine kinases, and src kinase inhibitory activity when administered to human cells.

Materials and Methods

[00398] *Macrocycle Synthesis:* Carboxamide-containing macrocycles were synthesized on multi-milligram scale using Fmoc solid-phase peptide synthesis as described in Kleiner et al.(Kleiner, Dumelin et al. 2010) Carboxylate-containing macrocycles were synthesized using standard macrocycle synthesis protocols substituting 2-chlorotrityl resin (EMD Biosciences) in place of Rink amide resin. Fluorescein-macrocycle conjugates were synthesized using standard macrocycle synthesis protocols substituting 1,6-diaminohexane trityl resin (EMD Biosciences) in place of Rink amide resin. After HPLC purification, the 6-aminohexane-conjugate was reacted with 5 equivalents of 5-carboxyfluorescein N-succinimidyl ester (Sigma-Aldrich) and 10 equivalents of DIPEA in DMF. The fluorescein-macrocycle was then purified by reverse-phase HPLC using a C18 stationary phase and eluting with a gradient of water/acetonitrile.

[00399] *Protein Purification:* Kinase domain constructs of human c-Abl (residues 229-512), chicken c-Src (residues 251-533 and residues 83-533), murine Lck (residues 227-509), and human Hck (residues 166-445) were expressed as previously described (Seeliger, Young et al. 2005; Seeliger, Nagar et al. 2007; the entire contents of each of which are incorporated by reference herein). Mutations were introduced into chicken c-Src (residues 251-533) (T338I, C277Q, L297M) by site-directed mutagenesis and verified by DNA sequencing.

[00400] *Kinase Activity Assays*: In vitro kinase inhibition assays were performed using either a continuous spectrophotometric assay or a FRET-based endpoint assay. For the continuous assay (Barker, Kassel et al. 1995), 100 μM of a Src optimal substrate peptide (AEEEIYGEFAKKK, SEQ ID NO: 16) or 200 μM of an Abl optimal substrate peptide (EAIYAAPFAKKK, SEQ ID NO: 17) (Songyang and Cantley 1995; Songyang, Carraway et al. 1995) were combined with 50 μM ATP, unless stated otherwise. Titrations of **MC1**, **MC1.2**, **MC4**, **MC4.1**, and **MC9** (ranging from 0 μM to 83.3 μM) were performed at 30°C as described before for imatinib (Seeliger, Nagar et al. 2007) to determine the concentration at which 50% of the initial kinase activity is inhibited (IC50).

[00401] For the FRET-based endpoint assay, kinase assays were performed using Invitrogen's Z'-LYTE kinase assay using the Tyr 02 substrate peptide (based upon the optimal Abl substrate peptide EAIYAAPF) according to the manufacturer's instructions. Assays were performed at near KM ATP concentrations for full-length c-Src and full-length c-Src T338I - 50 μM and 7 μM, respectively.

[00402] *KM Assays*: The Michaelis constant (KM) of the kinases for ATP, Src optimal peptide and Abl optimal peptide were determined for each of the kinases through use of the continuous spectrophotometric assay (Barker, Kassel et al. 1995). Concentrations of ATP ranged from 0 to 250 μM and ranged from 0 to 1.2 mM for Src optimal peptide and 0 to 2.4 mM for Abl optimal peptide. All experiments were performed in triplicate.

[00403] *Anisotropy Binding Assay*: The change in fluorescence anisotropy of fluorescein-labeled **MC1**, **MC4** and **MC9** at 518 nm upon excitation at 492 nm was monitored with a HORIBA Jobin Yvon FluoroMax-4 (Edison, NJ) spectrofluorimeter. Kinase was titrated to 0.5 μM of the fluorescently labeled macrocycle in 100 mM Tris pH 8.0, 10 mM MgCl2 at 25°C. After equilibration, the increase in the fluorescence anisotropy of the fluorescently labeled ligand was recorded and fitted against a quadratic binding equation in Kaleidagraph (Synergy Software, Reading PA) to yield the dissociation constant KD.

[00404] *Cell Culture Assays and Western Blots*: Cells were cultured in 48-well tissue culture plates in DMEM + 10% FBS until they reached confluence. After removing serum-containing medium, cells were washed with serum-free DMEM, and small molecule inhibitor in serum-free DMEM with 2% DMSO was added to each well and incubated overnight. After incubation with compound, cells were washed once with PBS and lysed in Cell Extraction Buffer (Invitrogen), a modified RIPA buffer, supplemented with PMSF and protease inhibitors according to the manufacturer's instruction. Cell lysates were quantified by BCA assay (Pierce Biotechnology) and an equivalent amount of lysate was loaded into each lane of a 4-12% polyacrylamide gel, and separated by SDS-PAGE. Separated proteins were transferred onto a PVDF membrane, and blocked for one hour with 5% BSA in TBST buffer. The membrane was then incubated overnight at 4 °C with 4G10 anti-phosphotyrosine antibody (Millipore) and a β-tubulin antibody (Sigma) as a loading control. Fluorescent anti-mouse and anti-rabbit secondary antibodies (LI-COR) were added prior to imaging the membrane.

Results and Discussion

[00405] *Development of Src-inhibiting macrocycles with improved potency*: Since ATP-competitive kinase inhibitors must compete with millimolar ATP concentrations in the cell, high in vitro potency, *e.g.*, an IC_{50} in the low nM range, is often required for a kinase inhibitor to demonstrate cellular efficacy at micromolar concentrations (Knight and Shokat 2005). It was therefore sought to improve the potency of the inhibitors identified herein, for example, of the pyrazine-containing **MC1** (MC stands for "macrocycle," sometimes also referred to as "macro" herein; **MC1** is also referred to as cis-A11-B1-C5-D7 herein) and *p*-nitrophenylalanine-containing **MC4** (also referred to as trans-A10-B1-C5-D6 herein) by the systematic replacement of macrocycle amino acids with modified building blocks. Figure 19, upper panel displays the structures of some exemplary macrocycles mentioned herein.

[00406] Derivatives of **MC4** were synthesized by Fmoc solid-phase peptide synthesis and assayed against full-length chicken c-Src using Invitrogen's Z'-LYTE kinase assay. Improvement of building blocks at the B and C positions were investigated first (**Figure 19**, middle panel). Holding the amino acids at the A and C positions constant, variants of **MC4** containing phenylalanine, cyclohexylalanine, pentafluorophenylalanine, and N-methyl-phenylalanine at the B position in place of furylalanine were synthesized and assayed. Substituting pentafluorophenylalanine and N-methylphenylalanine at this position abolished activity (IC_{50} > 10 μM), and cyclohexylalanine resulted in a ~10-fold decrease in potency. The phenylalanine derivative **MC4.1**, also referred to herein as trans-A10-Phe-C5-D6, wherein Phe is phenylalanine, in contrast, resulted in a 3-fold increase in Src inhibition potency to IC_{50} = 80 nM.

[00407] With this improved macrocycle in hand, building blocks at the C position were varied next. Holding the A and B positions constant as *p*-nitrophenylalanine and phenylalanine, respectively, phenylalanine, diphenylalanine, 1-naphthylalanine, and cyclohexylalanine were substituted at the C position in place of cyclopropylalanine (**Figure 19**, middle panel). Of these derivatives, phenylalanine and naphthylalanine decreased Src inhibition potency by ~ 3-5-fold, and diphenylalanine decreased inhibition potency by ~16-fold. The cyclohexylalanine derivative, **MC4.2**, also referred to herein as trans-A10-Phe-Cha-D6, wherein Cha refers to cyclohexylalanine, as illustrated in **Figure 19**, middle panel, on the right, however, exhibited an inhibition potency that was improved by >10-fold, with Src IC_{50} = 6 nM. These results collectively establish that modest modifications in the size and shape of the macrocycle side chains can result in significant gains in potency. Changes to the peptide backbone, such as N-methylation, however, did not appear to be tolerated, as

substitution of N-methyl amino acids at some positions resulted in a decrease in inhibition potency. Notably, none of the amino acids that increased potency of Src inhibition were present at the appropriate position in the DNA-templated macrocycle library from which the initial macrocyclic Src inhibitors were discovered, consistent with the quality of the structure-activity relationships resulting from the in vitro selection for Src binding .(Tse, Snyder et al. 2008)

[00408] Next, more subtly altered building blocks were installed into the partially optimized macrocycle structures. The importance of *p*-nitrophenylalanine at the A position of **MC4.2** was probed by replacing the nitro group at the para position with a methyl, chloro, bromo, trifluoromethyl, cyano, carbamoyl, or tert-butyl substituent (**Figure 19**, middle panel). Remarkably, all but the electronically similar p-cyanophenylalanine exhibited substantial reductions in potency (see Table in **Figure 19**, middle panel).

[00409] A similar series of subtle changes was introduced to the optimized B position building block, phenylalanine, of **MC4.2** by replacing it with tyrosine, *p*-fluorophenylalanine or *p*-methylphenylalanine. Introduction of a methyl or hydroxyl group onto the phenyl ring resulted in a substantial decreases in Src inhibition. In contrast, the *p*-fluorophenylalanine derivative, also referred to herin as **MC4.3**, or trans-A10-[4-F-Phe]-Cha-D6, wherein 4-F-Phe is p-fluorophenylalanine, a very conservative change, retained all of the parent compound activity.

[00410] Surprisingly, the analogous building block substitutions in the pyrazine-containing macrocycle structure **MC1**, changing furylalanine to phenylalanine (**MC1.1**) and cyclopropylalanine to cyclohexylalanine, resulted in a macrocycle, **MC1.2** (cis-A11-Phe-Cha-D7), with >150-fold greater potency against Src kinase than the parent compound (**Figure 19**, lower panel). The potency of **MC1.2** could not accurately be measured since its IC_{50} of ≤ 4 nM was lower than the enzyme concentration required by our in vitro kinase assay. These findings support a similar mode of binding for the B and C building blocks in **MC1** and **MC4** derivatives.

[00411] *Exceptionally specific inhibitors of Src kinase with an ATP and substrate peptide competitive mode of inhibition.* The exceptional specificity with which some of the macrocycles described herein inhibited Src kinase activity without inhibiting the activity of the closely related Src family kinase Hck or the activity of human c-Abl kinase, as described in Kleiner, Dumelin et al. 2010, is intriguing. To understand the origin of this unusual specificity, the activity and specificity of the following compounds was characterized: **MC1**,

MC4 and **MC9** (**MC9** is also referred to herein as cis-All-B8-C10-D7, see, *e.g.*, **Figure 5**) as well as three second-generation compounds with greatly improved potency - **MC1.2**, **MC4.1** and **MC4.3**. **MC1**, **MC1.2** and **MC9** share a diaminobutyric acid scaffold, cis-olefin stereochemistry, and a pyrazine group in position A, whereas **MC4**, **MC4.1**, and **MC4.3** contain an ornithine scaffold and nitrophenylalanine in position A.

[00412] The original characterization of kinase inhibition was performed with a commercial kinase assay that relies on a fluorescently labeled peptide kinase substrate (Z-LYTE, Invitrogen). For further characterization, a continuous spectrophotometric kinase activity assay was employed that is easily adapted to different substrate peptides as well as a wider range of peptide and ATP concentrations. Using this assay, we found that the original compounds **MC1**, **MC4** and **MC9** inhibited 50% of Src kinase domain activity (IC_{50}) at 16 μM, 11 μM and 69 μM respectively (**Figure 20A**). The IC_{50} values of these compounds for Hck, Lck and Abl kinase were over 100 μM and could not be determined accurately. Consistent with their improved potency in the Z'-LYTE assay, the second generation compounds **MC1.2**, **MC4.1**, and **MC4.3** exhibited more than 200-fold higher potency than the first generation compounds. **MC1.2** and **MC4.2** inhibited Src kinase domain with IC_{50}-values of 0.07 μM and 0.6 μM, respectively (**Figure 20B**). This overall increase in potency resulted in micromolar potency of **MC1.2** and **MC4.1** against Hck (IC_{50} of 0.36 μM and 0.87 μM, respectively) and Lck (IC_{50} of 4 μM and 5.2 μM, respectively).

[00413] While these results are in qualitative agreement with the Z'-LYTE assays (Kleiner, Dumelin et al. 2010), the absolute IC_{50} values observed in the spectrophotometric assay are approximately 10-fold higher. To address this discrepancy, the two major differences between the fluorescent peptide-based Z'-LYTE assay and the continuous spectrophotometric assay employed here were investigated: the kinase construct used (full-length kinase in the Z-LYTE assay vs. kinase domain in the current study) and the peptide identity and concentration (5 μM fluorescent Abl-optimal substrate peptide in the previous study vs. 100 μM Src-optimal substrate peptide in the current study). Surprisingly, it was discovered that the macrocycle compounds inhibited longer Src kinase constructs including the regulatory SH3 and SH2 domains (chicken c-Src 83-533) approximately 10-fold more potently than isolated kinase domains (chicken c-Src 248-533)(**Figure 20B**). The IC_{50} values determined for **MC1.2** and **MC4.1** were close to the concentration of the kinase used in the assay and therefore these values only represent an upper estimate of the real potency of these compounds.

[00414] The effect of substrate peptide on inhibitory potency of the macrocycle

compounds was investigated next. An Src optimal substrate peptide (AEEEIYGEFAKKK, SEQ ID NO: 16 (Songyang and Cantley 1995; Songyang, Carraway et al. 1995)) was employed in the assays at 100 μM concentration (**Figure 20C**). Upon increasing the substrate peptide concentration 3-fold to 300 μM a 50% increase in **IC50** was measured for **MC1** and **MC1.2**, indicating that the macrocycle compounds could be substrate peptide competitive. For comparison, when the ATP concentration was raised 50-fold, for all macrocycles a 2-fold to 5-fold increase in IC_{50} was measured consistent with the ATP competitive behavior of the compounds **(Figure 20C)**.

[00415] The IC_{50} values of macrocycles MC1, MC1.2,MC44, and MC4.1 for Src, Hck, Lck, and Abl kinases in response to a 3-fold increase in Src optimal substrate, and a 50-fold increase in ATP were investigated next:

Macrocycle 1			
	5 μM ATP 100 μM Src optimal peptide	**5 μM ATP 300 μM Src optimal peptide**	**250 μM ATP 300 μM Src optimal peptide**
Src	16 μM	26 μM	89 μM
Hck	> 100 μM	> 100 μM	> 100 μM
Lck	> 100 μM	> 100 μM	> 100 μM
Abl	> 100 μM	> 100 μM	> 100 μM
Macrocycle 1.2			
	5 μM ATP 100 μM Src optimal peptide	**5 μM ATP 300 μM Src optimal peptide**	**250 μM ATP 300 μM Src optimal peptide**
Src	0.07 μM*	0.12 μM	0.14 μM
Hck	0.36 μM*	0.46 μM	1.0 μM
Lck	0.87 μM*	1.9 μM	2.4 μM
Abl	> 100 μM	> 100 μM	> 100 μM
Macrocycle 4			
	5 μM ATP 100 μM Src optimal peptide	**5 μM ATP 300 μM Src optimal peptide**	**250 μM ATP 300 μM Src optimal peptide**
Src	11 μM	11 μM	21 μM
Hck	> 100 μM	> 100 μM	> 100 μM
Lck	> 100 μM	> 100 μM	> 100 μM
Abl	> 100 μM	> 100 μM	> 100 μM
Macrocycle 4.1			
	5 μM ATP 100 μM Src optimal peptide	**5 μM ATP 300 μM Src optimal peptide**	**250 μM ATP 300 μM Src optimal peptide**
Src	0.6 μM	0.8 μM	1.8 μM
Hck	4.6 μM	6.3 μM	11 μM
Lck	5.2 μM	5.5 μM	10 μM
Abl	> 100 μM	> 100 μM	> 100 μM

TABLE 11: IC_{50} values of Macrocycles 1, 1.2, 4, and 4.1 for Src, Hck, Lck, and Abl kinases in response to a 3-fold increase in Src optimal substrate, and a 50-fold increase in ATP. *values are an upper estimate of the IC^{50} because it is 2-fold less than the concentration of kinase used.

[00416] To clarify the inhibitory mechanism of the compounds further, the binding affinity of fluorescently labeled **MC1**, **MC4**, and **MC9** to Src kinase domain was determined in a fluorescence anisotropy assay (**Figure 21**). An exemplary structure of a fluorescently labeled macrocycle is provided in **Figure 21A**. It was found that both MC1 and MC4

inhibition potency decreased with increased ATP concentrations (**Figure 21A**). It was further observed that Src kinase domain binds **MC1**, **MC4** and **MC9** with a dissociation constant (KD) of 2.1 μM, 0.3 μM and 3.6 μM, respectively (**Figure 21B**). Binding affinity of the compounds decreased in the presence of 250 μM of the non-hydrolyzable ATP analog AMP-PNP to 3.3μM, 1.7μM and 12.6μM consistent with ATP competitive inhibitor characteristics (**Figure 21B**). Consistent with the decrease in IC_{50} observed in the kinase assay, the presence of 300 μM Src optimal substrate peptide decreased the apparent affinities of the fluorescent-macrocycle conjugates up to 10-fold (**Figure 21B**, lowest column in each group). From these results, it was concluded that the macrocycle compounds are both ATP-competitive and substrate peptide-competitive kinase inhibitors.

[00417] *Macrocycles are active against the Src gatekeeper mutation Thr338Ile.*

[00418] The emergence of resistance of an aberrant kinase to a therapeutic kinase inhibitor after therapy has commenced is a common problem in kinase inhibition therapy. For example ~30% of all CML patients undergoing imatinib treatment develop resistance mutations in the kinase domain of the BCR-Abl fusion protein. Some mutated kinases that are resistant to inhibition by imatinib, can be inhibited with certain second generation kinase inhibitors such as nilotinib or dasatinib. However, some of the most common mutations conferring resistance against kinase inhibition, so-called gatekeeper residue mutations, e.g., Thr315Ile (human Abl1a numbering), Thr338Ile in chicken c-Src, or Thr341Ile, cannot be overcome with currently available kinase inhibitor therapeutics. The gatekeeper residue in Src kinase regulates access to a hydrophobic binding pocket adjacent to the ATP binding pocket. This particular residue is of great interest because it is mutated to a threonine residue in almost all isoforms of viral Src, because it renders the mutated Src resistant to most ATP-competitive inhibitors (including GLEEVEC™, IRESSA™, and TARCEVA™), and because the corresponding mutation in Abl kinase (Thr315Ile, human Abl 1a numbering) is a common basis for IMATINIB™ resistance among CML patients. Currently, no small-molecule kinase inhibitor is available in the clinic to treat patients with this particular resistance mutation.

[00419] It was observed that that the gatekeeper mutation had a stronger impact on the potency of macrocycles based on the diaminobutyric acid scaffold, e.g., compounds MC1 and MC1.2, which were at least 100-fold less potent against the gatekeeper mutant than against Src wild-type (Figure 22, left panel, showing data for MC1). In contrast, MC9 inhibited Src Thr338Ile containing SH3 and SH2 domains with high potency (IC50 1.0 μM), which was only about 3-fold lower than the inhibition potency of MC9 against the corresponding Src

wild-type construct (IC50 0.39 μM) (**Figure 22**, right panel). Similarly, MC4 and its derivatives, e.g., MC4.2 and MC4.3 retained their inhibition potency against the Src Thr338Ile gatekeeper mutation.

[00420] *Src Kinase Inhibition in Cell Culture.* Having obtained macrocycle Src kinase inhibitors with nanomolar in vitro potency, the potency for inhibition of Src kinase activity in living cells was assessed. Since intracellular Src kinase signaling is complex and tightly regulated, a cell culture line transfected with a constitutively active form of murine c-Src was chosen. 3T3 cells (src -/-) stably transfected with c-Src Y529F were compared to 3T3 cells (src -/-) transfected with empty vector. Anti-phosphotryosine Western blotting with the 4G10 antibody (Millipore) indicated a marked difference between global phosphotyrosine levels in the c-Src Y529F transfected cells compared to those transfected with empty vector; in fact, almost all detectable intracellular phosphotyrosine in the Src-transfected cell line was a result of c-Src Y529F and downstream signaling (**Figure 23**, upper panel, compare lanes 2 and 3, transfected and trated with DMSO, against lanes 8 and 9, transfected with empty vector). In addition, treatment of the c-Src Y529F expressing cells with 10 μM PP2 (Hanke, Gardner et al. 1996) a known Src- family kinase inhibitor, prior to Western blotting resulted in reduction of global phosphotyrosine to levels comparable with those observed in the cells transfected with empty vector (**Figure 23**, compare lanes 6 and 7, treated with PP2, with lanes 8 and 9). These results implied that depletion of global tyrosine phosphorylation in the cells transfected with c-Src Y529F was indicative of specific inhibition of Src kinase activity in this cell line.

[00421] The effect of the most potent macrocycles, **MC1.2**, **MC4.2**, and **MC4.3** were assayed in the c-Src Y529F-transfected cells. Briefly, cells were seeded in a 48-well plate and grown to confluence in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Individual wells were then treated with 10 or 100 μM concentration of macrocycle in serum-free DMEM overnight. After removing the small molecule and washing with phosphate-buffered saline, cells were lysed in radioimmunoprecipitation assay (RIPA) buffer, and global phosphotyrosine levels were quantified by Western blot. After overnight treatment with the *p*-nitrophenylalanine-containing **MC4.2** global phosphotyrosine levels showed a modest reduction, with an EC_{50} of >100 μM (**Figure 23**, upper panel, lanes 4 and 5). Surprisingly, treating cells with **MC4.3** (**Figure 23**, lower panel), which differs in structure from **MC4.2** by a single fluorine atom (boxed in the figure), resulted in higher levels of tyrosine phosphorylation inhibition at comparable small molecule concentrations. The pyrazine-containing **MC1.2** did not reduce

global tyrosine phosphorylation at 100 μM concentration, possibly because of low membrane permeability.

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OTHER EMBODIMENTS

[00422] All publications, patents and sequence database entries mentioned herein, including those items listed below, are hereby incorporated by reference in their entirety for disclosure of the teachings relevant to this invention, as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of the present specification and a document incorporated by reference including conflicting disclosure, the present specification shall control.

[00423] While several embodiments of the present invention have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other methods and/or structures for performing the functions and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the present invention. More generally, those skilled in the art will readily appreciate that all structures, reagents, methods, features, parameters, materials, and configurations described herein are meant to be exemplary and that the actual structures, reagents, methods, features, parameters, materials, and configurations will depend upon the specific application or applications for which the teachings of the present invention is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, the invention may be practiced otherwise than as specifically described and claimed. The present invention is directed to each individual structure, reagent, method, feature, parameter, material, and configuration described herein. In addition, any combination of two or more such structures, reagents, methods, features, parameters, materials, and configurations, if such are not mutually inconsistent, is included within the scope of the present invention.

[00424] All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

[00425] The indefinite articles “a” and “an”, as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

[00426] The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “ either or both” of the elements so conjoined, i.e., elements

that are conjunctively present in some cases and disjunctively present in other cases. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified unless clearly indicated to the contrary. Thus, as a non-limiting example, a reference to "A and/or B", when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A without B (optionally including elements other than B); in another embodiment, to B without A (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

[00427] As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as " only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (i.e. "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of." "Consisting essentially of", when used in the claims, shall have its ordinary meaning as used in the field of patent law.

[00428] As used herein in the specification and in the claims, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently, "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

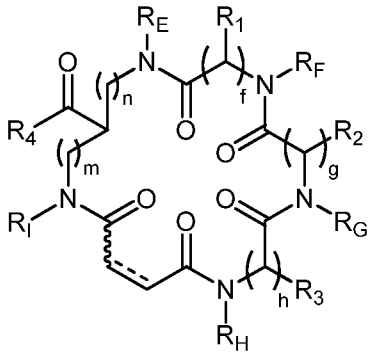
[00429] It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one act, the order of the acts of the method is not necessarily limited to the order in which the acts of the method are recited.

[00430] The foregoing has been a description of certain non–limiting preferred embodiments of the invention. Those of ordinary skill in the art will appreciate that various changes and modifications to this description may be made without departing from the spirit or scope of the present invention, as defined in the following claims.

CLAIMS

What is claimed is:

1. A compound of Formula (I):



(I)

or a pharmaceutically acceptable salt thereof;

wherein:

n is 0 or an integer between 1-4, inclusive;

m is 0 an integer between 1-4, inclusive;

f is an integer between 1-3, inclusive;

g is an integer between 1-3, inclusive;

h is an integer between 1-3, inclusive;

------ is a single or double C-C bond, wherein when ------ is a double C-C bond, then ∿∿∿ indicates that the adjacent C-C double bond is in a *cis* or *trans* configuration;

each instance of R_1 is independently hydrogen; halogen; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; acyl; $-OR_A$; $-N(R_A)_2$; $-SR_A$; =O; -CN; $-NO_2$; -SCN; $-SOR_A$; or $-SO_2R_A$; wherein each occurrence of R_A is independently hydrogen; a protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; acyl; substituted or unsubstituted aryl; or substituted or unsubstituted heteroaryl;

each instance of R_2 is independently hydrogen; halogen; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; acyl; $-OR_B$; $-N(R_B)_2$; $-SR_B$; =O; -CN; $-NO_2$; -SCN; $-SOR_B$; or $-SO_2R_B$; wherein each occurrence of R_B independently hydrogen; a protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; acyl; substituted or unsubstituted aryl; or substituted or unsubstituted heteroaryl;

each instance of R_3 is independently hydrogen; halogen; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; acyl; $-OR_C$; $-N(R_C)_2$; $-SR_C$; =O; -CN; $-NO_2$; -SCN; $-SOR_C$; or $-SO_2R_C$; wherein each occurrence of R_C is independently hydrogen; a protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; acyl; substituted or unsubstituted aryl; or substituted or unsubstituted heteroaryl;

R_4 is substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; $-N(R_D)_2$; $-OR_D$; or $-SR_D$; wherein each occurrence of R_D is independently independently hydrogen; a protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; acyl; substituted or unsubstituted aryl; or substituted or unsubstituted heteroaryl; or two R_D groups are joined to form a substituted or unsubstituted heterocyclic group; optionally wherein R^4 further comprises a label, resin, or therapeutic agent attached thereto; and

each instance of R_E, R_F, R_G, R_H, and R_I is independently hydrogen; acyl; a nitrogen protecting group; substituted or unsubstituted aliphatic; substituted or unsubstituted heteroaliphatic; substituted or unsubstituted aryl; substituted or unsubstituted heteroaryl; substitute or unsubstituted hydroxyl; substituted or unsubstituted thiol; substituted or unsubstituted amino; or halogen; optionally wherein an R_1 group and R^F are joined to form a substituted or unsubstituted heterocyclic ring; an R_2 group and R^G are joined to form a substituted or unsubstituted heterocyclic ring; and/or an R_3 group and R^H are joined to form a substituted or unsubstituted heterocyclic ring.

2. The compound of claim 1, wherein R_4 is $-N(R_D)_2$, and wherein each instance of R^D is independently selected from the group consisting of hydrogen; substituted aliphatic, substituted heteroaliphatic, substituted aryl, or substituted heteroaryl, wherein the substituent covalently or non-covalently attached thereto is a resin, a label, or therapeutic agent; a label; a resin; and a therapeutic agent.

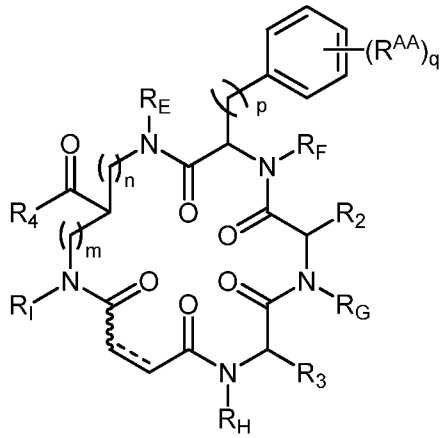
3. The compound of claim 1, wherein each occurrence of R_E, R_F, R_G, R_H, and R_I is independently hydrogen, a nitrogen protecting group, or substituted or unsubstituted alkyl.

4. The compound of claim 1, wherein n is an integer between 2-4, inclusive.

5. The compound of claim 1, wherein m is 0.

6. The compound of claim 1, wherein ====== represents a double bond in the *cis* or *trans* configuration.

7. The compound of claim 1, wherein the compound is of Formula (III):



(III)

or a pharmaceutically acceptable salt thereof,

wherein:

p is an integer between 1 and 6, inclusive;

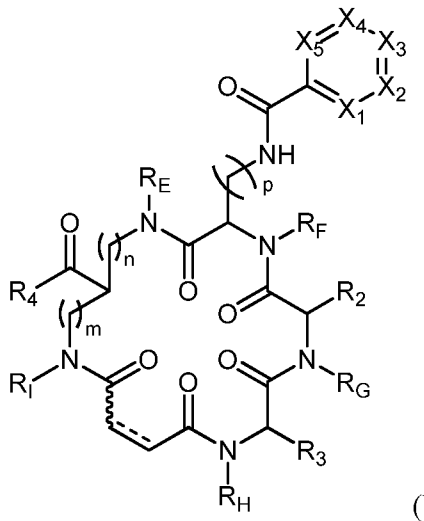
q is 0 or an integer between 1 and 5, inclusive; and

each instance of R^{AA} is independently halogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-OR^{A3}$, $-N(R^{A4})_2$, $-SR^{A3}$, $-C(=O)R^{A3}$, $-C(=O)OR^{A3}$, $-C(=O)SR^{A3}$, $-C(=O)N(R^{A4})_2$, $-OC(=O)R^{A3}$, $-OC(=O)OR^{A3}$, $-OC(=O)SR^{A3}$, $-OC(=O)N(R^{A4})_2$, $-NR^{A4}C(=O)R^{A4}$, $-NR^{A4}C(=O)OR^{A3}$, $-NR^{A4}C(=O)SR^{A3}$, $-NR^{A4}C(=O)N(R^{A4})_2$, $-SC(=O)R^{A3}$, $-SC(=O)OR^{A3}$, $-SC(=O)SR^{A3}$, $-SC(=O)N(R^{A4})_2$, $-C(=NR^{A4})R^{A3}$, $-C(=NR^{A4})OR^{A3}$, $-C(=NR^{A4})SR^{A3}$, $-C(=NR^{A4})N(R^{A4})_2$, $-OC(=NR^{A4})R^{A3}$, $-OC(=NR^{A4})OR^{A3}$, $-OC(=NR^{A4})SR^{A3}$, $-OC(=NR^{A4})N(R^{A4})_2$, $-NR^{A4}C(=NR^{A4})R^{A2}$, $-NR^{A4}C(=NR^{A4})OR^{A3}$, $-NR^{A4}C(=NR^{A4})SR^{A3}$, $-NR^{A4}C(=NR^{A4})N(R^{A4})_2$, $-SC(=NR^{A4})R^{A3}$, $-SC(=NR^{A4})OR^{A3}$, $-SC(=NR^{A4})SR^{A3}$, $-SC(=NR^{A4})N(R^{A4})_2$, $-C(=S)R^{A3}$, $-C(=S)OR^{A3}$, $-C(=S)SR^{A3}$, $-C(=S)N(R^{A4})_2$, $-OC(=S)R^{A3}$, $-OC(=S)OR^{A3}$, $-OC(=S)SR^{A3}$, $-OC(=S)N(R^{A4})_2$, $-NR^{A4}C(=S)R^{A4}$, $-NR^{A4}C(=S)OR^{A3}$, $-NR^{A4}C(=S)SR^{A3}$, $-NR^{A4}C(=S)N(R^{A4})_2$, $-SC(=S)R^{A3}$, $-SC(=S)OR^{A3}$, $-SC(=S)SR^{A3}$, -

$SC(=S)N(R^{A4})_2$, $-S(=O)R^{A3}$, $-SO_2R^{A3}$, $-NR^{A4}SO_2R^{A3}$, $-SO_2N(R^{A4})_2$, $-N_3$, -CN, -SCN, and $-NO_2$,

wherein each occurrence of R^{A3} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and each occurrence of R^{A4} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or a nitrogen protecting group, or two R^{A4} groups are joined to form an substituted or unsubstituted heterocyclic ring.

8. The compound of claim 1, wherein the compound is of Formula (VI):



(VI)

or a pharmaceutically acceptable salt thereof,

wherein:

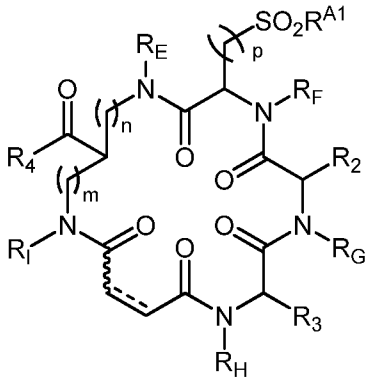
p is an integer between 1 and 6, inclusive;

each instance of X^1, X^2, X^3, X^4, and X^5 is independently selected from N, CH, or CR^{AA}; and each instance of R^{AA} is independently halogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-OR^{A3}$, $-N(R^{A4})_2$, $-SR^{A3}$, $-C(=O)R^{A3}$, $-C(=O)OR^{A3}$, $-C(=O)SR^{A3}$, $-C(=O)N(R^{A4})_2$, $-OC(=O)R^{A3}$, $-OC(=O)OR^{A3}$, $-OC(=O)SR^{A3}$, $-OC(=O)N(R^{A4})_2$, $-NR^{A4}C(=O)R^{A4}$, $-NR^{A4}C(=O)OR^{A3}$, $-NR^{A4}C(=O)SR^{A3}$, $-NR^{A4}C(=O)N(R^{A4})_2$, $-SC(=O)R^{A3}$, $-SC(=O)OR^{A3}$, $-SC(=O)SR^{A3}$, $-SC(=O)N(R^{A4})_2$, $-C(=NR^{A4})R^{A3}$, $-C(=NR^{A4})OR^{A3}$, -

$C(=NR^{A4})SR^{A3}$, $-C(=NR^{A4})N(R^{A4})_2$, $-OC(=NR^{A4})R^{A3}$, $-OC(=NR^{A4})OR^{A3}$, $-OC(=NR^{A4})SR^{A3}$, $-OC(=NR^{A4})N(R^{A4})_2$, $-NR^{A4}C(=NR^{A4})R^{A2}$, $-NR^{A4}C(=NR^{A4})OR^{A3}$, $-NR^{A4}C(=NR^{A4})SR^{A3}$, $-NR^{A4}C(=NR^{A4})N(R^{A4})_2$, $-SC(=NR^{A4})R^{A3}$, $-SC(=NR^{A4})OR^{A3}$, $-SC(=NR^{A4})SR^{A3}$, $-SC(=NR^{A4})N(R^{A4})_2$, $-C(=S)R^{A3}$, $-C(=S)OR^{A3}$, $-C(=S)SR^{A3}$, $-C(=S)N(R^{A4})_2$, $-OC(=S)R^{A3}$, $-OC(=S)OR^{A3}$, $-OC(=S)SR^{A3}$, $-OC(=S)N(R^{A4})_2$, $-NR^{A4}C(=S)R^{A4}$, $-NR^{A4}C(=S)OR^{A3}$, $-NR^{A4}C(=S)SR^{A3}$, $-NR^{A4}C(=S)N(R^{A4})_2$, $-SC(=S)R^{A3}$, $-SC(=S)OR^{A3}$, $-SC(=S)SR^{A3}$, $-SC(=S)N(R^{A4})_2$, $-S(=O)R^{A3}$, $-SO_2R^{A3}$, $-NR^{A4}SO_2R^{A3}$, $-SO_2N(R^{A4})_2$, $-N_3$, -CN, -SCN, and $-NO_2$,

wherein each occurrence of R^{A3} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and each occurrence of R^{A4} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or a nitrogen protecting group, or two R^{A4} groups are joined to form an substituted or unsubstituted heterocyclic ring.

9. The compound of claim 1, wherein the compound is of Formula (IX):



(IX)

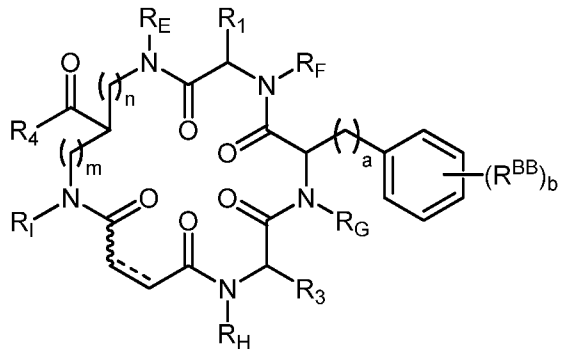
or a pharmaceutically acceptable salt thereof,

wherein:

p is an integer between 1 and 6, inclusive; and

R^{A1} is substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

10. The compound of claim 1, wherein the compound is of Formula (XII):



(XII)

or a pharmaceutically acceptable salt thereof,

wherein:

a is an integer between 1 and 6, inclusive;

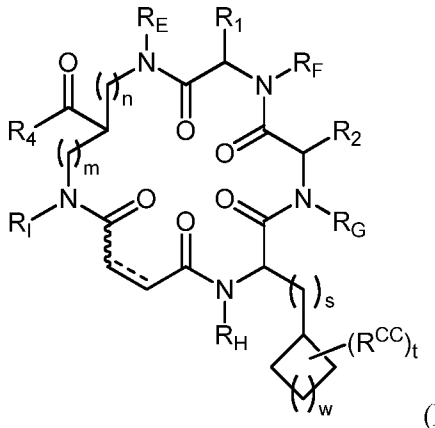
b is 0 or an integer between 1 and 5, inclusive; and

each instance of R^{BB} is independently selected from the group consisting of halogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-OR^{B3}$, $-N(R^{B4})_2$, $-SR^{B3}$, $-C(=O)R^{B3}$, $-C(=O)OR^{B3}$, $-C(=O)SR^{B3}$, $-C(=O)N(R^{B4})_2$, $-OC(=O)R^{B3}$, $-OC(=O)OR^{B3}$, $-OC(=O)SR^{B3}$, $-OC(=O)N(R^{B4})_2$, $-NR^{B4}C(=O)R^{B4}$, $-NR^{B4}C(=O)OR^{B3}$, $-NR^{B4}C(=O)SR^{B3}$, $-NR^{B4}C(=O)N(R^{B4})_2$, $-SC(=O)R^{B3}$, $-SC(=O)OR^{B3}$, $-SC(=O)SR^{B3}$, $-SC(=O)N(R^{B4})_2$, $-C(=NR^{B4})R^{B3}$, $-C(=NR^{B4})OR^{B3}$, $-C(=NR^{B4})SR^{B3}$, $-C(=NR^{B4})N(R^{B4})_2$, $-OC(=NR^{B4})R^{B3}$, $-OC(=NR^{B4})OR^{B3}$, $-OC(=NR^{B4})SR^{B3}$, $-OC(=NR^{B4})N(R^{B4})_2$, $-NR^{B4}C(=NR^{B4})R^{B2}$, $-NR^{B4}C(=NR^{B4})OR^{B3}$, $-NR^{B4}C(=NR^{B4})SR^{B3}$, $-NR^{B4}C(=NR^{B4})N(R^{B4})_2$, $-SC(=NR^{B4})R^{B3}$, $-SC(=NR^{B4})OR^{B3}$, $-SC(=NR^{B4})SR^{B3}$, $-SC(=NR^{B4})N(R^{B4})_2$, $-C(=S)R^{B3}$, $-C(=S)OR^{B3}$, $-C(=S)SR^{B3}$, $-C(=S)N(R^{B4})_2$, $-OC(=S)R^{B3}$, $-OC(=S)OR^{B3}$, $-OC(=S)SR^{B3}$, $-OC(=S)N(R^{B4})_2$, $-NR^{B4}C(=S)R^{B4}$, $-NR^{B4}C(=S)OR^{B3}$, $-NR^{B4}C(=S)SR^{B3}$, $-NR^{B4}C(=S)N(R^{B4})_2$, $-SC(=S)R^{B3}$, $-SC(=S)OR^{B3}$, $-SC(=S)SR^{B3}$, $-SC(=S)N(R^{B4})_2$, $-S(=O)R^{B3}$, $-SO_2R^{B3}$, $-NR^{B4}SO_2R^{B3}$, $-SO_2N(R^{B4})_2$, $-N_3$, -CN, -SCN, and $-NO_2$,

wherein each occurrence of R^{B3} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and each occurrence of R^{B4} is

independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or a nitrogen protecting group, or two R^{B4} groups are joined to form an substituted or unsubstituted heterocyclic ring.

11. The compound of claim 1, wherein the compound is of Formula (XIV):



(XIV)

or a pharmaceutically acceptable salt thereof,

wherein:

s is an integer between 1 and 6, inclusive;

t is 0, 1, or 2;

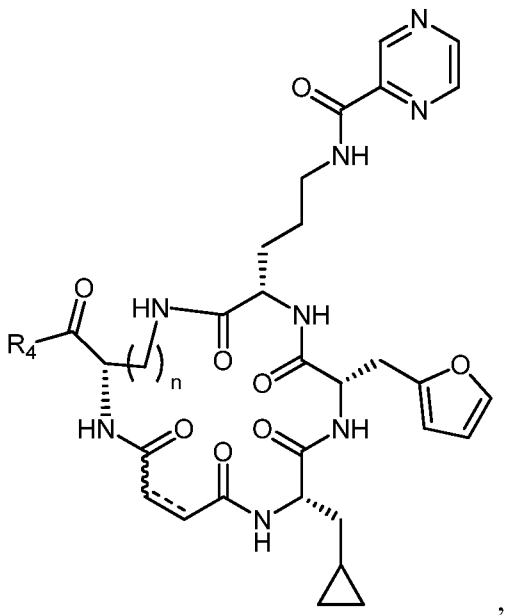
w is 0 or an integer between 1 and 4, inclusive; and

each instance of R^{CC} is independently halogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-OR^{C3}$, $-N(R^{C4})_2$, $-SR^{C3}$, $-C(=O)R^{C3}$, $-C(=O)OR^{C3}$, $-C(=O)SR^{C3}$, $-C(=O)N(R^{C4})_2$, $-OC(=O)R^{C3}$, $-OC(=O)OR^{C3}$, $-OC(=O)SR^{C3}$, $-OC(=O)N(R^{C4})_2$, $-NR^{C4}C(=O)R^{C4}$, $-NR^{C4}C(=O)OR^{C3}$, $-NR^{C4}C(=O)SR^{C3}$, $-NR^{C4}C(=O)N(R^{C4})_2$, $-SC(=O)R^{C3}$, $-SC(=O)OR^{C3}$, $-SC(=O)SR^{C3}$, $-SC(=O)N(R^{C4})_2$, $-C(=NR^{C4})R^{C3}$, $-C(=NR^{C4})OR^{C3}$, $-C(=NR^{C4})SR^{C3}$, $-C(=NR^{C4})N(R^{C4})_2$, $-OC(=NR^{C4})R^{C3}$, $-OC(=NR^{C4})OR^{C3}$, $-OC(=NR^{C4})SR^{C3}$, $-OC(=NR^{C4})N(R^{C4})_2$, $-NR^{C4}C(=NR^{C4})R^{C2}$, $-NR^{C4}C(=NR^{C4})OR^{C3}$, $-NR^{C4}C(=NR^{C4})SR^{C3}$, $-NR^{C4}C(=NR^{C4})N(R^{C4})_2$, $-SC(=NR^{C4})R^{C3}$, $-SC(=NR^{C4})OR^{C3}$, $-SC(=NR^{C4})SR^{C3}$, $-SC(=NR^{C4})N(R^{C4})_2$, $-C(=S)R^{C3}$, $-C(=S)OR^{C3}$, $-C(=S)SR^{C3}$, $-C(=S)N(R^{C4})_2$, $-OC(=S)R^{C3}$, $-OC(=S)OR^{C3}$, $-OC(=S)SR^{C3}$, $-OC(=S)N(R^{C4})_2$, $-NR^{C4}C(=S)R^{C4}$, $-NR^{C4}C(=S)OR^{C3}$, $-NR^{C4}C(=S)SR^{C3}$, $-NR^{C4}C(=S)N(R^{C4})_2$, $-SC(=S)R^{C3}$, $-SC(=S)OR^{C3}$, $-SC(=S)SR^{C3}$, -

$SC(=S)N(R^{C4})_2$, $-S(=O)R^{C3}$, $-SO_2R^{C3}$, $-NR^{C4}SO_2R^{C3}$, $-SO_2N(R^{C4})_2$, $-N_3$, -CN, -SCN, and $-NO_2$,

wherein each occurrence of R^{C3} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and each occurrence of R^{C4} is independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or a nitrogen protecting group, or two R^{C4} groups are joined to form an substituted or unsubstituted heterocyclic ring.

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



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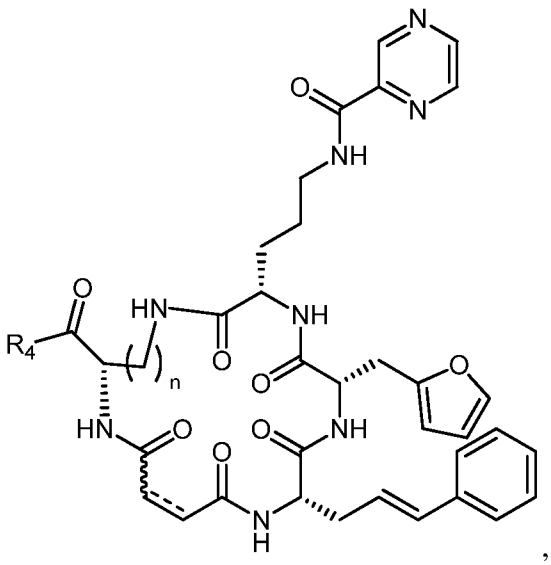

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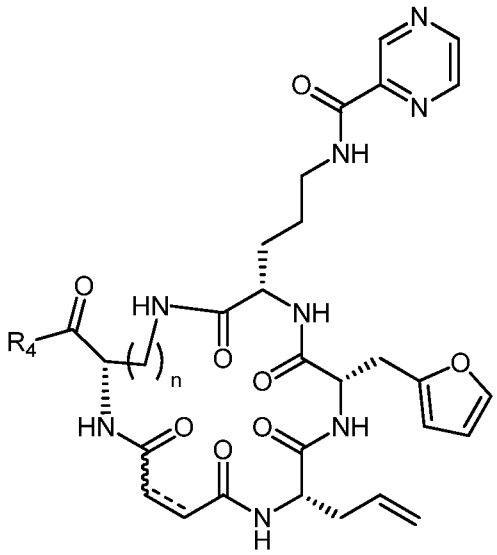

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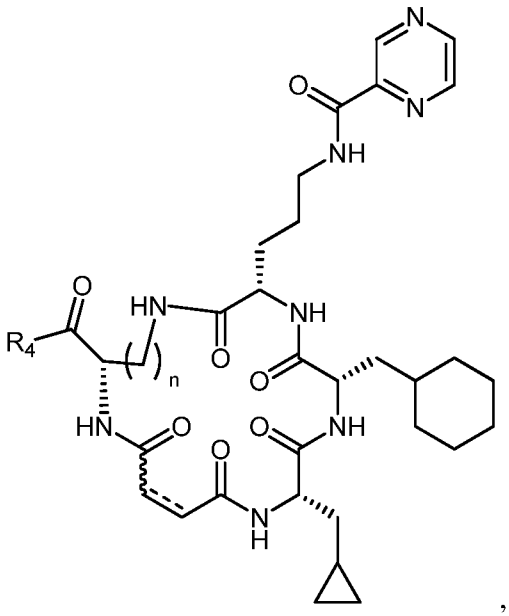

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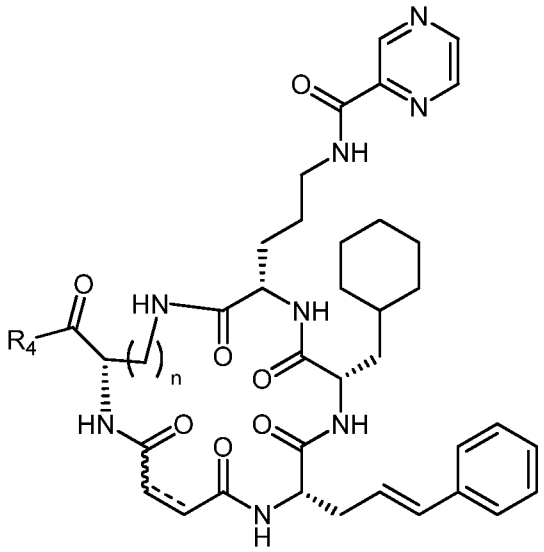

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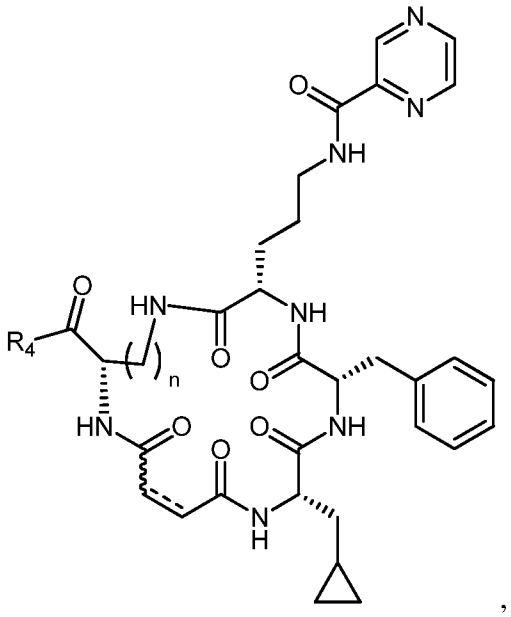

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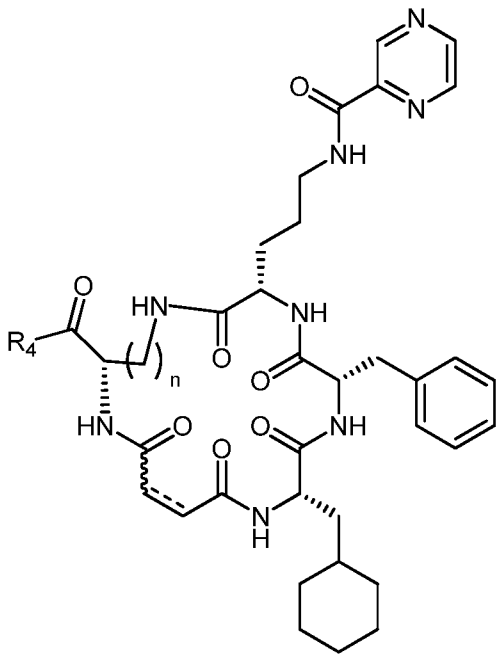

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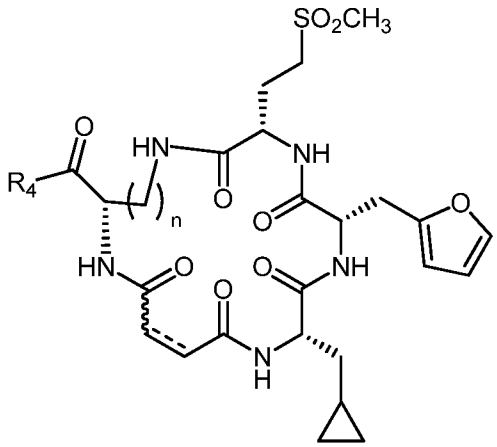

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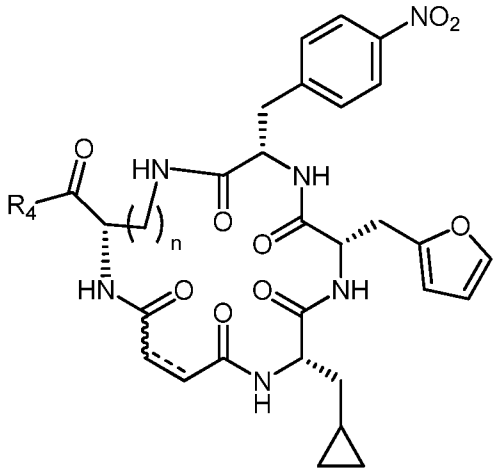

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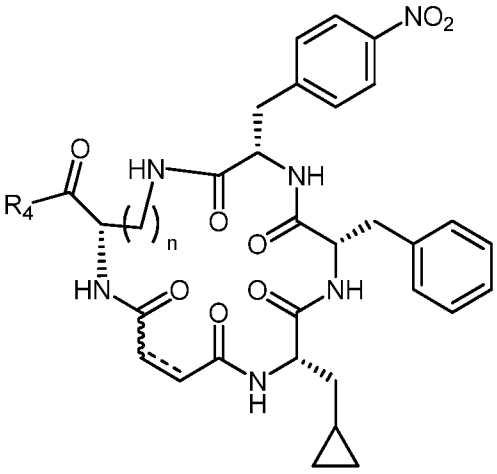

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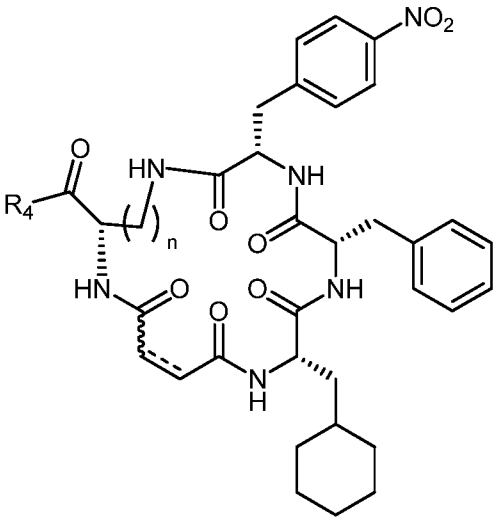

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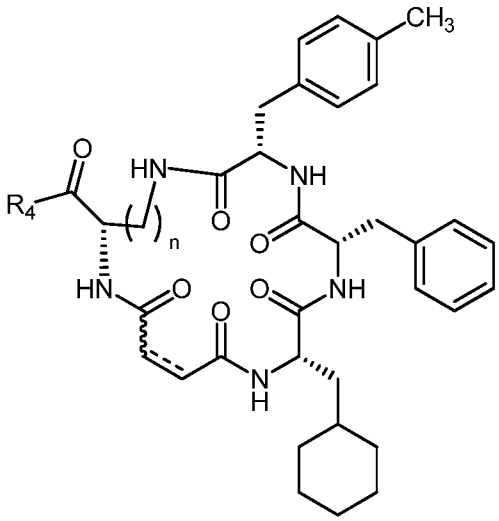

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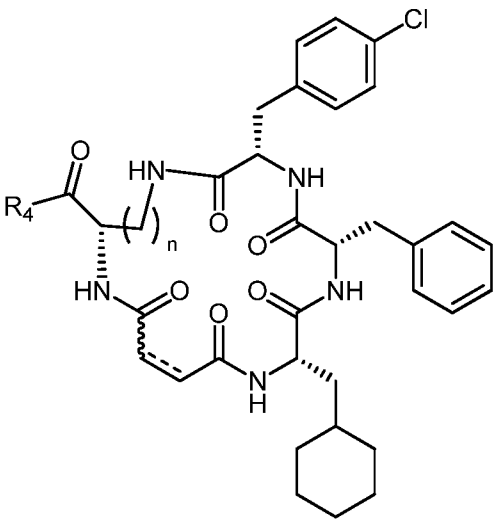

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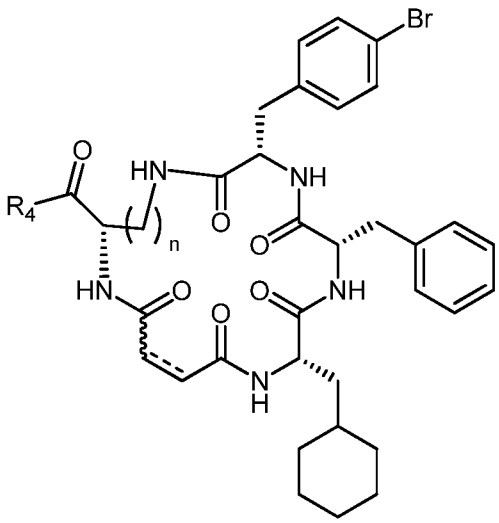

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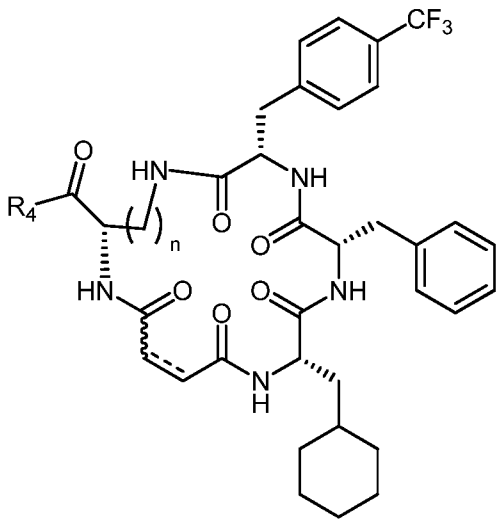

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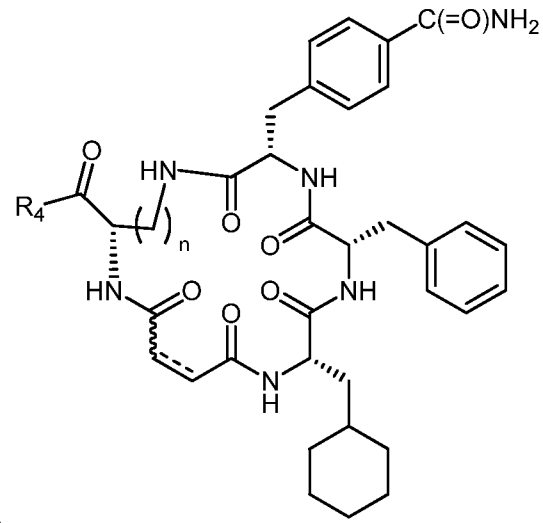

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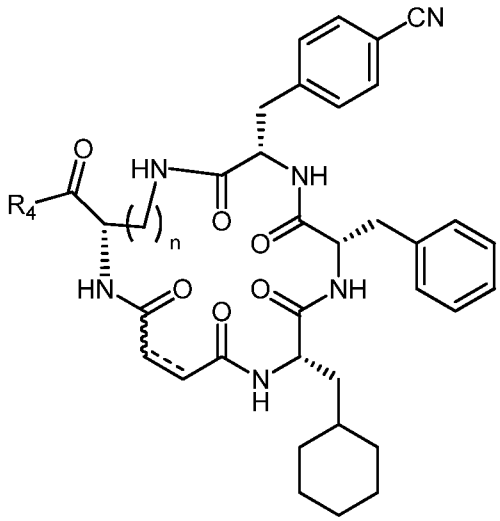

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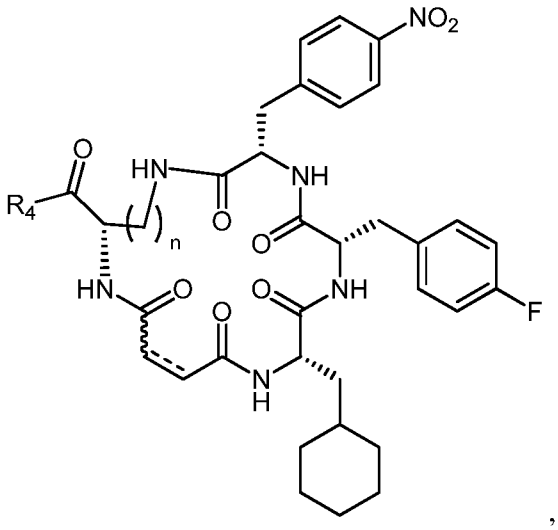

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and pharmaceutically acceptable salts thereof.

13. A pharmaceutical composition comprising a therapeutically effective amount of a compound of any of claims 1-12 and a pharmaceutically acceptable carrier.

14. A method of treating a proliferative disease, the method comprising administering a therapeutically effective amount of a compound of any of claims 1-12, or a pharmaceutically acceptable salt thereof, to a subject in need thereof.

15. The method of claim 14, wherein the compound is administered based on the subject being diagnosed with harboring a cell or a tissue expressing an increased level of the kinase or exhibiting increased levels of kinase activity.

16. An *in vitro* or *in vivo* method of modulating the activity of a kinase, the method comprising contacting a kinase with a compound of any of claims 1-12.

17. The method of claim 16, wherein the modulating is inhibiting.

18. The method of claim 15 or 17, wherein the kinase is Src, Akt3, AMPK, ERB4, MK2, p38α, MKK6, or Pim1 kinase.

19. The method of claim 18, wherein the kinase is Src and comprises a gatekeeper mutation.

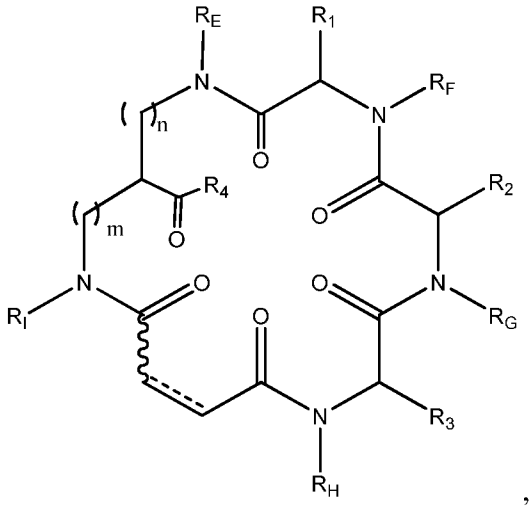
20. The method of claim 19, wherein the gatekeeper mutation is a T338I or a T341I mutation, or a mutation homologous thereto.

21. The method of claim 15 or 17, wherein the kinase is a p38α-MAPKAP2 kinase complex or MAPKAP2 kinase in a conformation essentially identical to that of MAPKAP2 in the p38α-MAPKAP2 kinase complex.

22. The method of claim 17, wherein modulating is activating.

23. The method of claim 22, wherein the kinase is VEGFR2.

24. A compound of the formula:



,

wherein

n is an integer between 0-4, inclusive;

m is an integer between 0-4, inclusive;

------ is a single or double C-C bond;

∿∿∿ indicates that the adjacent C-C double bond in a cis or trans configuration;

R_1 is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; $-OR_C$; =O; $-C(=O)R_A$; $-CO_2R_A$; -CN; -SCN; $-SR_A$; $-SOR_A$; $-SO_2R_A$; $-NO_2$; $-N(R_A)_2$; $-NHC(O)R_A$; or $-C(R_A)_3$; wherein each occurrence of R_A is independently hydrogen, a protecting group, aliphatic, heteroaliphatic, acyl, aryl, heteroaryl, alkoxy, aryloxy, alkylthio, arylthio, amino, alkylamino, dialkylamino, heteroaryloxy, or heteroarylthio;

R_2 is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; $-OR_B$; =O; $-C(=O)R_B$; $-CO_2R_B$; -CN; -SCN; $-SR_B$; $-SOR_B$; $-SO_2R_B$; $-NO_2$; $-N(R_B)_2$; $-NHC(O)R_B$; or $-C(R_B)_3$; wherein each occurrence of R_B is independently hydrogen, a protecting group, aliphatic, heteroaliphatic, acyl, aryl, heteroaryl, alkoxy,

aryloxy, alkylthio, arylthio, amino, alkylamino, dialkylamino, heteroaryloxy, or heteroarylthio;

R_3 is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; $-OR_C$; =O; $-C(=O)R_C$; $-CO_2R_C$; -CN; -SCN; $-SR_C$; $-SOR_C$; $-SO_2R_C$; $-NO_2$; $-N(R_C)_2$; $-NHC(O)R_C$; or $-C(R_C)_3$; wherein each occurrence of R_C is independently hydrogen, a protecting group, aliphatic, heteroaliphatic, acyl, aryl, heteroaryl, alkoxy, aryloxy, alkylthio, arylthio, amino, alkylamino, dialkylamino, heteroaryloxy, or heteroarylthio;

R_4 is cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; $-N(R_D)_2$; $-OR_D$; $-SR_D$; or $-C(R_D)_3$; wherein each occurrence of R_D is independently hydrogen, a protecting group, aliphatic, heteroaliphatic, acyl, aryl, heteroaryl, alkoxy, aryloxy, alkylthio, arylthio, amino, alkylamino, dialkylamino, heteroaryloxy, or heteroarylthio; and wherein two or more R_D groups of each $-N(R_D)_2$ or $-C(R_D)_3$ can optionally be joined to form a cyclic group; and

each occurrence of R_E, R_F, R_G, R_H, and R_I is independently hydrogen, a protecting group, aliphatic, heteroaliphatic, acyl, aryl, heteroaryl, alkoxy, aryloxy, alkylthio, arylthio, amino, alkylamino, dialkylamino, heteroaryloxy, or heteroarylthio.

25. The compound of claim 24, wherein R_4 is $-N(R_D)_2$.

26. The compound of claim 24, wherein R_4 is $-NH(R_D)$.

27. The compound of claim 24, wherein R_4 is $-NH_2$.

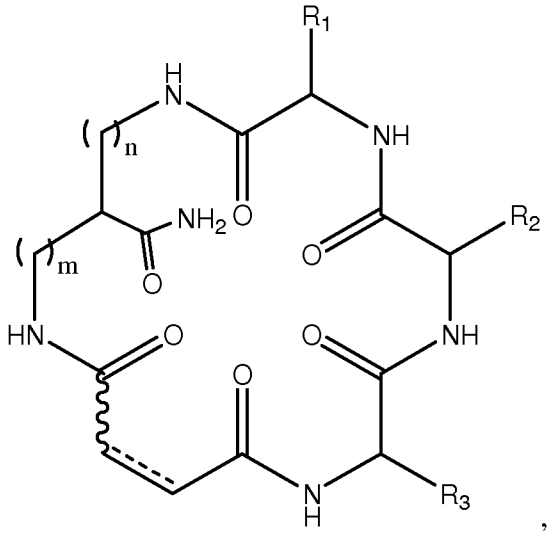
28. The compound of claim 24, wherein R_4 is $-OR_D$.

29. The compound of claim 24, wherein R_4 is -OH.

30. The compound of claim 24, wherein each occurrence of R_E, R_F, R_G, R_H, and R_I is independently alkyl or hydrogen.

31. The compound of claim 24, wherein each occurrence of R_E, R_F, R_G, R_H, and R_I is independently methyl or hydrogen.

32. The compound of claim 24, wherein the compound is a compound of the formula:



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wherein

n is an integer between 0-4, inclusive;

m is an integer between 0-4, inclusive;

====== is a single or double C-C bond;

∿∿∿ indicates that the adjacent C-C double bond in a cis or trans configuration;

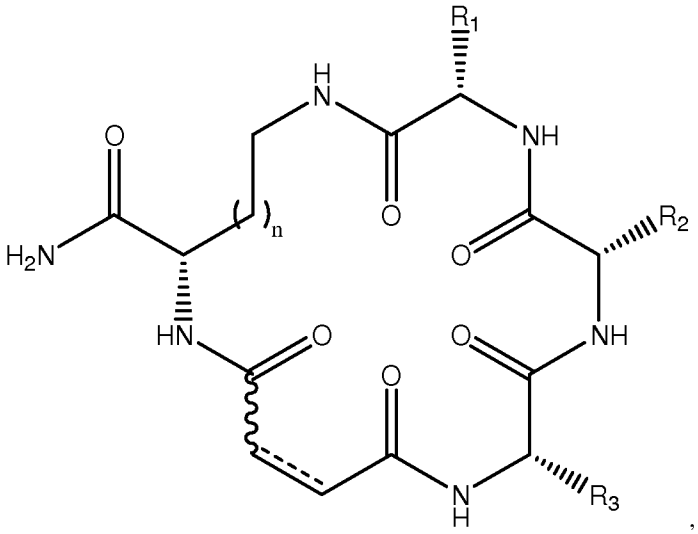
R_1 is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; $-OR_C$; =O; $-C(=O)R_A$; $-CO_2R_A$; -CN; -SCN; $-SR_A$; $-SOR_A$; $-SO_2R_A$; $-NO_2$; $-N(R_A)_2$; $-NHC(O)R_A$; or $-C(R_A)_3$; wherein each occurrence of R_A is independently hydrogen, a protecting group, aliphatic, heteroaliphatic, acyl, aryl, heteroaryl, alkoxy, aryloxy, alkylthio, arylthio, amino, alkylamino, dialkylamino, heteroaryloxy, or heteroarylthio;

R_2 is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or

unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; $-OR_B$; =O; $-C(=O)R_B$; $-CO_2R_B$; -CN; -SCN; $-SR_B$; $-SOR_B$; $-SO_2R_B$; $-NO_2$; $-N(R_B)_2$; $-NHC(O)R_B$; or $-C(R_B)_3$; wherein each occurrence of R_B is independently hydrogen, a protecting group, aliphatic, heteroaliphatic, acyl, aryl, heteroaryl, alkoxy, aryloxy, alkylthio, arylthio, amino, alkylamino, dialkylamino, heteroaryloxy, or heteroarylthio; and

R_3 is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; $-OR_C$; =O; $-C(=O)R_C$; $-CO_2R_C$; -CN; -SCN; $-SR_C$; $-SOR_C$; $-SO_2R_C$; $-NO_2$; $-N(R_C)_2$; $-NHC(O)R_C$; or $-C(R_C)_3$; wherein each occurrence of R_C is independently hydrogen, a protecting group, aliphatic, heteroaliphatic, acyl, aryl, heteroaryl, alkoxy, aryloxy, alkylthio, arylthio, amino, alkylamino, dialkylamino, heteroaryloxy, or heteroarylthio.

33. The compound of claim 24, wherein the compound is a compound of the formula:



wherein

n is an integer between 0-4, inclusive;

------ is a single or double C-C bond;

∿∿∿ indicates that the adjacent C-C double bond in a cis or trans configuration;

R_1 is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or

unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; -OR_C; =O; -$C(=O)R_A$; -CO_2R_A; -CN; -SCN; -SR_A; -SOR_A; -SO_2R_A; -NO_2; -$N(R_A)_2$; -$NHC(O)R_A$; or -$C(R_A)_3$; wherein each occurrence of R_A is independently hydrogen, a protecting group, aliphatic, heteroaliphatic, acyl, aryl, heteroaryl, alkoxy, aryloxy, alkylthio, arylthio, amino, alkylamino, dialkylamino, heteroaryloxy, or heteroarylthio;

R_2 is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; -OR_B; =O; -$C(=O)R_B$; -CO_2R_B; -CN; -SCN; -SR_B; -SOR_B; -SO_2R_B; -NO_2; -$N(R_B)_2$; -$NHC(O)R_B$; or -$C(R_B)_3$; wherein each occurrence of R_B is independently hydrogen, a protecting group, aliphatic, heteroaliphatic, acyl, aryl, heteroaryl, alkoxy, aryloxy, alkylthio, arylthio, amino, alkylamino, dialkylamino, heteroaryloxy, or heteroarylthio; and

R_3 is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; -OR_C; =O; -$C(=O)R_C$; -CO_2R_C; -CN; -SCN; -SR_C; -SOR_C; -SO_2R_C; -NO_2; -$N(R_C)_2$; -$NHC(O)R_C$; or -$C(R_C)_3$; wherein each occurrence of R_C is independently hydrogen, a protecting group, aliphatic, heteroaliphatic, acyl, aryl, heteroaryl, alkoxy, aryloxy, alkylthio, arylthio, amino, alkylamino, dialkylamino, heteroaryloxy, or heteroarylthio.

34. The compound of claim 24, wherein $n + m \leq 4$.

35. The compound of claim 24, wherein n is 0.

36. The compound of claim 24, wherein n is 1.

37. The compound of claim 24, wherein n is 2.

38. The compound of claim 24, wherein n is 3.

39. The compound of claim 24, wherein n is 4.

40. The compound of claim 24, wherein m is 0.

41. The compound of claim 24, wherein m is 1.

42. The compound of claim 24, wherein m is 2.

43. The compound of claim 24, wherein m is 3.

44. The compound of claim 24, wherein m is 4.

45. The compound of claim 24, wherein n is an integer between 2 and 4, inclusive.

46. The compound of claim 24, wherein R_1 is substituted aliphatic.

47. The compound of claim 24, wherein R_1 is substituted alkyl.

48. The compound of claim 24, wherein R_1 is substituted heteroaliphatic.

49. The compound of claim 24, wherein R_1 is substituted heteroalkyl.

50. The compound of claim 24, wherein R_1 is heteroaryl-heteroaliphatic.

51. The compound of claim 24, wherein R_1 is acyl-heteroapliphatic.

52. The compound of claim 24, wherein R_1 is substituted aryl-alkyl.

53. The compound of claim 24, wherein R_1 is substituted phenyl-alkyl.

54. The compound of claim 24, wherein R_1 is nitro-substituted phenyl-alkyl.

55. The compound of claim 24, wherein R_1 is sulfonyl-substituted aliphatic.

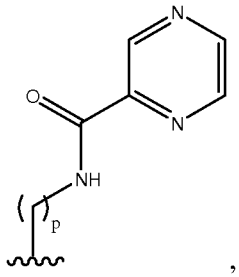
56. The compound of claim 24, wherein R_1 is methyl-sulfonyl-substituted aliphatic.

57. The compound of claim 24, wherein R_1 is alkyl substituted by -$NHCOR_A$, wherein R_A is aryl or heteroaryl.

58. The compound of claim 24, wherein R_A is phenyl.

59. The compound of claim 24, wherein R_A is pyrazinyl.

60. The compound of claim 24, wherein R_1 is of the formula:



,

wherein p is an integer between 1 and 6, inclusive.

61. The compound of claim 60, wherein p is 1.

62. The compound of claim 60, wherein p is 2.

63. The compound of claim 60, wherein p is 3.

64. The compound of claim 60, wherein p is 4.

65. The compound of claim 60, wherein p is 5.

66. The compound of claim 60, wherein p is 6.

67. The compound of claim 24, wherein R_1 is substituted or unsubstituted arylalkyl.

68. The compound of claim 24, wherein R_1 is phenylalkyl.

69. The compound of claim 24, wherein R_1 is substituted phenylalkyl.

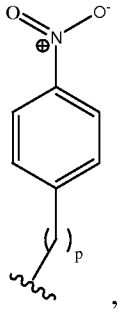
70. The compound of claim 24, wherein R_1 is heteroarylalkyl.

71. The compound of claim 24, wherein R_1 is substituted heteroarylalkyl.

72. The compound of claim 24, wherein R_1 is alkyl substituted by aryl or heteroaryl.

73. The compound of claim 24, wherein R_1 is alkyl substituted by phenyl.

74. The compound of claim 24, wherein R_1 is of the formula:



and p is an integer between 1 and 6, inclusive.

75. The compound of claim 74, wherein p is 1.

76. The compound of claim 52, wherein p is 2.

77. The compound of claim 52, wherein p is 3.

78. The compound of claim 52, wherein p is 4.

79. The compound of claim 52, wherein p is 5.

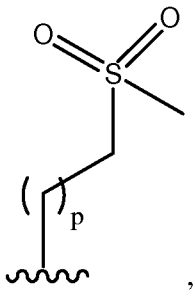
80. The compound of claim 52, wherein p is 6.

81. The compound of claim 24, wherein R_1 is alkyl substituted by $-SO_2R_A$, wherein R_A is hydrogen, halogen, alkyl, or aliphatic.

82. The compound of claim 24, wherein R_A is alkyl, alkenyl, or alkynyl.

83. The compound of claim 24, wherein R_A is unbranched aliphatic or unbranched heteroaliphatic.

84. The compound of claim 24, wherein R_1 is of the formula:



and p is an integer between 1 and 6, inclusive.

85. The compound of claim 63, wherein p is 1.

86. The compound of claim 63, wherein p is 2.

87. The compound of claim 63, wherein p is 3.

88. The compound of claim 63, wherein p is 4.

89. The compound of claim 63, wherein p is 5.

90. The compound of claim 63, wherein p is 6.

91. The compound of claim 24, wherein R_2 is substituted aliphatic.

92. The compound of claim 24, wherein R_2 is aliphatic-heteroaryl.

93. The compound of claim 24, wherein R_2 is substituted alkyl.

94. The compound of claim 24, wherein R_2 is alkyl-heteroaryl.

95. The compound of claim 24, wherein R_2 is alkyl-cycloaliphatic.

96. The compound of claim 24, wherein R_2 is arylalkyl.

97. The compound of claim 24, wherein R_2 is heteroarylalkyl.

98. The compound of claim 24, wherein R_2 is 5-membered heteroarylalkyl.

99. The compound of claim 24, wherein R_2 is 6-membered heteroarylalkyl.

100. The compound of claim 24, wherein R_2 is 7-membered heteroarylalkyl.

101. The compound of claim 24, wherein R_2 is phenylalkyl.

102. The compound of claim 24, wherein R_2 is furanylalkyl.

103. The compound of claim 24, wherein R_2 is 2-furanylalkyl.

104. The compound of claim 24, wherein R_2 is 3-furanylalkyl.

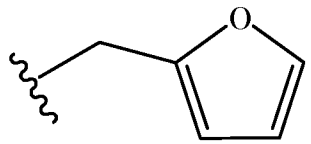
105. The compound of claim 24, wherein R_2 is carbocyclicalkyl.

106. The compound of claim 24, wherein R_2 is cyclohexylalkyl.

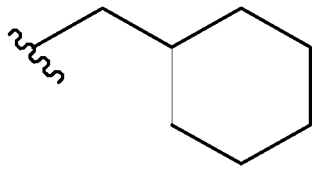
107. The compound of claim 24, wherein R_2 is cyclopentylalkyl.

108. The compound of claim 24, wherein R_2 is cycloheptylalkyl.

109. The compound of claim 24, wherein R_2 is of the formula:



110. The compound of claim 24, wherein R_2 is of the formula:



111. The compound of claim 24, wherein R_3 is substituted aliphatic.

112. The compound of claim 24, wherein R_3 is substituted alkyl.

113. The compound of claim 24, wherein R_3 is substituted alkenyl.

114. The compound of claim 24, wherein R_3 is alkenyl-aryl.

115. The compound of claim 24, wherein R_3 is arylalkyl.

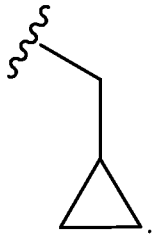
116. The compound of claim 24, wherein R_3 is arylalkenyl.

117. The compound of claim 24, wherein R_3 is alkyl.

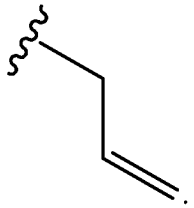
118. The compound of claim 24, wherein R_3 is alkenyl.

119. The compound of claim 24, wherein R_3 is alkynyl.

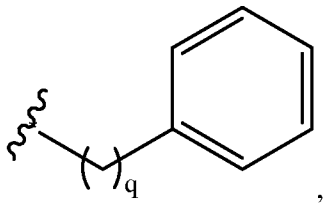
120. The compound of claim 24, wherein R_3 is of the formula:



121. The compound of claim 24, wherein R_3 is of the formula:



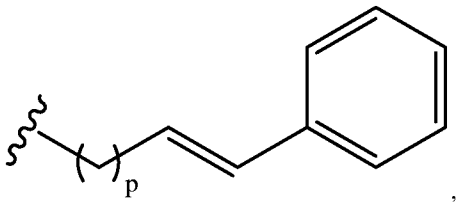
122. The compound of claim 24, wherein R_3 is of the formula:



wherein q is an integer between 0 and 10, inclusive.

123. The compound of claim 122, wherein q is an integer between 2 and 5, inclusive.

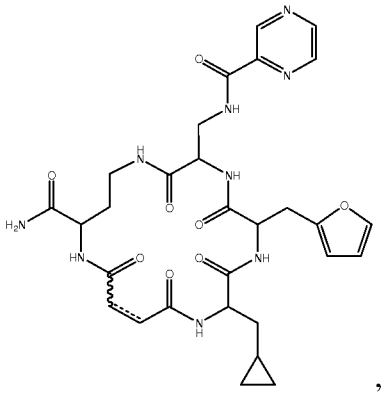
124. The compound of claim 24, wherein R_3 is of the formula:



wherein p is an integer between 0 and 10, inclusive.

125. The compound of claim 24, wherein p is an integer between 2 and 5, inclusive.

126. The compound of claim 24, wherein the compound is a compound according to any of the following formulae: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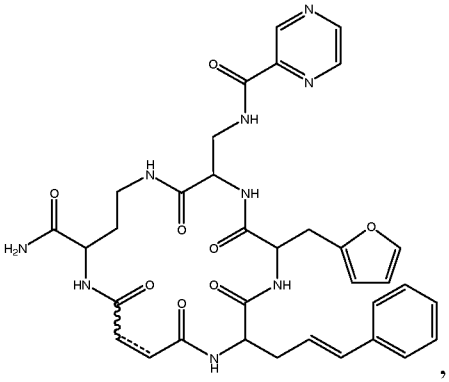
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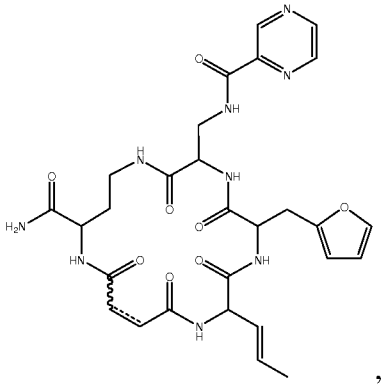
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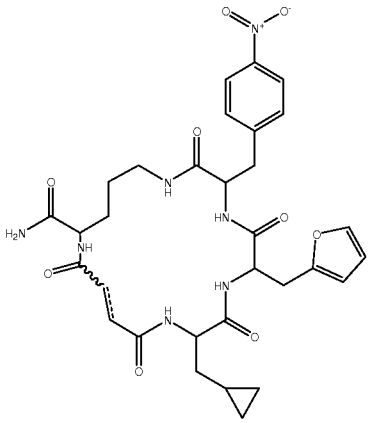
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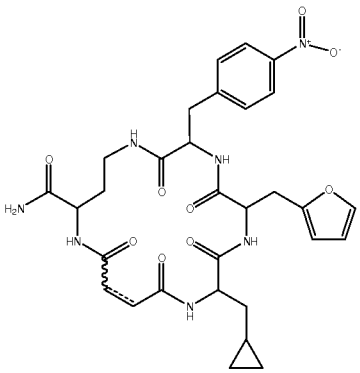
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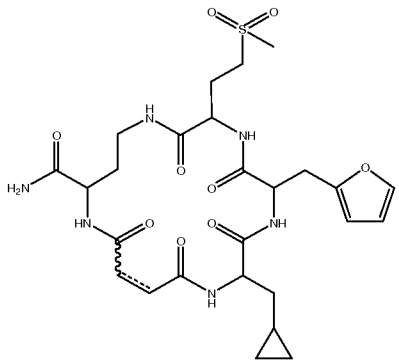
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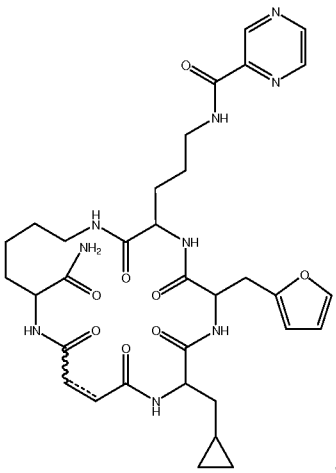
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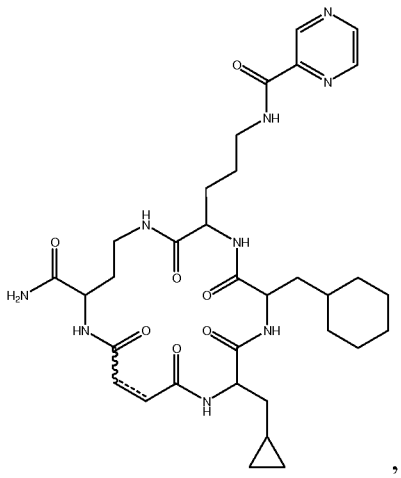
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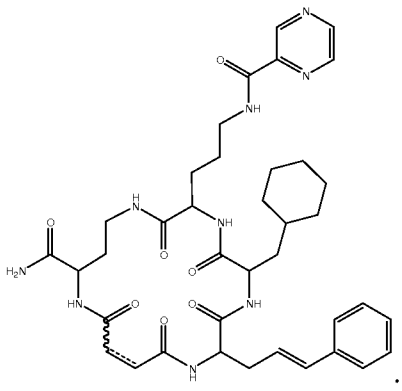
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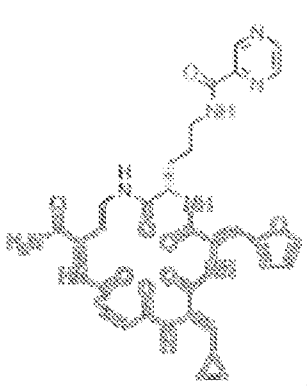
127. The compound of claim 24, wherein ====== is a double C-C bond.

128. The compound of claim 127, wherein ====== is a double C-C bond in the cis configuration.

129. The compound of claim 127, wherein ====== is a double C-C bond in the cis configuration.

130. The compound of claim 127, wherein ====== is a single C-C bond.

131. The compound of claim 24, wherein the compound is a compound of any of the following formulae: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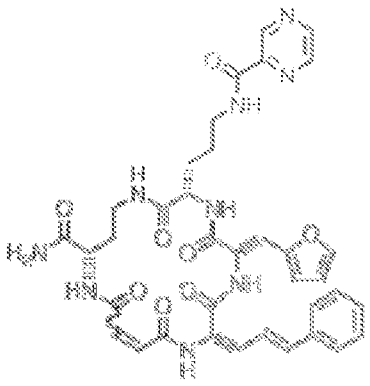
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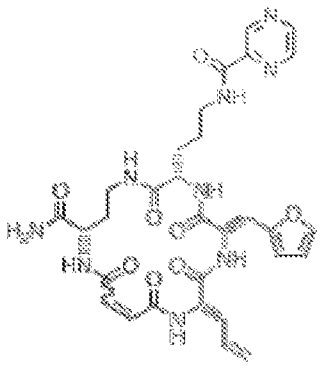
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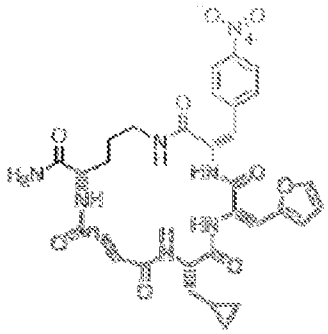
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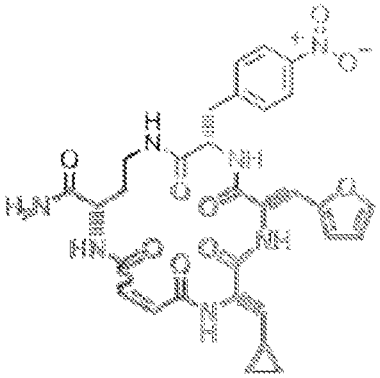
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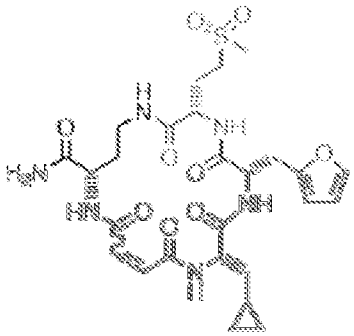
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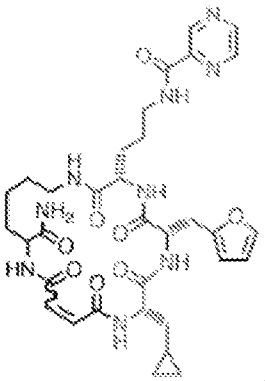
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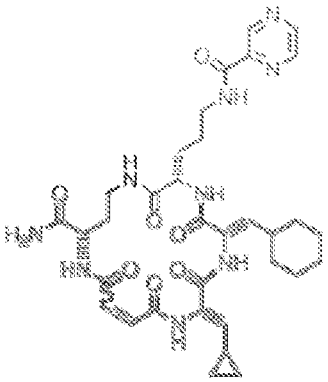
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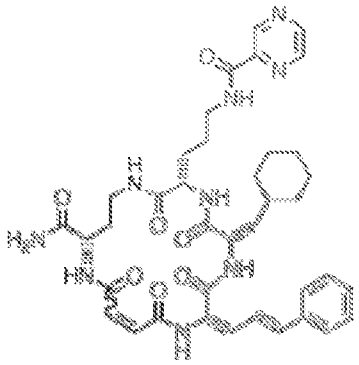
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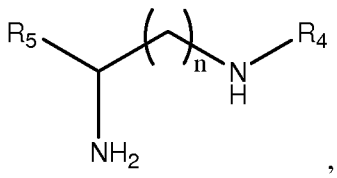
132. A method of synthesizing the compound of any of claims 24-131, the method comprising:

(a) providing a differentially protected diamino acid macrocyclization precursor of the formula:



,

wherein

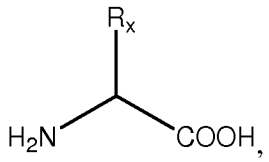
n is an integer between 0 and 10, inclusive;

R_4 is a protecting group;

R_5 is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; $-OR_D$; =O; $-C(=O)R_D$; $-CO_2R_D$; -CN; -SCN; $-SR_D$; $-SOR_D$; $-SO_2R_D$; $-NO_2$; $-N(R_D)_2$; $-NHC(O)R_D$; or $-C(R_D)_3$; wherein each occurrence of R_D is independently hydrogen, a protecting group, aliphatic, heteroaliphatic, acyl; aryl; heteroaryl; alkoxy; aryloxy; alkylthio; arylthio; amino, alkylamino, dialkylamino, heteroaryloxy; or heteroarylthio; and

optionally, wherein the macrocyclization precursor is coupled to a solid support via R_5;

(b) contacting the macrocyclization precursor provided under (a) with a building block of the formula:



under conditions suitable for the formation of a peptide bond between the carboxyl group of the building block provided under (b) with the unprotected amino group of the macrocyclization precursor,wherein

R_x, is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; $-OR_E$; =O; $-C(=O)R_E$; $-CO_2R_E$; -CN; -SCN; $-SR_E$; $-SOR_E$; $-SO_2R_E$; $-NO_2$; $-N(R_E)_2$; $-NHC(O)R_E$; or $-C(R_E)_3$; wherein each occurrence of R_E is independently hydrogen, a protecting group, aliphatic, heteroaliphatic, acyl; aryl; heteroaryl; alkoxy; aryloxy; alkylthio; arylthio; amino, alkylamino, dialkylamino, heteroaryloxy; or heteroarylthio;

(c) performing 1-5 additional steps of contacting the reaction product generated under (b) with an additional building block of the formula provided in (b), wherein Rx is defined for each building block separately and individually as under (b)

(d) optionally, cleaving the macrocyclization precursor from the solid support and/or purifying the macrocyclization precursor; and

(e) effecting cyclization of the macrocyclization precursor.

133. The method of claim 132, wherein n in the formula of the macrocyclization precursor provided under (a) is an integer between 1 and 4, inclusive.

134. The method of claim 132, wherein R_4 is an amino protective group.

135. The method of claim 132, wherein R_4 is isopropylidene-protected tartrate monomethyl ester.

136. The method of claim 132, wherein the macrocyclization precursor is coupled to an amide resin.

137. The method of claim 132, wherein the building blocks are added to the macrocyclization precursor by Fmoc synthesis.

138. The method of claim 132, wherein the method comprises generating a phosphonium salt of the macrocyclization precursor on resin.

139. The method of claim 132, wherein the macrocyclization precursor is cleaved from the solid support by treatment with a strong acid.

140. The method of claim 139, wherein the cleavage reaction generates a carboxamide at the C-terminus and deprotects a tartrate diol group.

141. The method of claim 132, wherein the macrocyclization precursor is purified before cyclization.

142. The method of claim 132, further comprising a step of oxidatively cleaving the diol group, thus generating an aldehyde group.

143. The method of claim 132, wherein the cyclization is a Wittig cyclization.

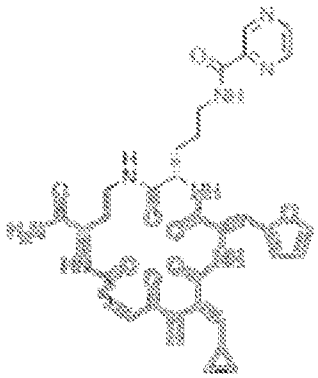
144. The method of claim 143, wherein the Wittig cyclization is effected by raising the pH to generate a phosphonium ylide.

145. A pharmaceutical composition, comprising:
a compound of any of claims 24-131, and
a pharmaceutically acceptable carrier.

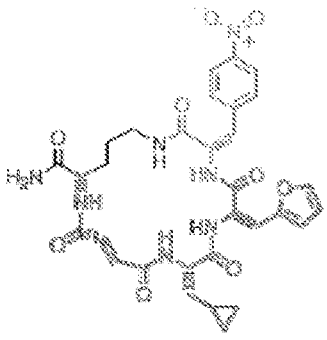
146. A method of modulating the activity of a kinase, the method comprising contacting a kinase with a compound of any of claims 24-131.

147. The method of claim 146, wherein the modulating is inhibiting, and the kinase is Src, Akt3, AMPK, ERB4, MK2, p38α, MKK6, or Pim1 kinase.

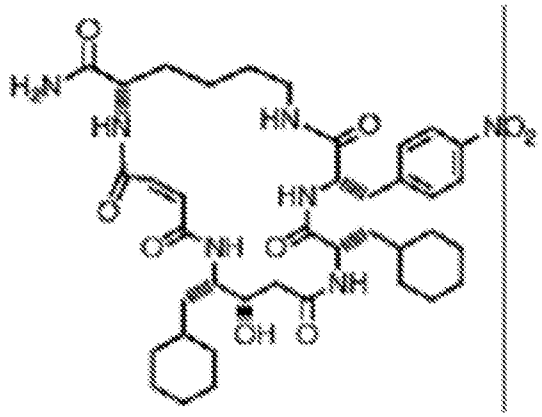
148. The method of claim 147, wherein the kinase is Src; and the compound is of the formula:



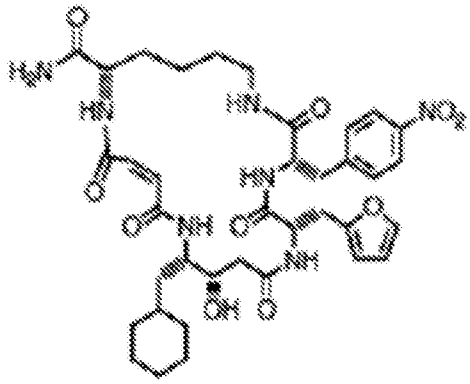
149. The method of claim 147, wherein the kinase is Src, and the compound is of the formula:



150. The method of claim 147, wherein the kinase is Akt3, Pim1, or MAPKAP2 kinase, and the compound is of the formula:

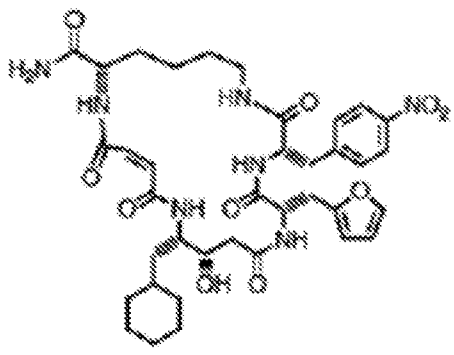


151. The method of claim 147, wherein the kinase is a p38α-MAPKAP2 kinase complex or MAPKAP2 kinase in a conformation essentially identical to that of MAPKAP2 in the p38α-MAPKAP2 kinase complex, and the compound is of the formula:



152. The method of claim 147, wherein modulating is activating, and the kinase is VEGFR2.

153. The method of claim 152, wherein the compound is of the formula:



154. The method of claim 146, wherein the kinase is contacted *in vitro*.

155. The method of claim 146, wherein the kinase is contacted in a subject *in vivo*.

156. The method of claim 146, wherein the kinase is in a cell.

157. The method of claim 156, wherein the cell is a cell overexpressing the kinase or exhibiting an increased level of kinase activity.

158. The method of claim 156, wherein the cell is overexpressing the kinase or exhibiting an increased level of the kinase as compared to the average expression level or the average activity level of the kinase in healthy cells of the same cell type or tissue of origin.

159. The method of claim 156 or 157, wherein the cell is a cell exhibiting an increased level of Src kinase activity.

160. The method of claim 146, wherein the contacting is effected by administering to the subject an effective dose of the compound.

161. The method of claim 160, wherein the compound is administered based on the subject being diagnosed with harboring a cell or a tissue expressing an increased level of the kinase or exhibiting increased levels of kinase activity.

162. The method of claim 161, wherein the compound is administered orally.

163. The method of claim 161, wherein the compound is administered parenterally.

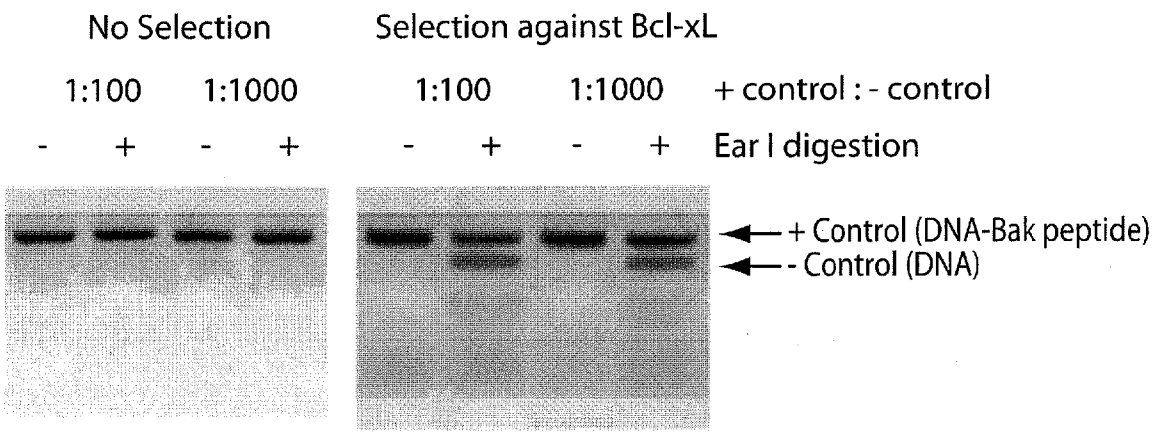

No Selection
Selection against Bcl-xL
1:100
1:1000
1:100
1:1000
+ control : - control
- + - + - + - +
Ear I digestion
+ Control (DNA-Bak peptide)
- Control (DNA)

Fig. 1

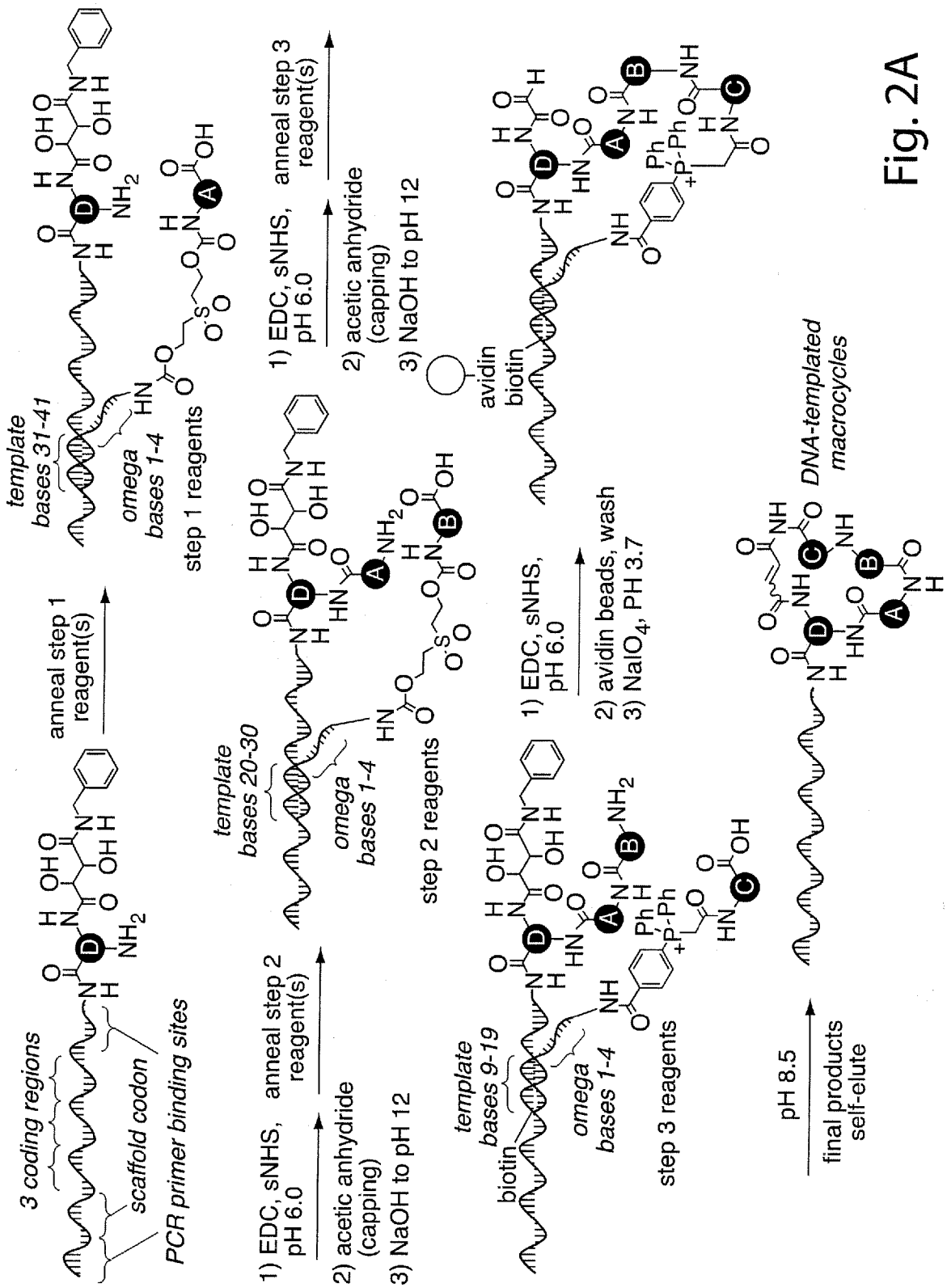

3 coding regions
scaffold codon
PCR primer binding sites
anneal step 1 reagent(s)
template bases 31-41
omega bases 1-4
step 1 reagents
1) EDC, sNHS, pH 6.0
2) acetic anhydride (capping)
3) NaOH to pH 12
anneal step 2 reagent(s)
template bases 20-30
omega bases 1-4
step 2 reagents
1) EDC, sNHS, pH 6.0
anneal step 3 reagent(s)
avidin
biotin
template bases 9-19
omega bases 1-4
step 3 reagents
1) EDC, sNHS, pH 6.0
2) avidin beads, wash
3) NaIO4, PH 3.7
pH 8.5
final products self-elute
DNA-templated macrocycles

Fig. 2A

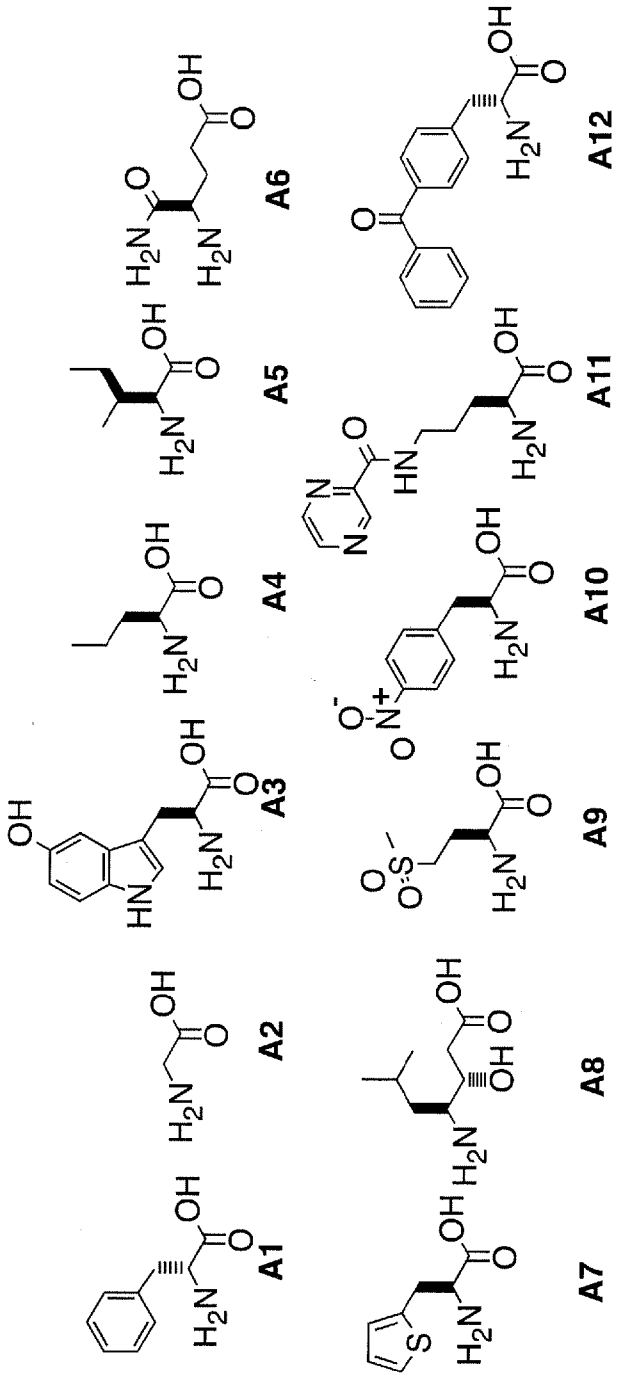


Fig. 2B-1

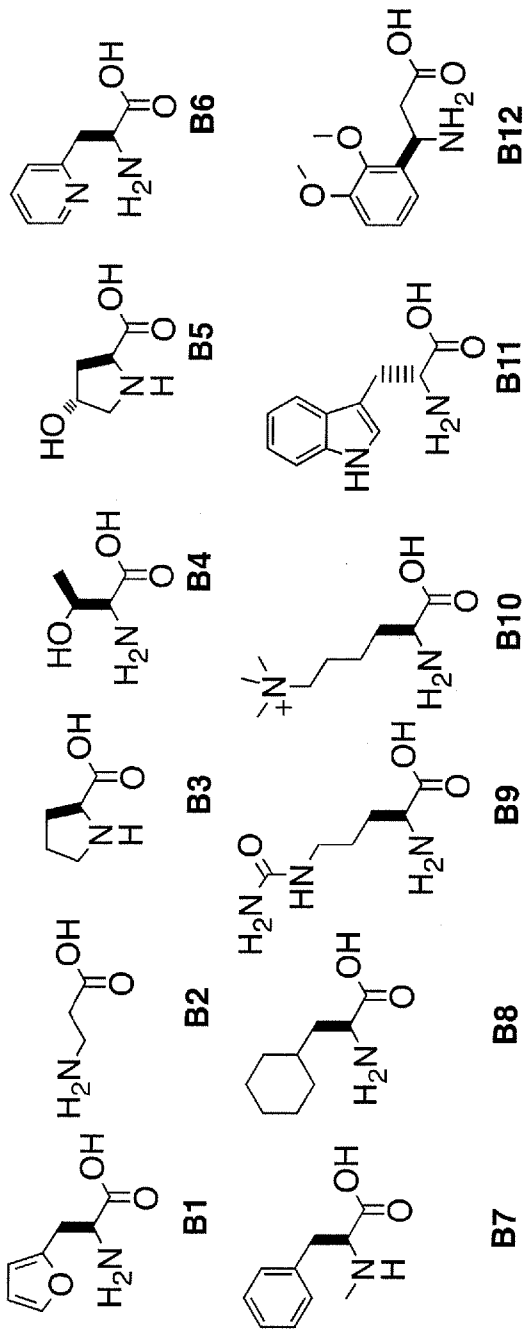


Fig. 2B-2

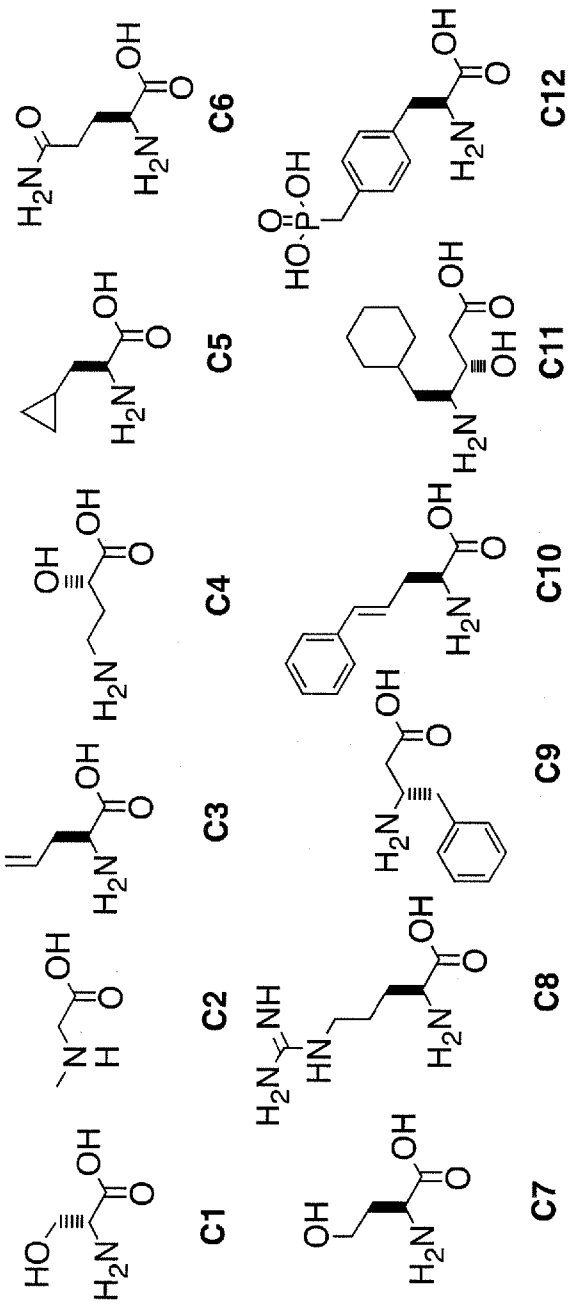


Fig. 2B-3

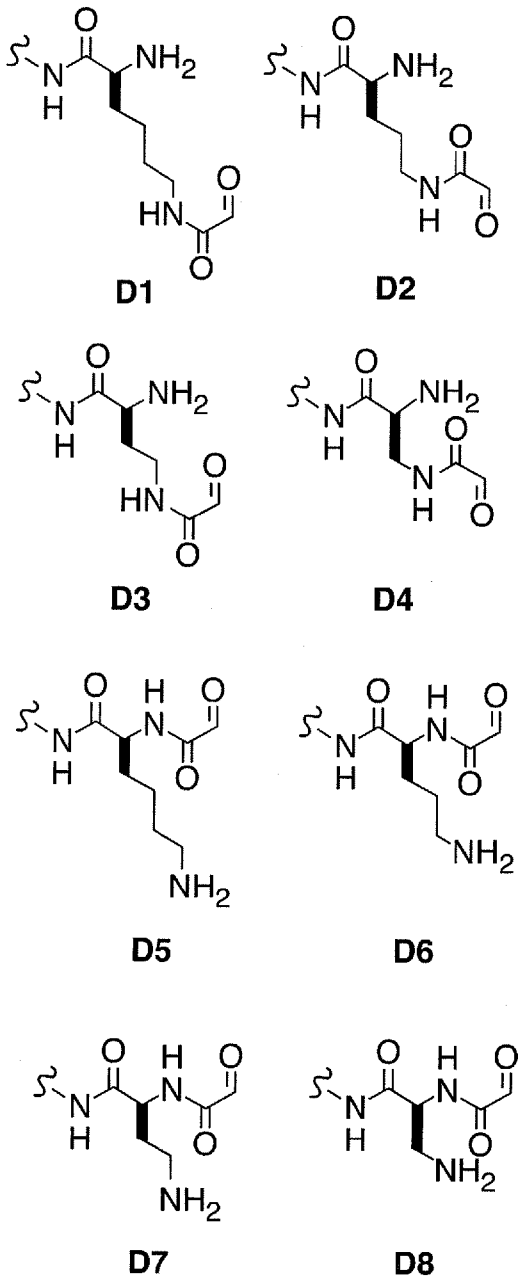


Fig. 2B-4

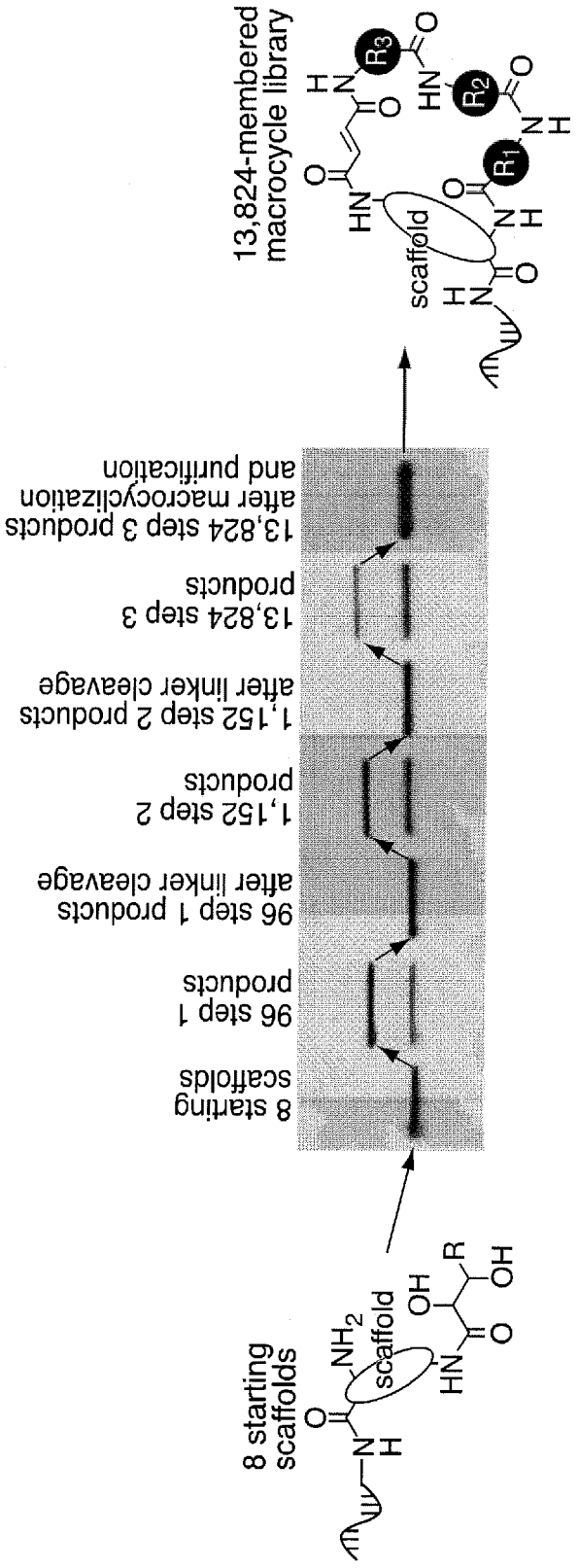

8 starting scaffolds
scaffold
13,824-membered macrocycle library
8 starting scaffolds
96 step 1 products
96 step 1 products after linker cleavage
1,152 step 2 products
1,152 step 2 products after linker cleavage
13,824 step 3 products
13,824 step 3 products after macrocyclization and purification

Fig. 2B-5

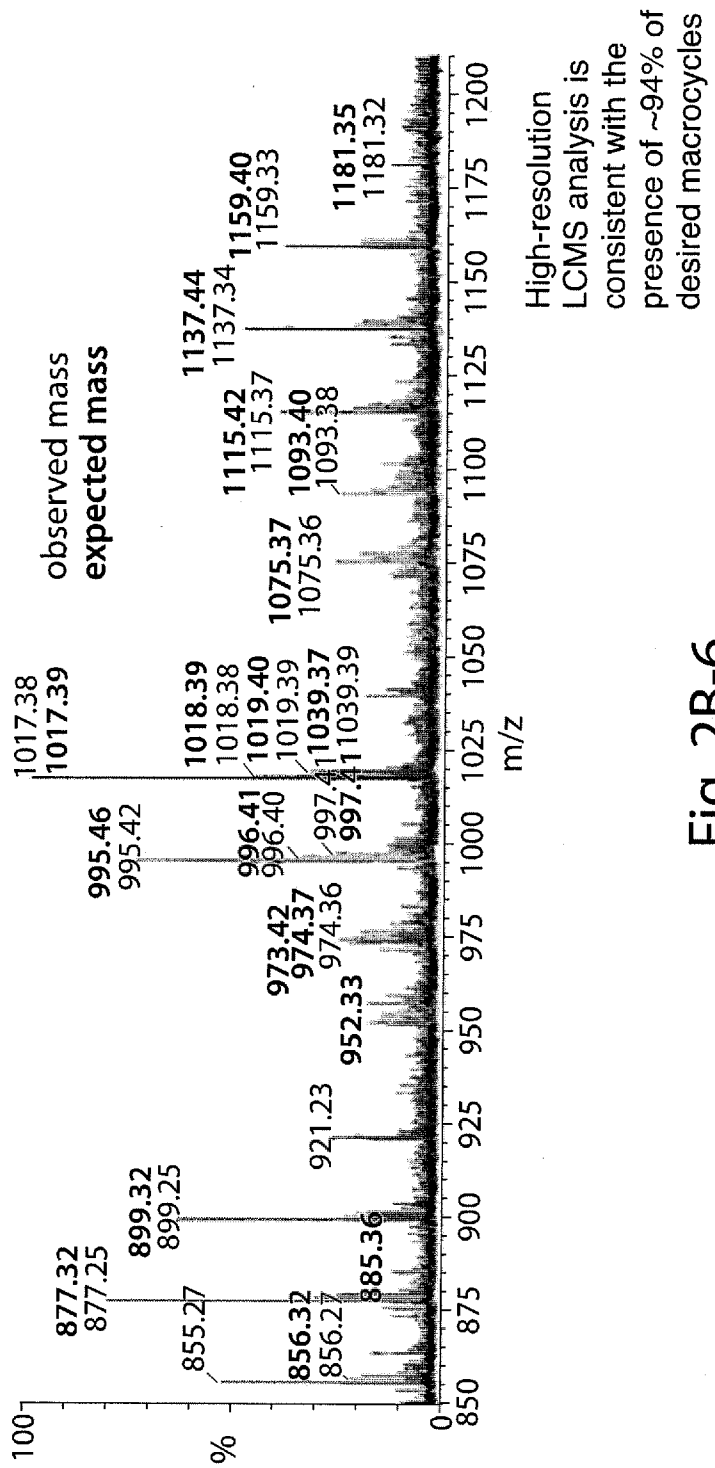

observed mass
expected mass
1017.38
1017.39
995.46
995.42
1018.39
1018.38
996.41
996.40
1019.40
1019.39
997.4
997.4
1039.37
1039.39
973.42
974.37
974.36
952.33
921.23
899.32
899.25
877.32
877.25
855.27
856.32
856.27
885.36
1075.37
1075.36
1115.42
1115.37
1093.40
1093.38
1137.44
1137.34
1159.40
1159.33
1181.35
1181.32
100
%
0
850
875
900
925
950
975
1000
1025
1050
1075
1100
1125
1150
1175
1200
m/z
High-resolution
LCMS analysis is
consistent with the
presence of ~94% of
desired macrocycles

Fig. 2B-6

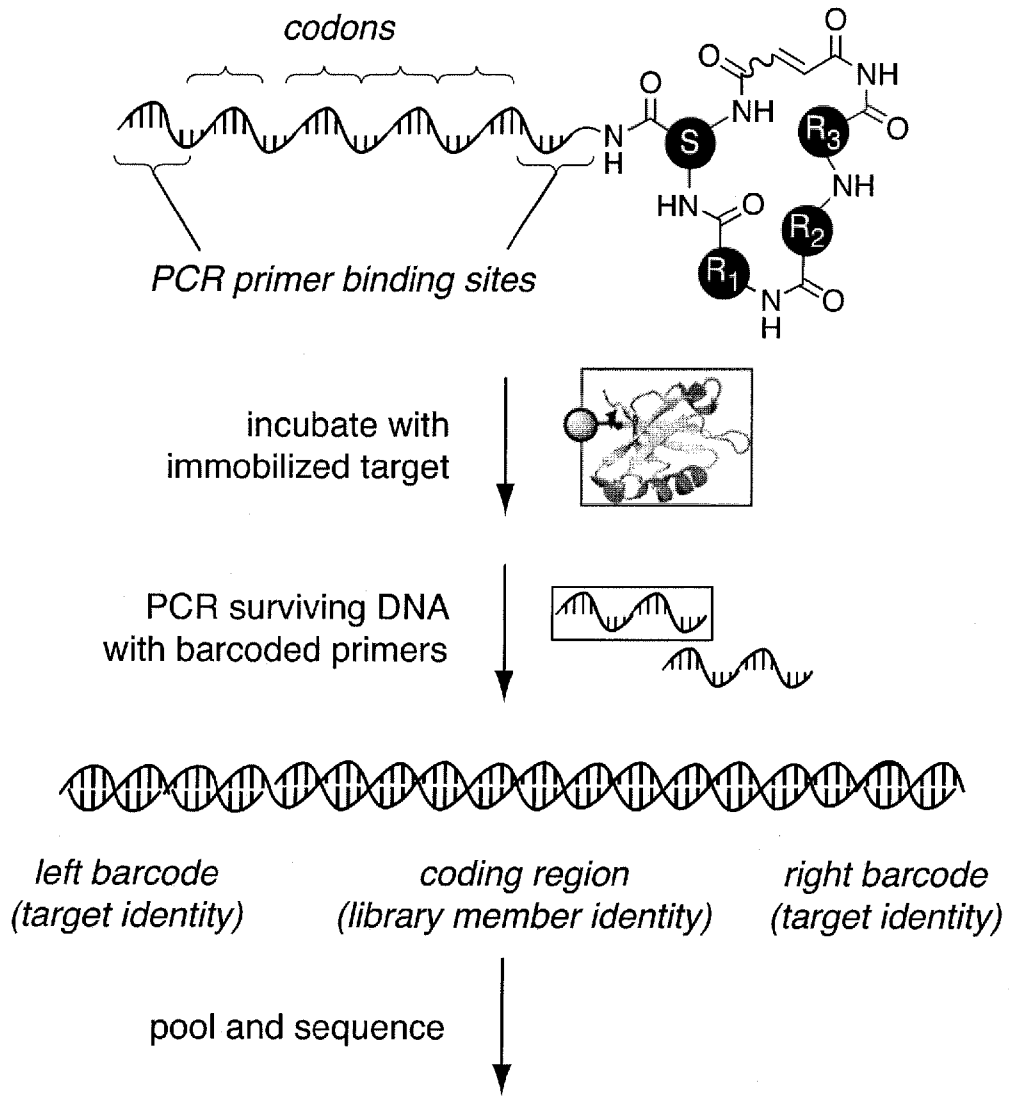

codons
PCR primer binding sites
incubate with
immobilized target
PCR surviving DNA
with barcoded primers
left barcode
(target identity)
coding region
(library member identity)
right barcode
(target identity)
pool and sequence
~12,400,000 sequence reads

Fig. 3

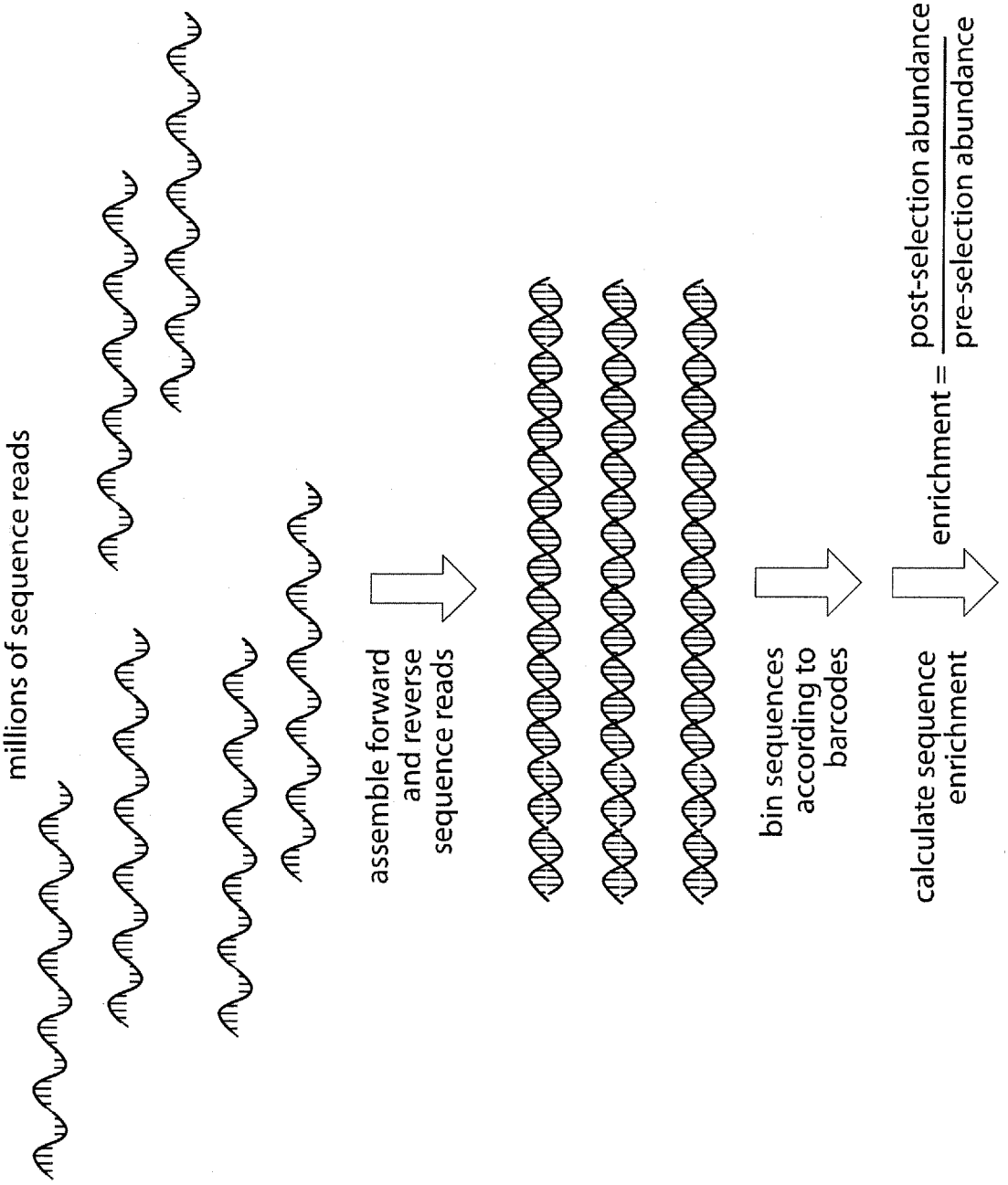

millions of sequence reads
assemble forward and reverse sequence reads
bin sequences according to barcodes
calculate sequence enrichment
enrichment = post-selection abundance / pre-selection abundance

Fig. 4A

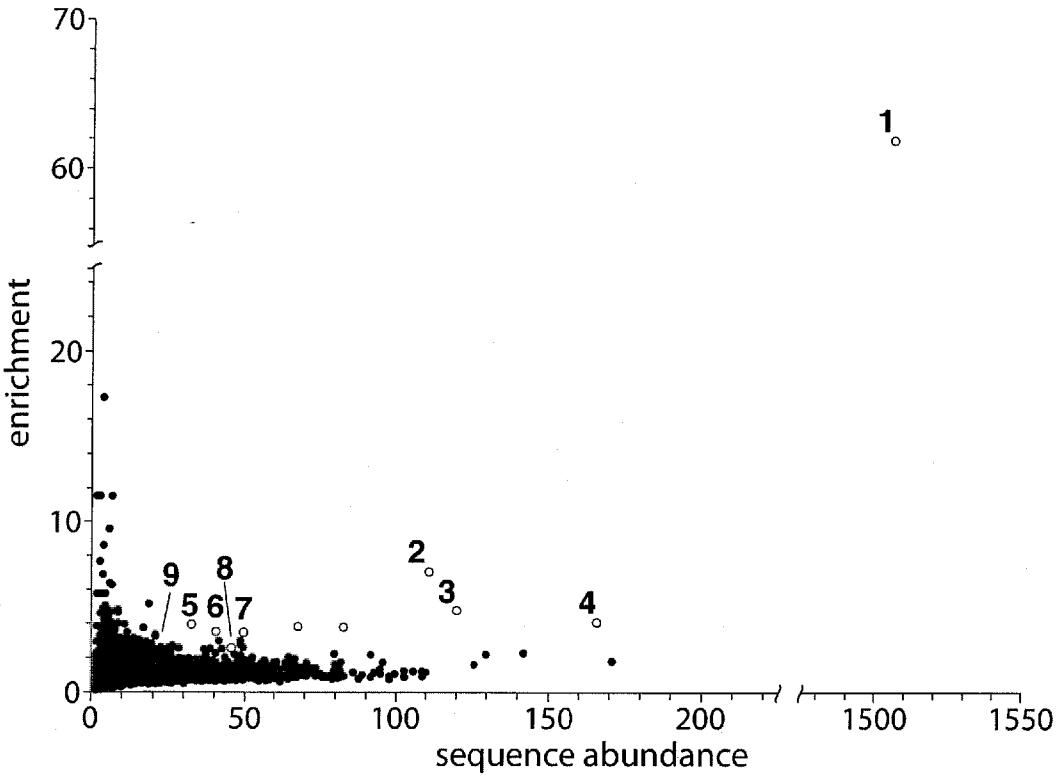

70
60
20
10
0
enrichment
1
2
3
4
5
6
7
8
9
0
50
100
150
200
1500
1550
sequence abundance

Fig. 4B-1

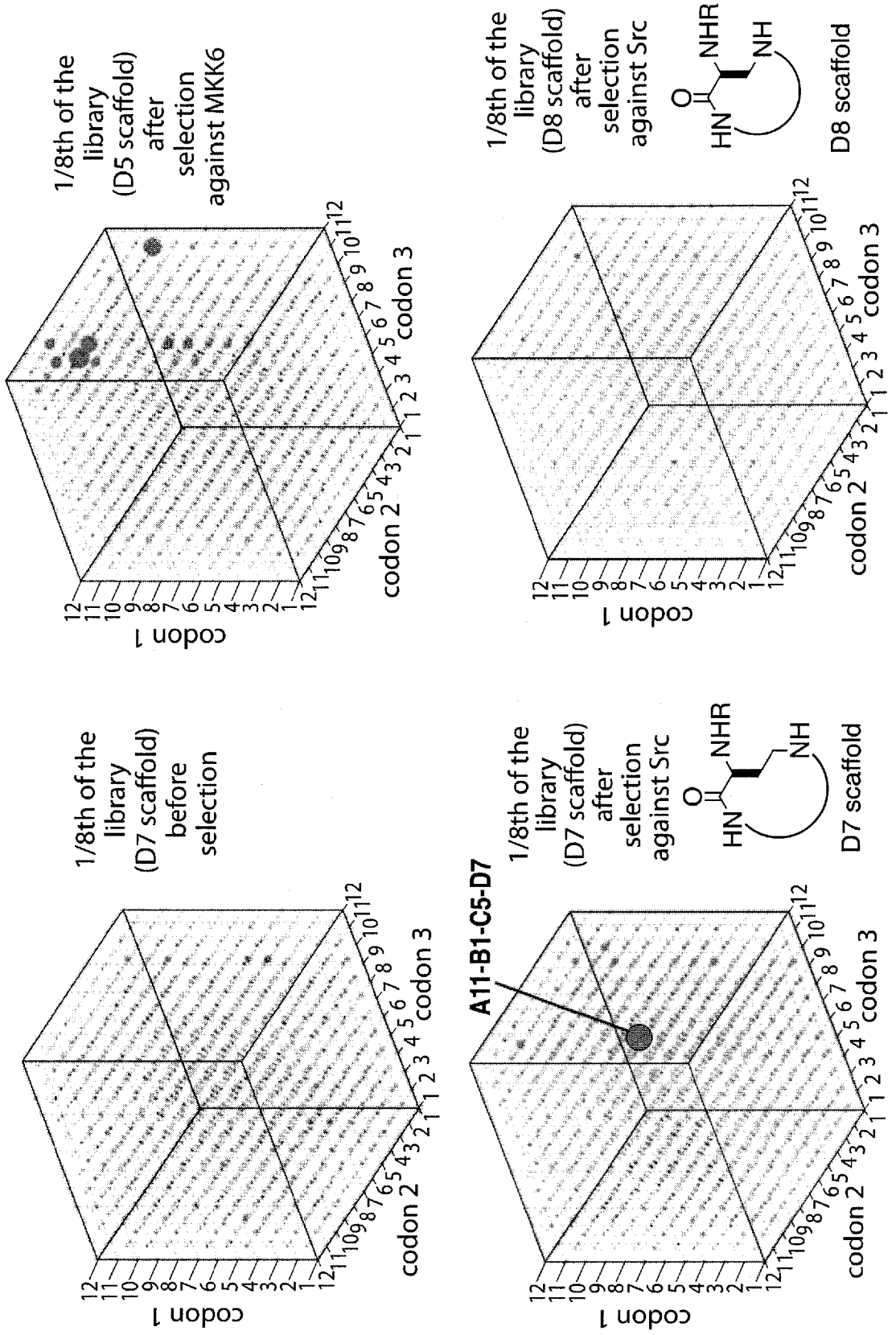
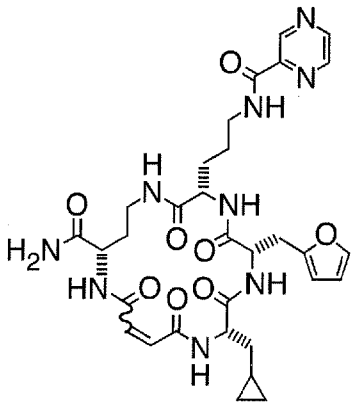
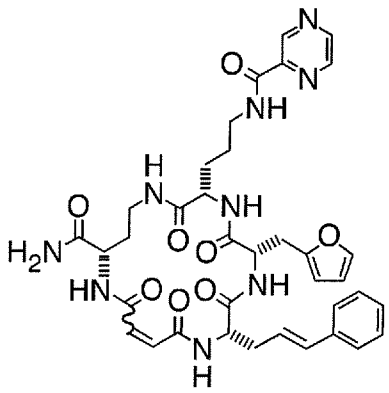

1/8th of the library (D5 scaffold) after selection against MKK6
1/8th of the library (D8 scaffold) after selection against Src
1/8th of the library (D7 scaffold) before selection
1/8th of the library (D7 scaffold) after selection against Src
A11-B1-C5-D7
D7 scaffold
D8 scaffold
codon 1
codon 2
codon 3
NHR
NH
HN
O

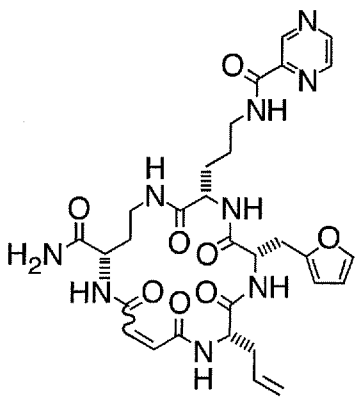
Fig. 4B-2



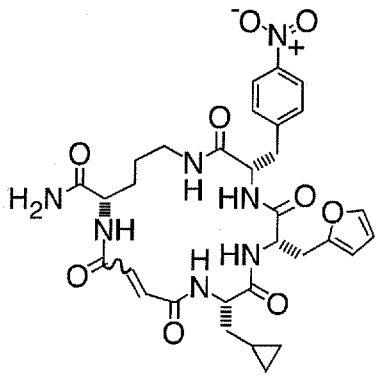
1 (A11-B1-C5-D7)



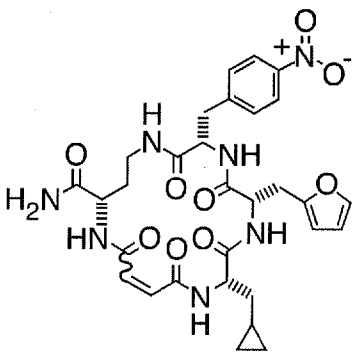
2 (A11-B1-C10-D7)



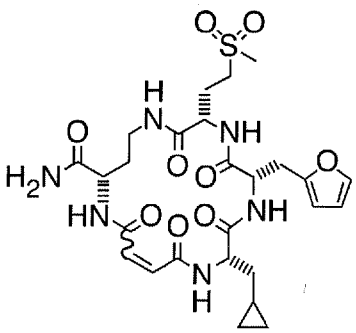
3 (A11-B1-C3-D7)



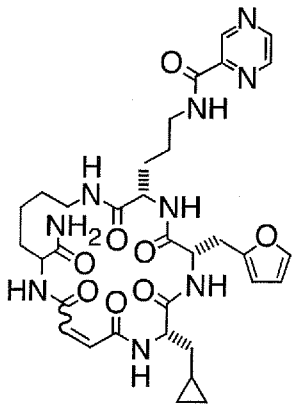
4 (A10-B1-C5-D6)



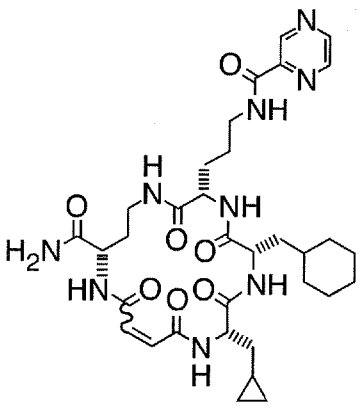
5 (A10-B1-C5-D7)



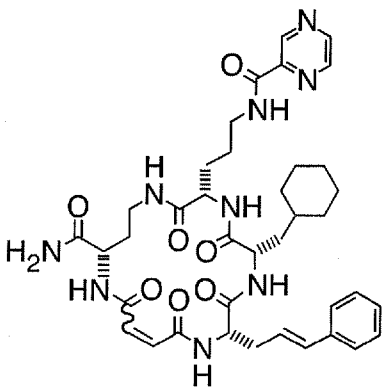
6 (A9-B1-C5-D7)



7 (A11-B1-C5-D5)



8 (A11-B8-C5-D7)



9 (A11-B8-C10-D7)

Fig. 5

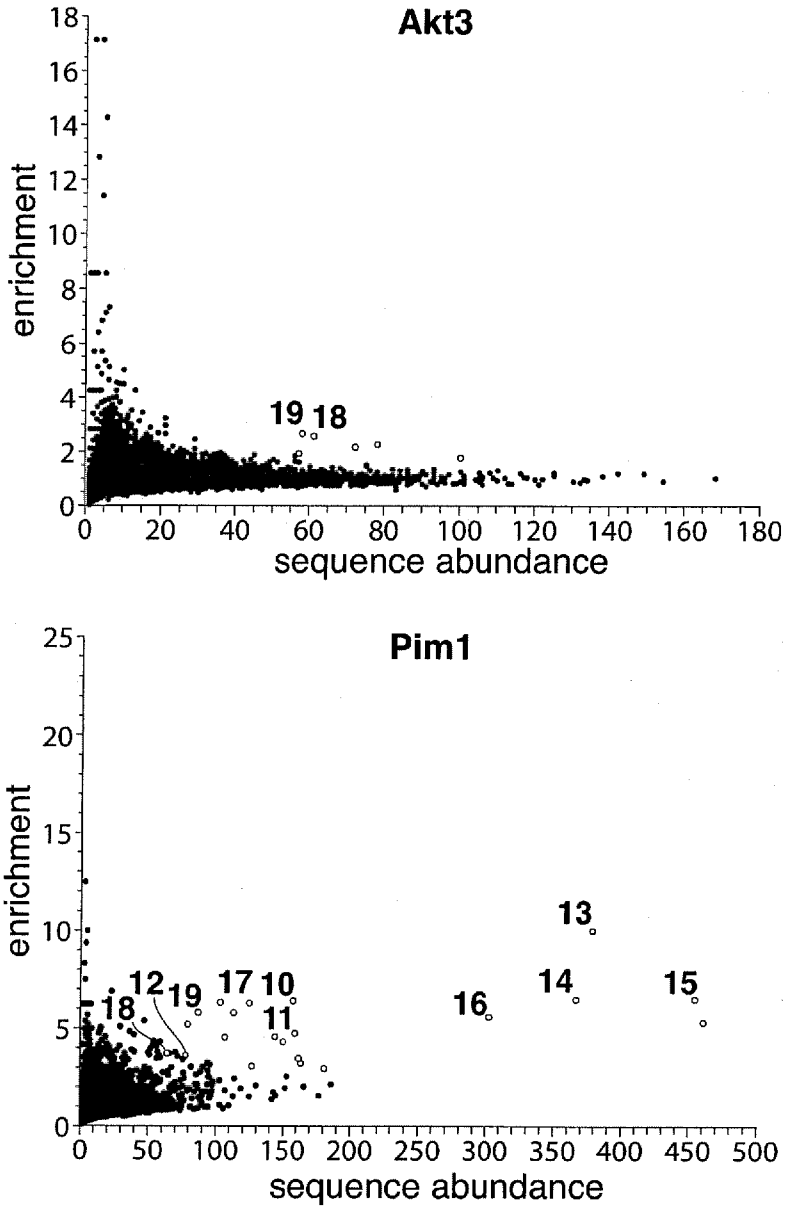

Akt3
enrichment
sequence abundance
19
18
Pim1
enrichment
sequence abundance
13
14
15
16
12
19
17
10
18
11

Fig. 6-1

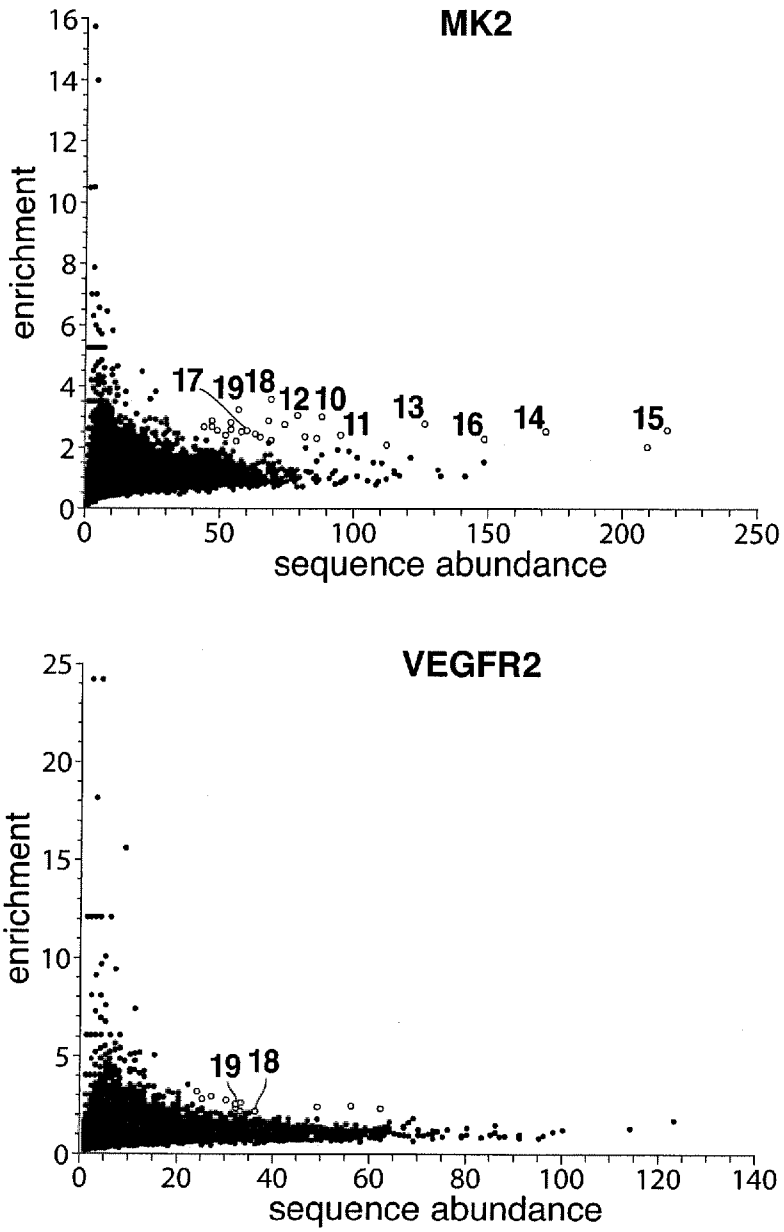
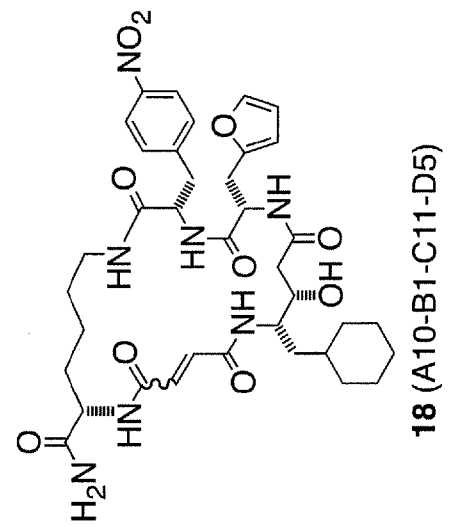
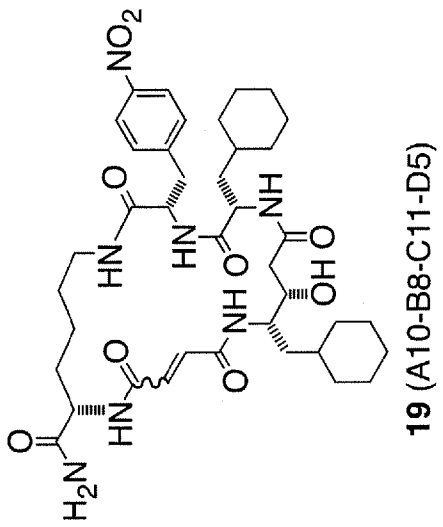

MK2
enrichment
sequence abundance
17
19
18
12
10
11
13
16
14
15
VEGFR2
enrichment
sequence abundance
19
18

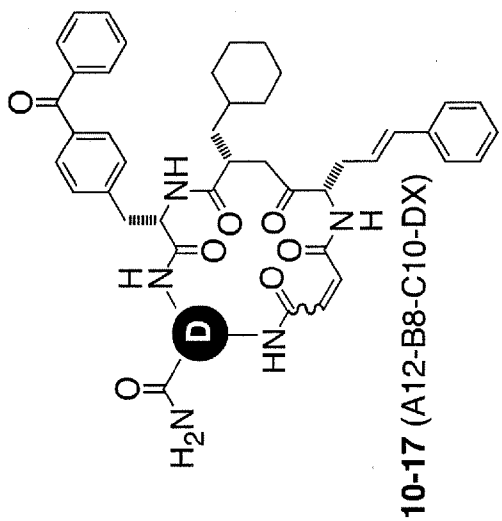
Fig. 6-2



18 (A10-B1-C11-D5)



19 (A10-B8-C11-D5)



10-17 (A12-B8-C10-DX)

D1-D4. "A" building block (red) attached to α amine

10 (D1 = lysine)
11 (D2 = ornithine)
12 (D3 = diaminobutyric acid)
13 (D4 = diaminopropionic acid)

D5-D8. "A" building block (red) attached to side-chain amine

14 (D5 = lysine)
15 (D6 = ornithine)
16 (D7 = diaminobutyric acid)
17 (D8 = diaminopropionic acid)

Fig. 6-3

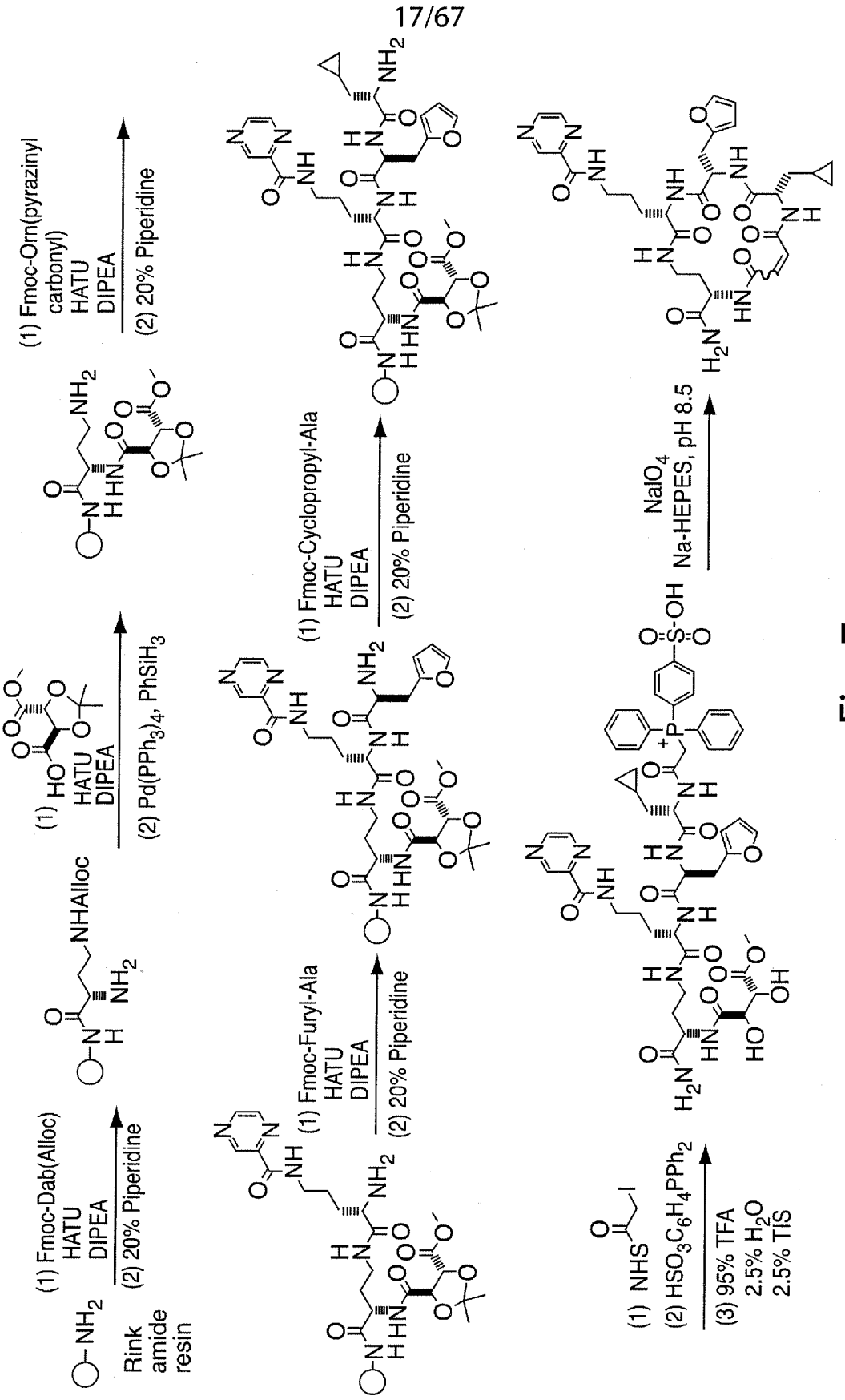

Rink amide resin
(1) Fmoc-Dab(Alloc) HATU DIPEA
(2) 20% Piperidine
(1) HO HATU DIPEA
(2) Pd(PPh3)4, PhSiH3
(1) Fmoc-Furyl-Ala HATU DIPEA
(2) 20% Piperidine
(1) Fmoc-Cyclopropyl-Ala HATU DIPEA
(2) 20% Piperidine
(1) Fmoc-Orn(pyrazinyl carbonyl) HATU DIPEA
(2) 20% Piperidine
(1) NHS
(2) HSO3C6H4PPh2
(3) 95% TFA 2.5% H2O 2.5% TIS
NaIO4 Na-HEPES, pH 8.5

Fig. 7

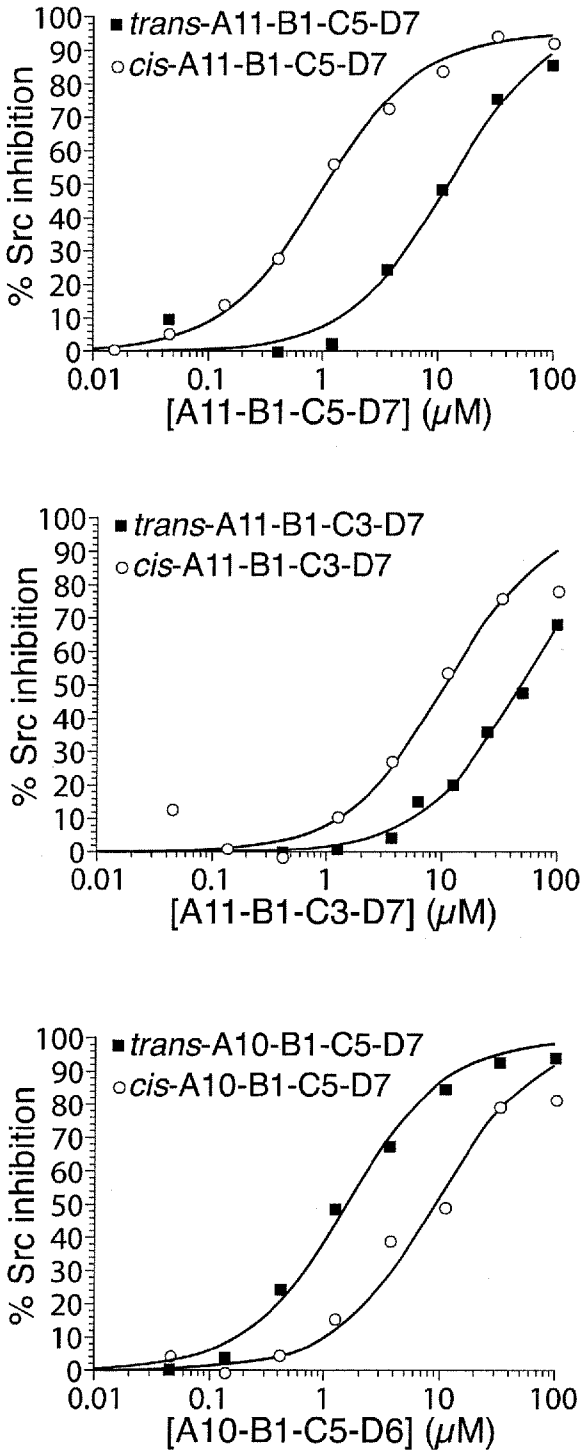

trans-A11-B1-C5-D7
cis-A11-B1-C5-D7
% Src inhibition
[A11-B1-C5-D7] (μM)
trans-A11-B1-C3-D7
cis-A11-B1-C3-D7
% Src inhibition
[A11-B1-C3-D7] (μM)
trans-A10-B1-C5-D7
cis-A10-B1-C5-D7
% Src inhibition
[A10-B1-C5-D6] (μM)

Fig. 8-1

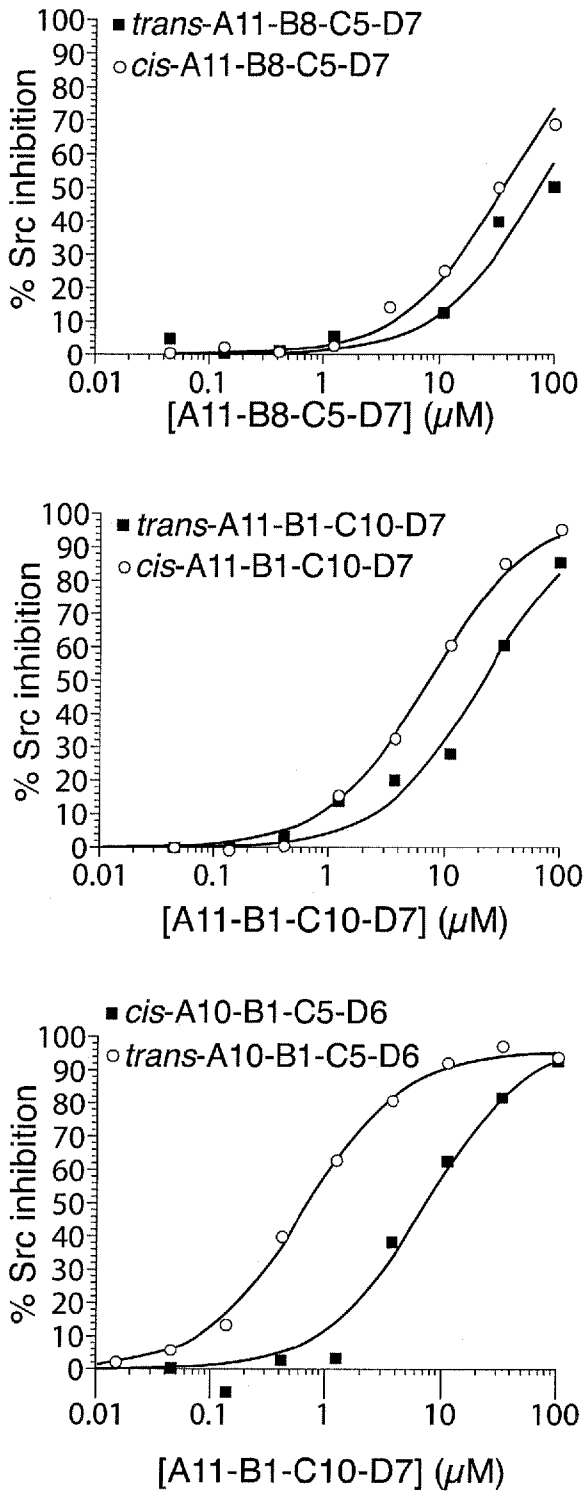

■ trans-A11-B8-C5-D7
○ cis-A11-B8-C5-D7
% Src inhibition
[A11-B8-C5-D7] (μM)
■ trans-A11-B1-C10-D7
○ cis-A11-B1-C10-D7
% Src inhibition
[A11-B1-C10-D7] (μM)
■ cis-A10-B1-C5-D6
○ trans-A10-B1-C5-D6
% Src inhibition
[A11-B1-C10-D7] (μM)

Fig. 8-2

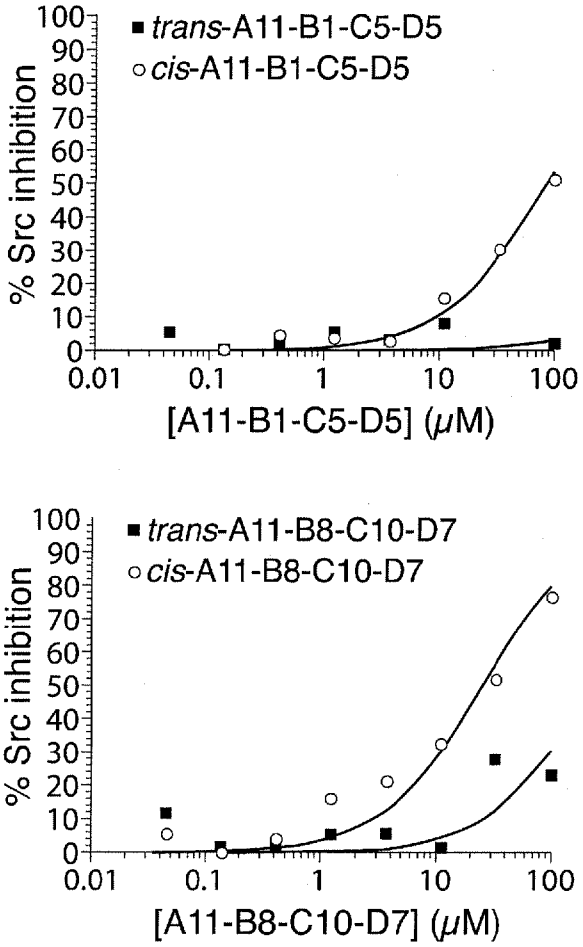

■ trans-A11-B1-C5-D5
○ cis-A11-B1-C5-D5
% Src inhibition
100
90
80
70
60
50
40
30
20
10
0
0.01
0.1
1
10
100
[A11-B1-C5-D5] (μM)
■ trans-A11-B8-C10-D7
○ cis-A11-B8-C10-D7
% Src inhibition
[A11-B8-C10-D7] (μM)

Fig. 8-3

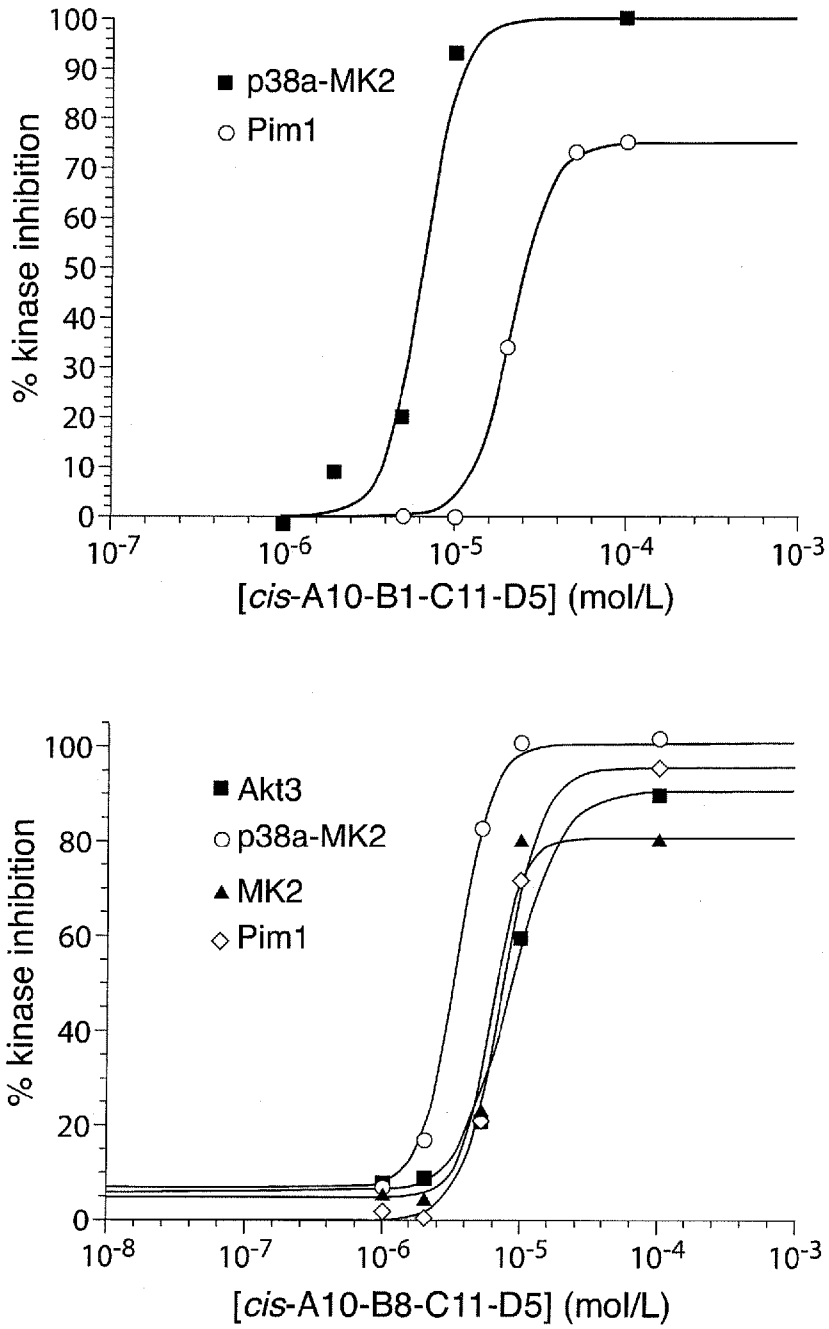

100
90
80
70
60
50
40
30
20
10
0
% kinase inhibition
■ p38a-MK2
○ Pim1
10-7
10-6
10-5
10-4
10-3
[cis-A10-B1-C11-D5] (mol/L)
100
80
60
40
20
0
% kinase inhibition
■ Akt3
○ p38a-MK2
▲ MK2
◇ Pim1
10-8
10-7
10-6
10-5
10-4
10-3
[cis-A10-B8-C11-D5] (mol/L)

Fig. 9

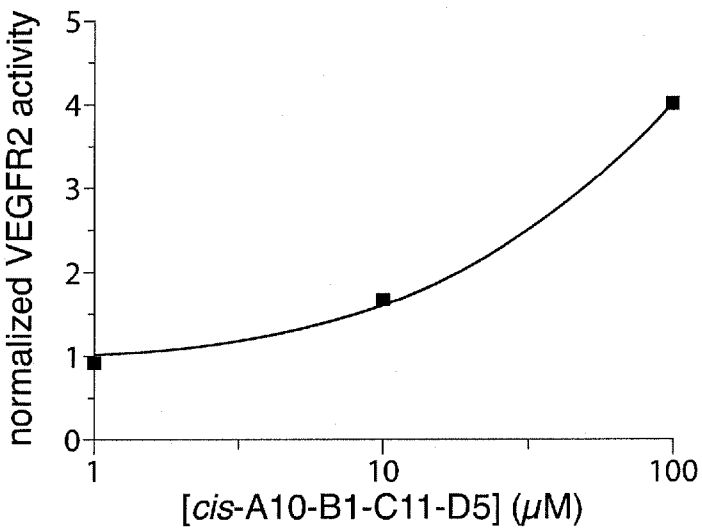

normalized VEGFR2 activity
0
1
2
3
4
5
1
10
100
[cis-A10-B1-C11-D5] (μM)

Fig. 10

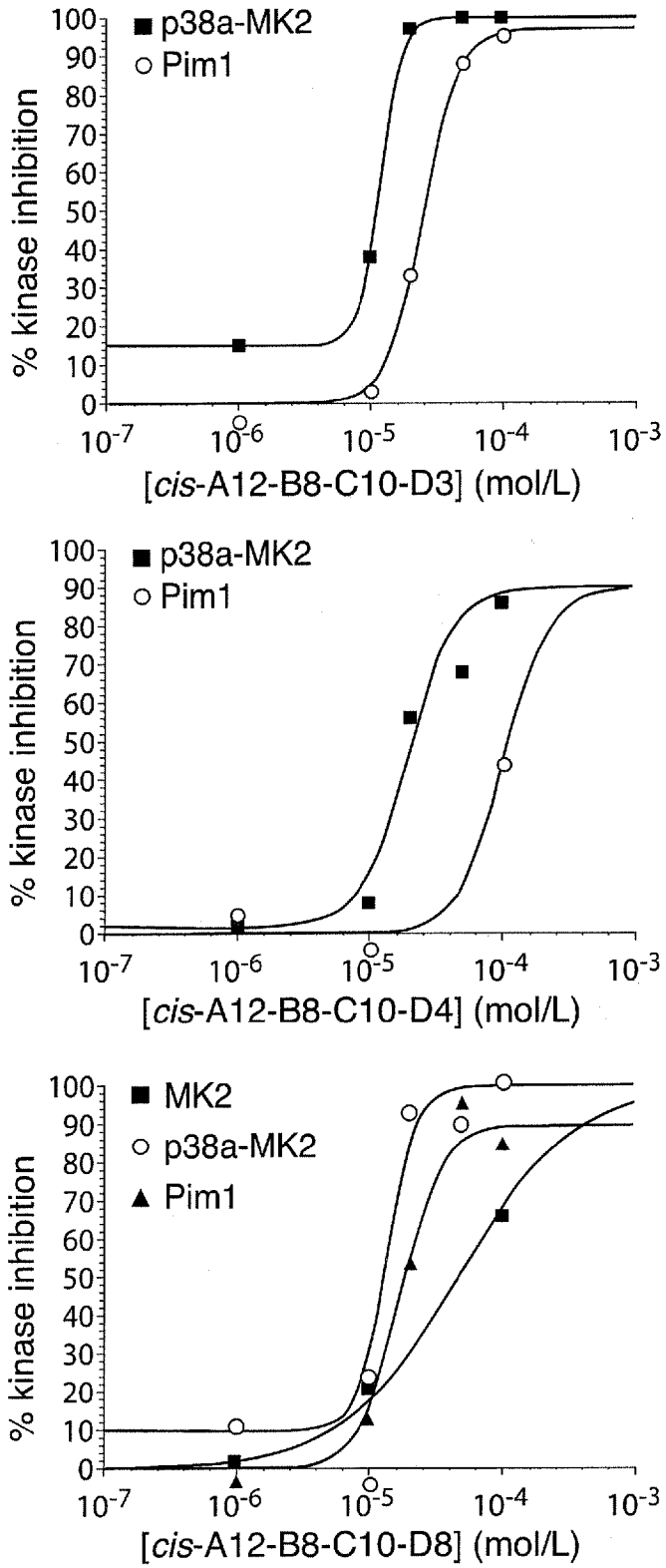

p38a-MK2
Pim1
% kinase inhibition
[cis-A12-B8-C10-D3] (mol/L)
p38a-MK2
Pim1
% kinase inhibition
[cis-A12-B8-C10-D4] (mol/L)
MK2
p38a-MK2
Pim1
% kinase inhibition
[cis-A12-B8-C10-D8] (mol/L)

Fig. 11

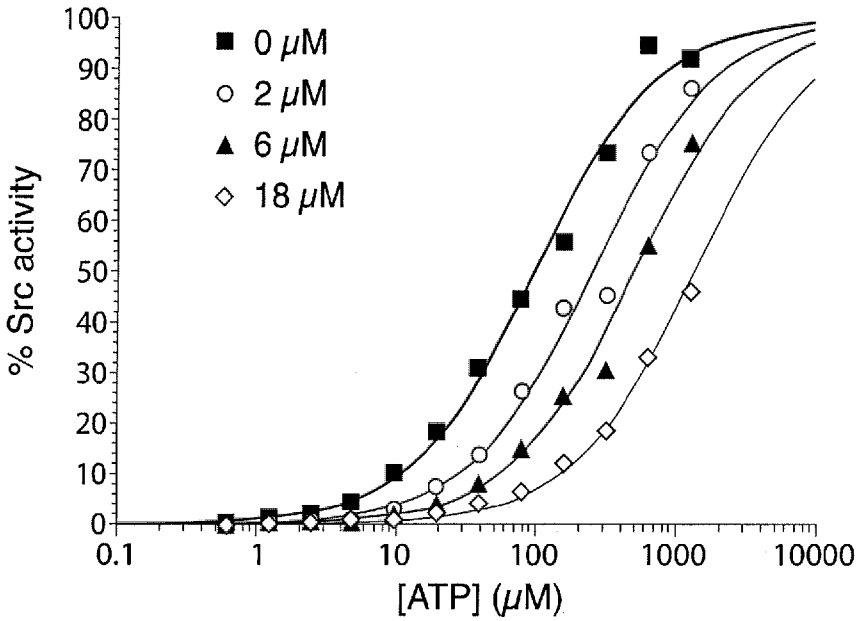


Fig. 12A

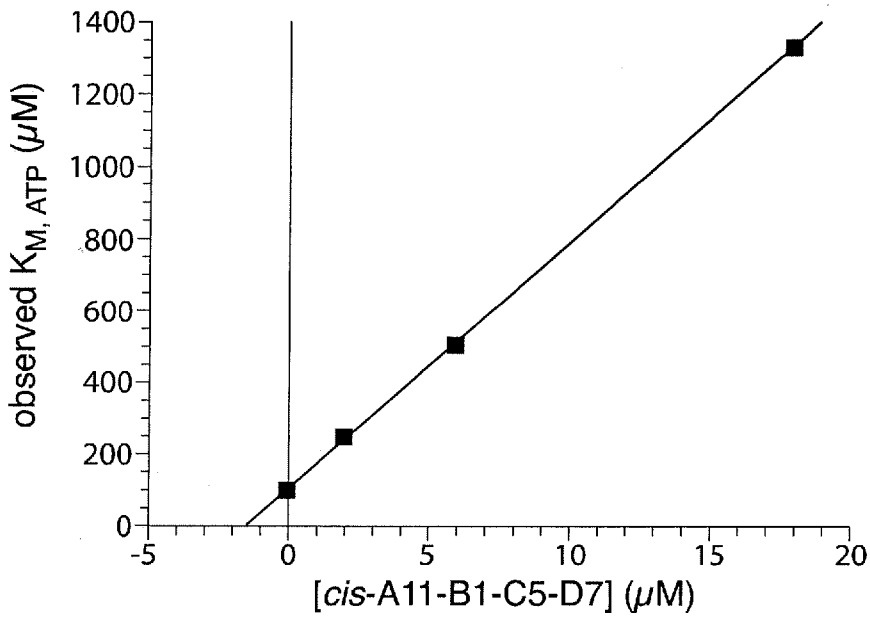


Fig. 12B

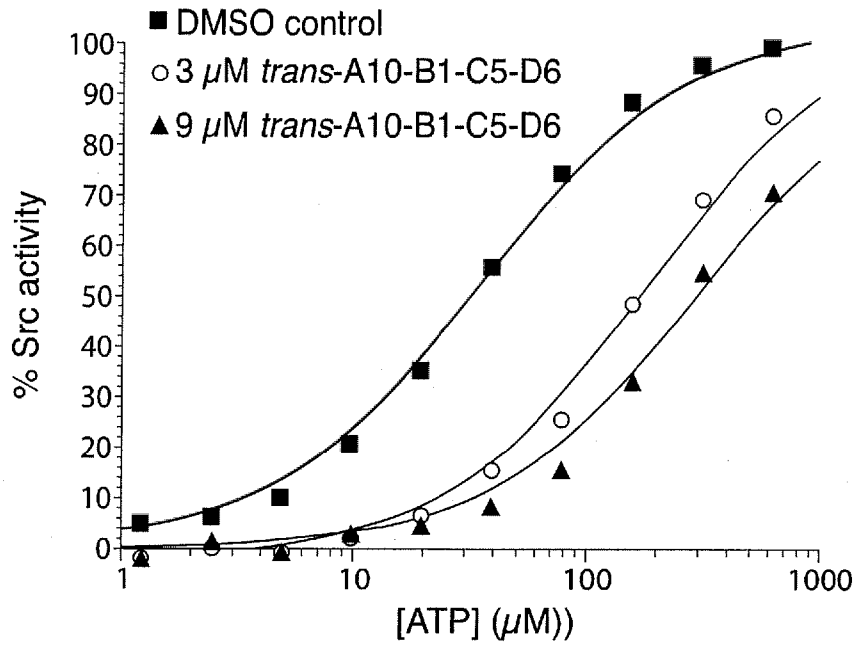
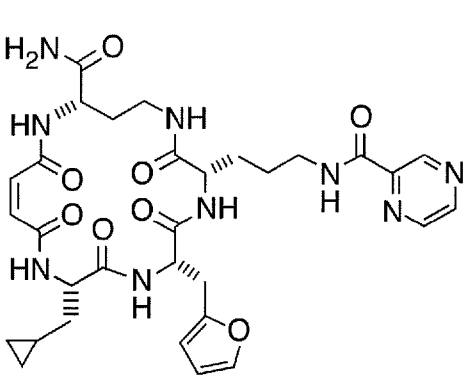
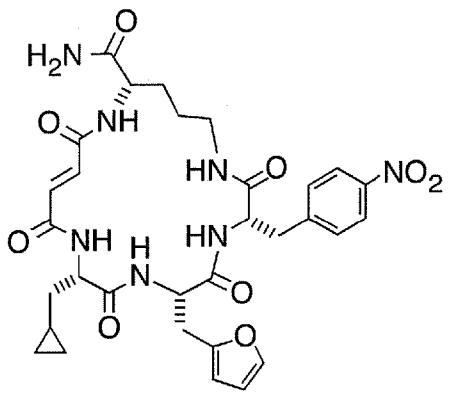

DMSO control
3 μM trans-A10-B1-C5-D6
9 μM trans-A10-B1-C5-D6
% Src activity
100
90
80
70
60
50
40
30
20
10
0
1
10
100
1000
[ATP] (μM))

Fig. 13



cis-**A11-B1-C5-D7**
Src IC_{50} = 960 nM



trans-**A10-B1-C5-D6**
Src IC_{50} = 680 nM

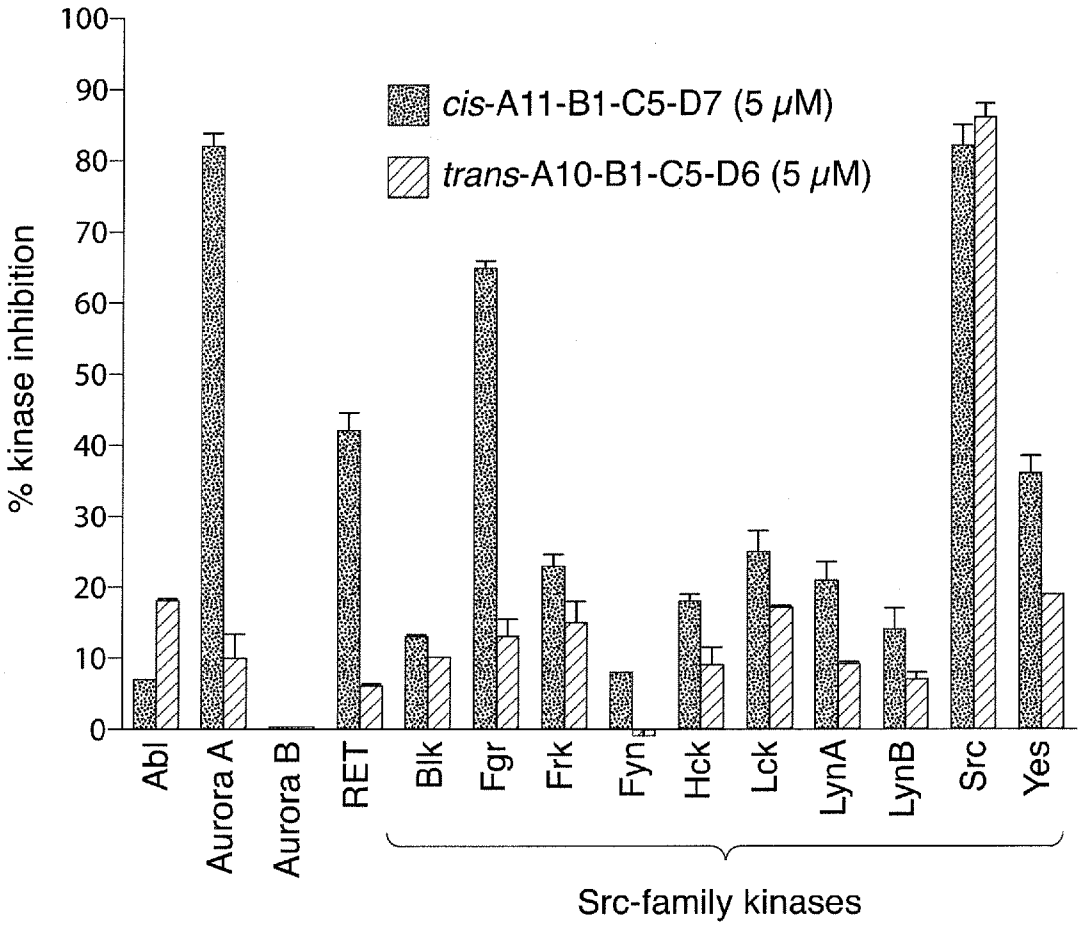


Fig. 14

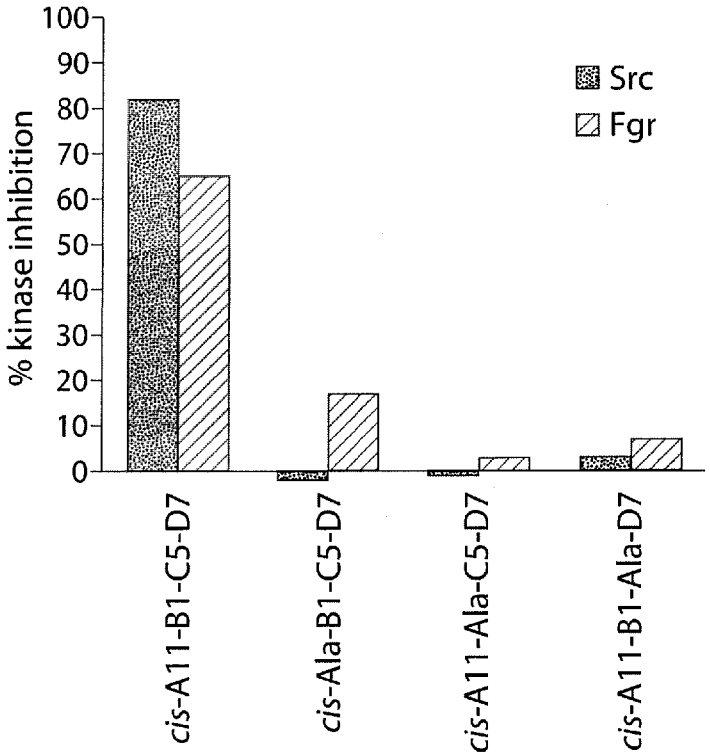
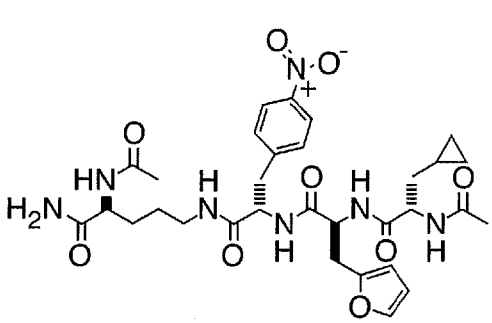
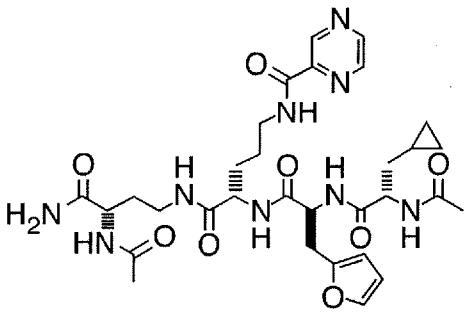


Fig. 15A



linear A10-B1-C5-D6
IC_{50} > 100 μM



linear A11-B1-C5-D7
IC_{50} > 100 μM

Fig. 15B

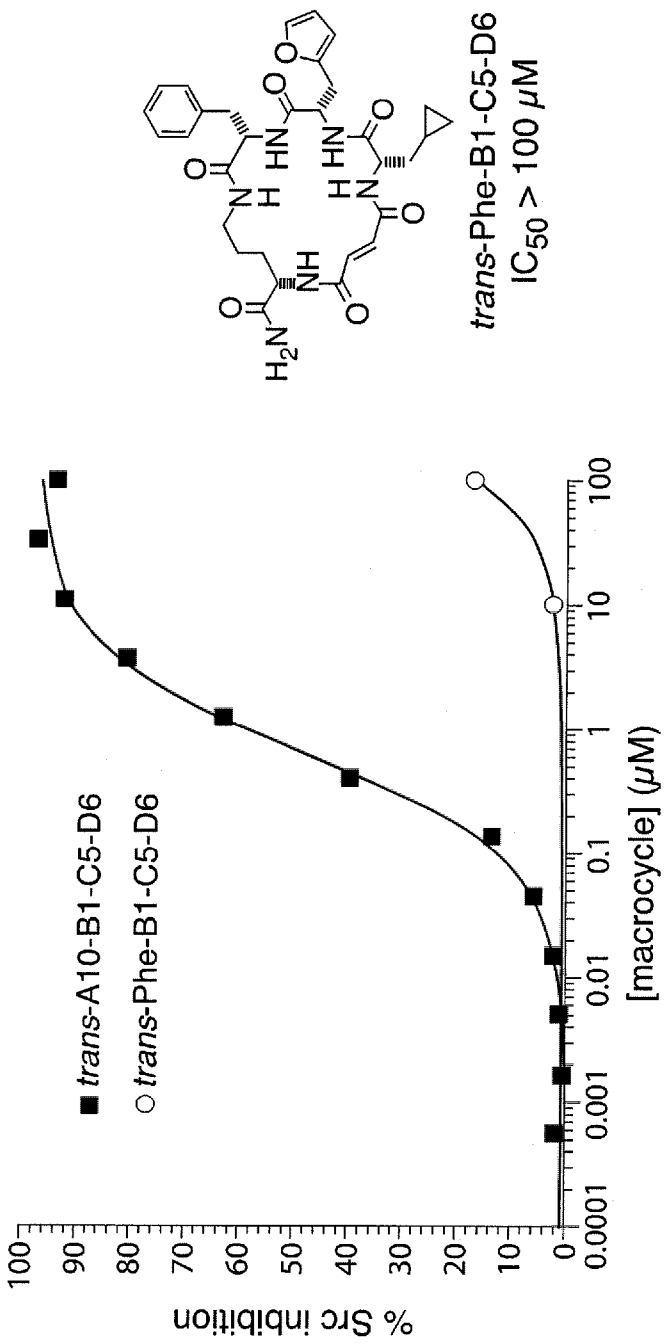

trans-Phe-B1-C5-D6
IC50 > 100 μM
% Src inhibition
[macrocycle] (μM)
trans-A10-B1-C5-D6
trans-Phe-B1-C5-D6

Fig. 15C

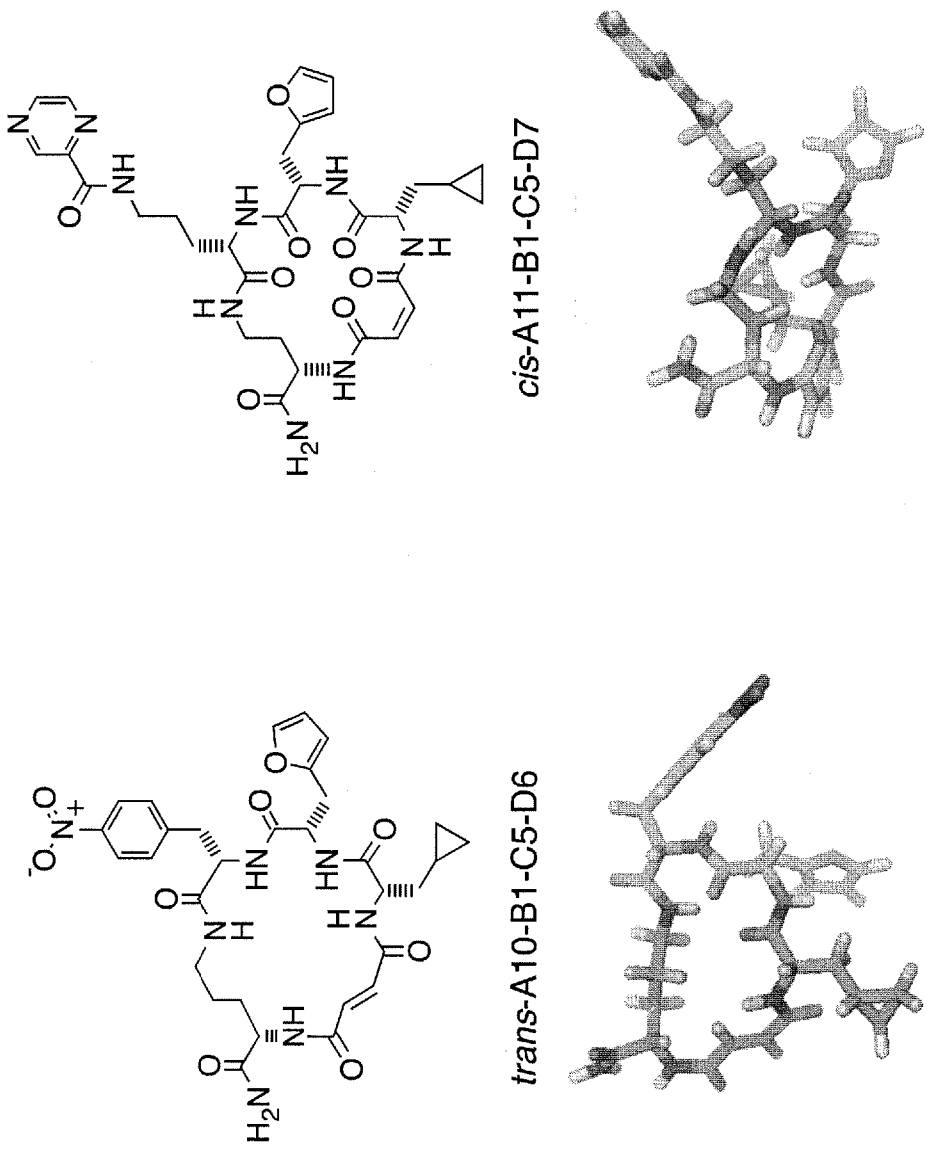


Fig. 16

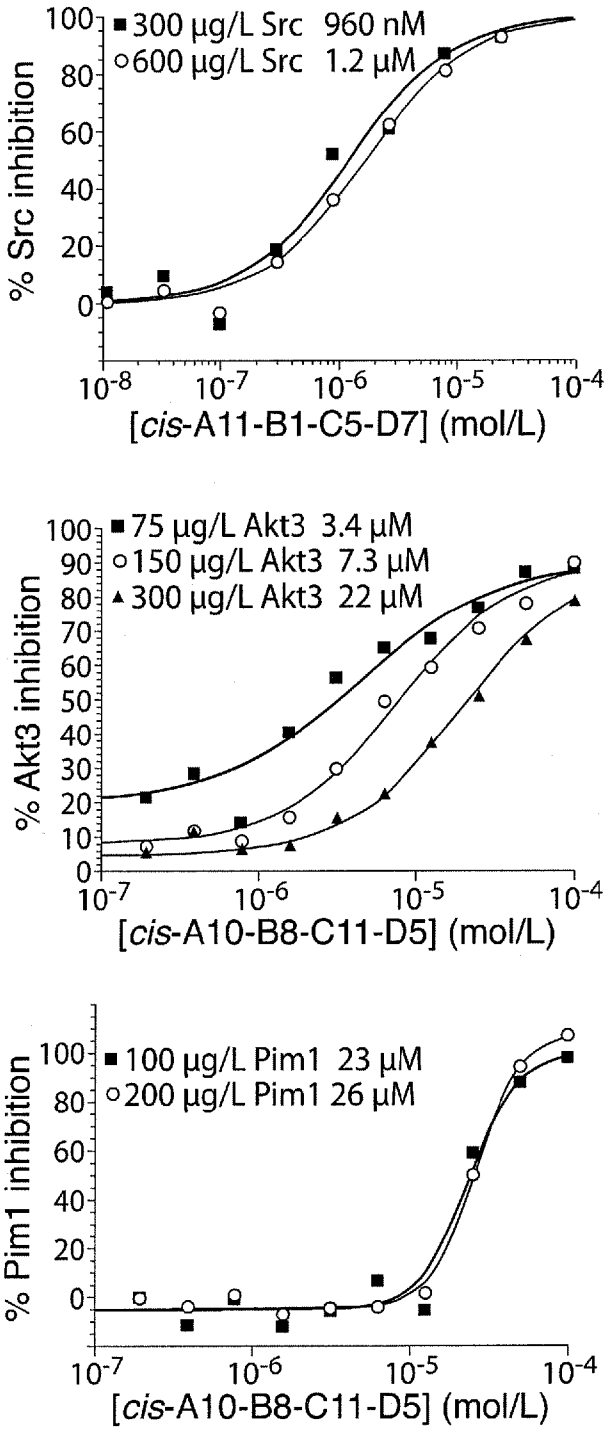

■ 300 µg/L Src 960 nM
○ 600 µg/L Src 1.2 µM
% Src inhibition
[cis-A11-B1-C5-D7] (mol/L)
■ 75 µg/L Akt3 3.4 µM
○ 150 µg/L Akt3 7.3 µM
▲ 300 µg/L Akt3 22 µM
% Akt3 inhibition
[cis-A10-B8-C11-D5] (mol/L)
■ 100 µg/L Pim1 23 µM
○ 200 µg/L Pim1 26 µM
% Pim1 inhibition
[cis-A10-B8-C11-D5] (mol/L)

Fig. 17-1

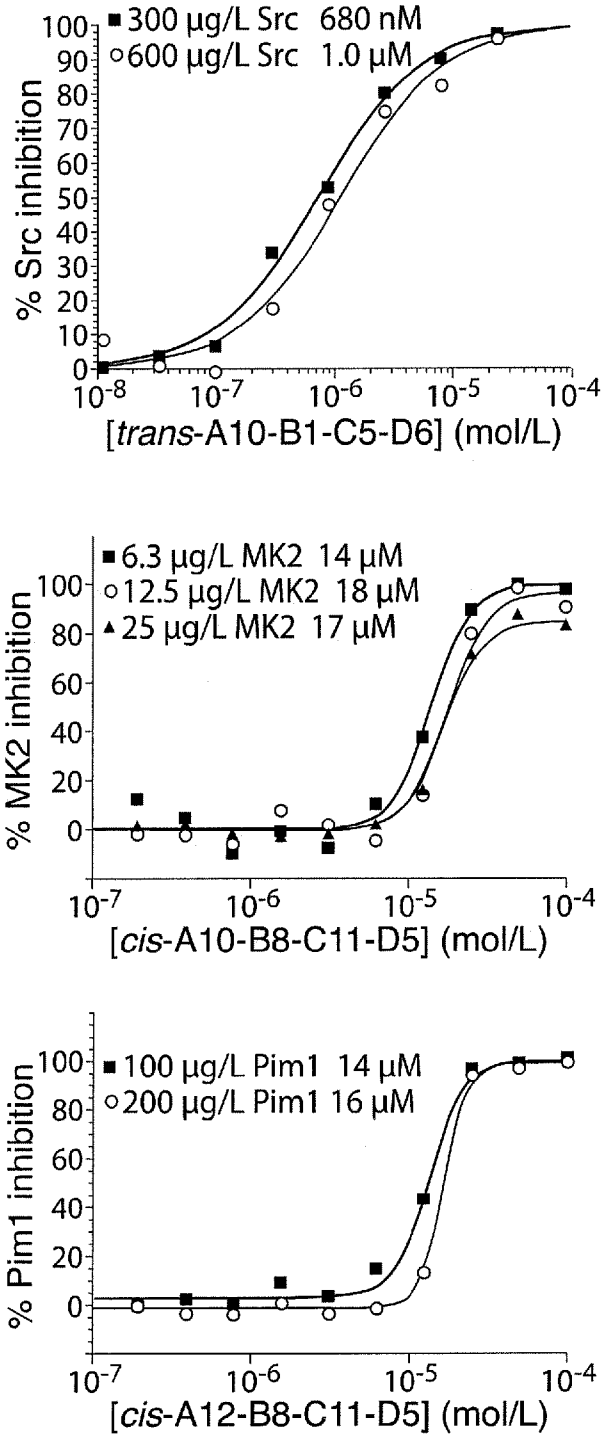

■ 300 µg/L Src 680 nM
○ 600 µg/L Src 1.0 µM
% Src inhibition
[trans-A10-B1-C5-D6] (mol/L)
■ 6.3 µg/L MK2 14 µM
○ 12.5 µg/L MK2 18 µM
▲ 25 µg/L MK2 17 µM
% MK2 inhibition
[cis-A10-B8-C11-D5] (mol/L)
■ 100 µg/L Pim1 14 µM
○ 200 µg/L Pim1 16 µM
% Pim1 inhibition
[cis-A12-B8-C11-D5] (mol/L)

Fig. 17-2

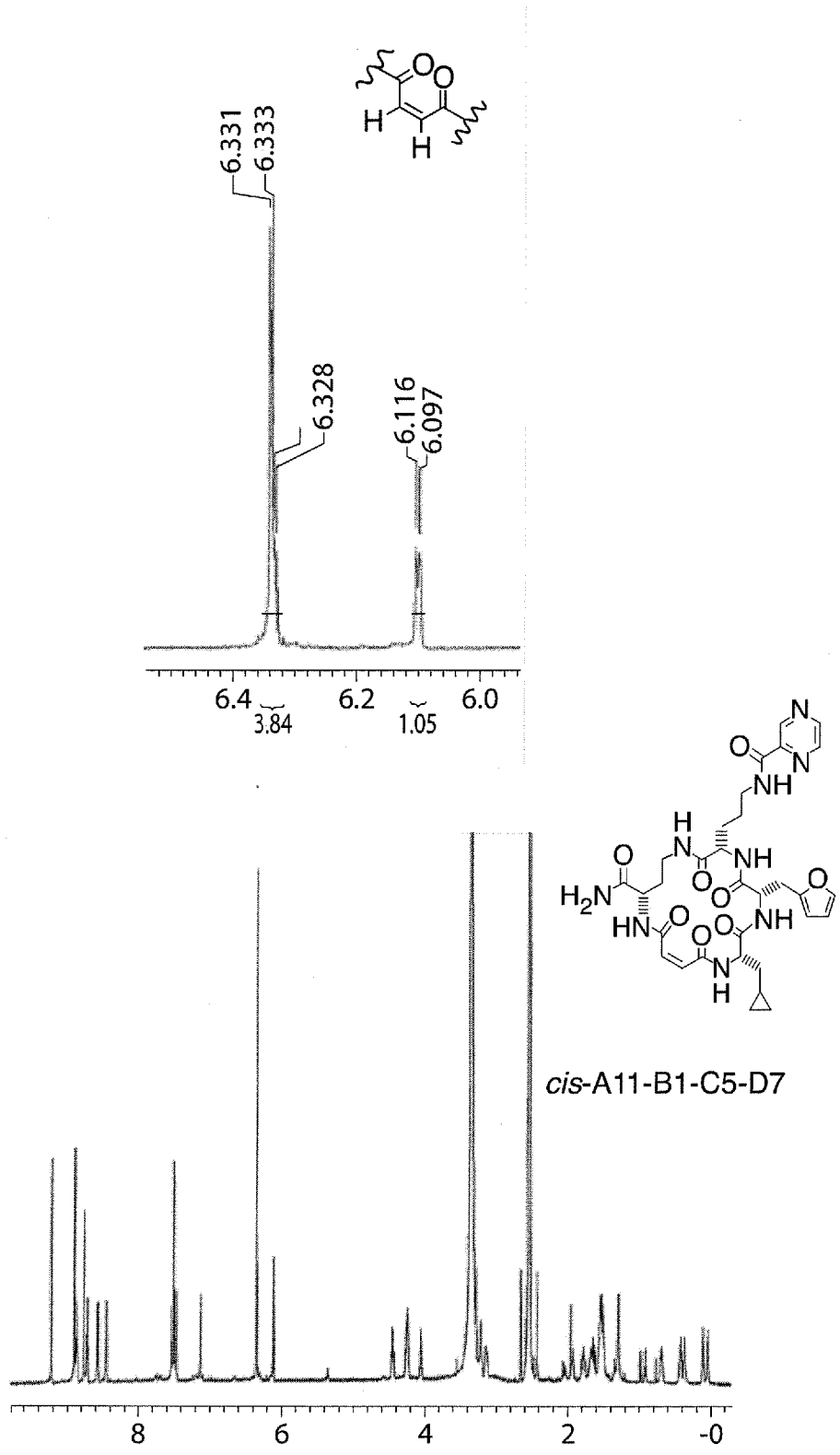


Fig. 18-1

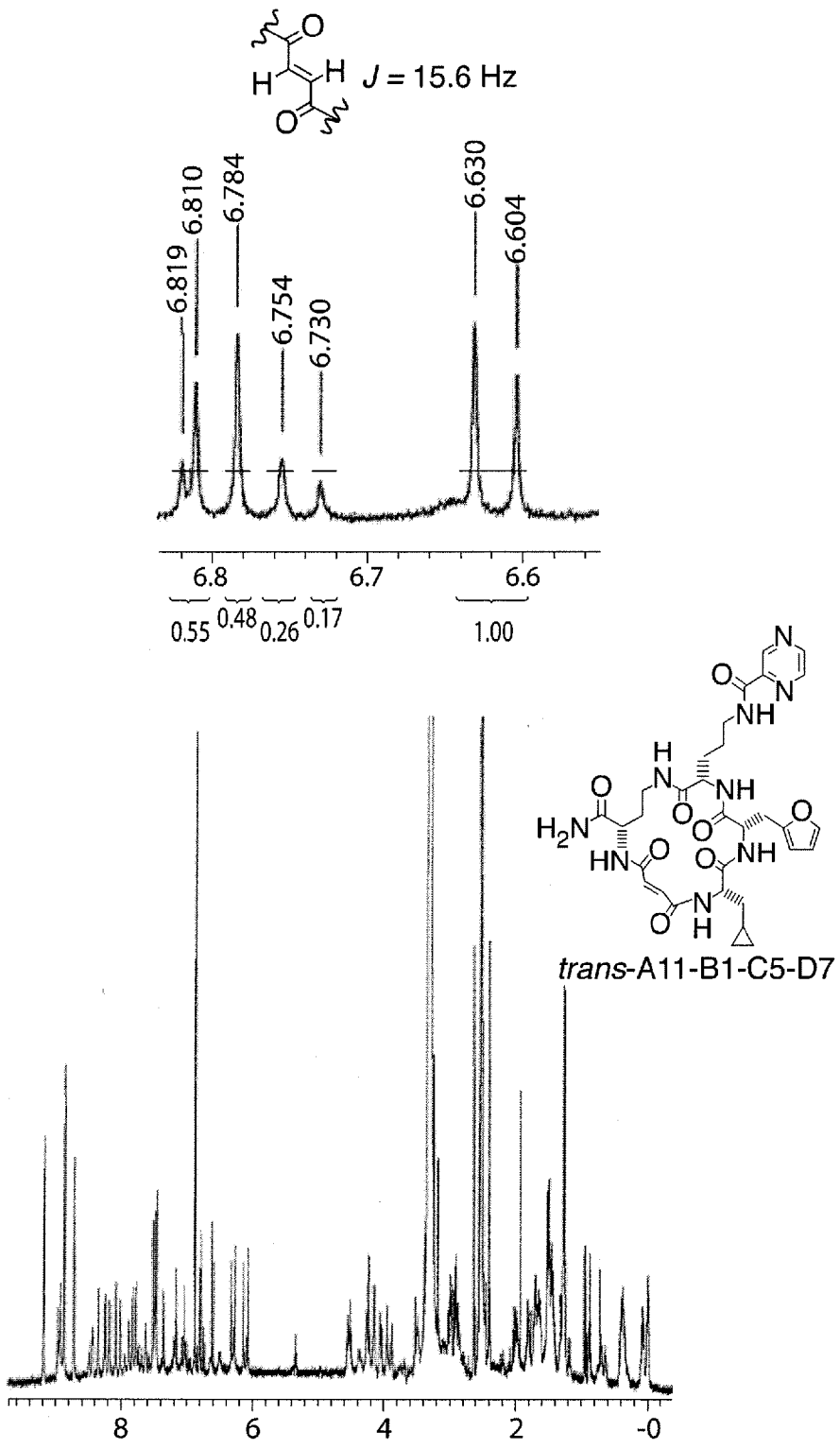

J = 15.6 Hz
6.819
6.810
6.784
6.754
6.730
6.630
6.604
6.8
6.7
6.6
0.55
0.48
0.26
0.17
1.00
trans-A11-B1-C5-D7
8
6
4
2
-0

Fig. 18-2

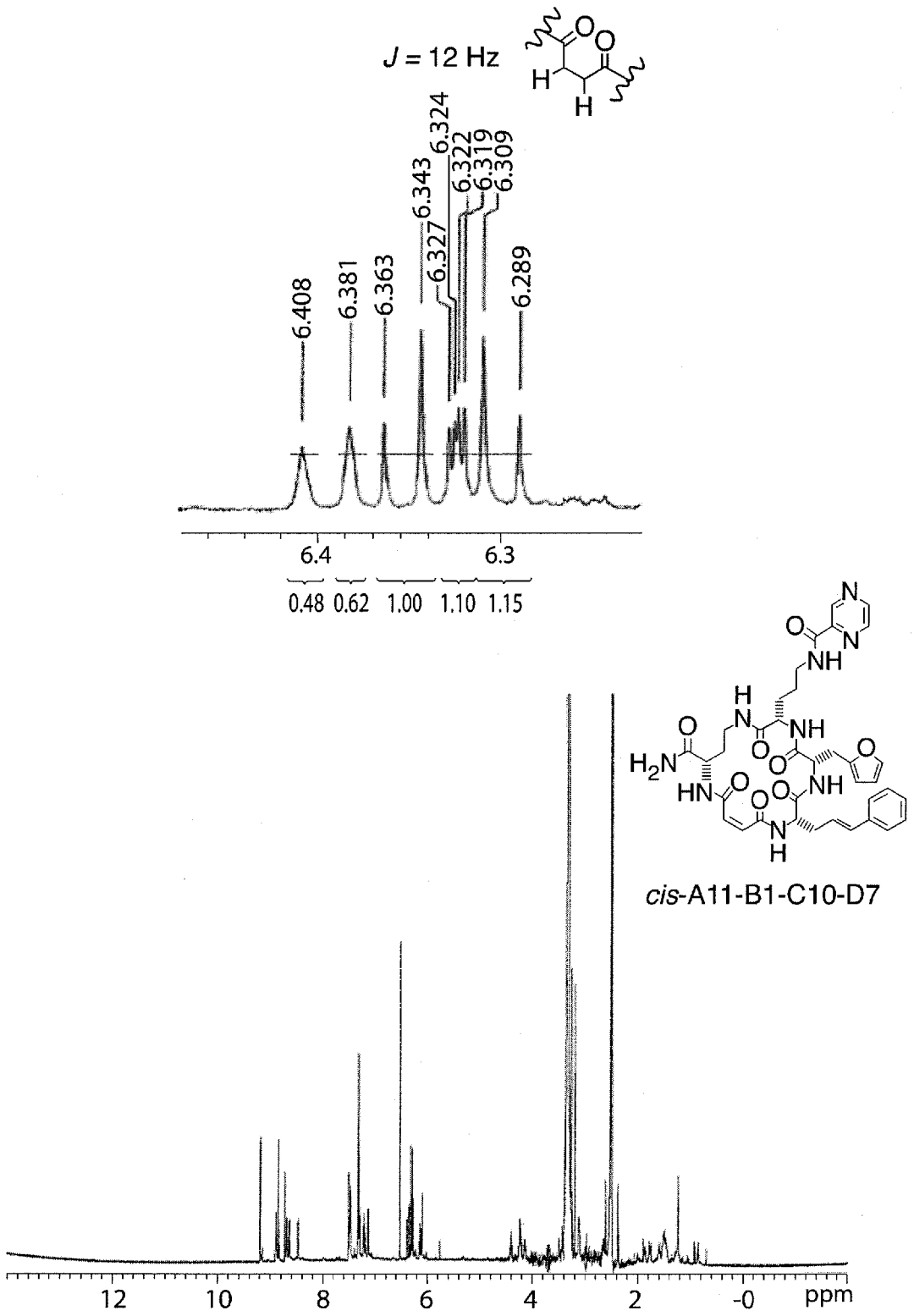

J = 12 Hz
6.408
6.381
6.363
6.343
6.327
6.324
6.322
6.319
6.309
6.289
6.4
6.3
0.48 0.62 1.00 1.10 1.15
cis-A11-B1-C10-D7
12
10
8
6
4
2
-0
ppm

Fig. 18-3

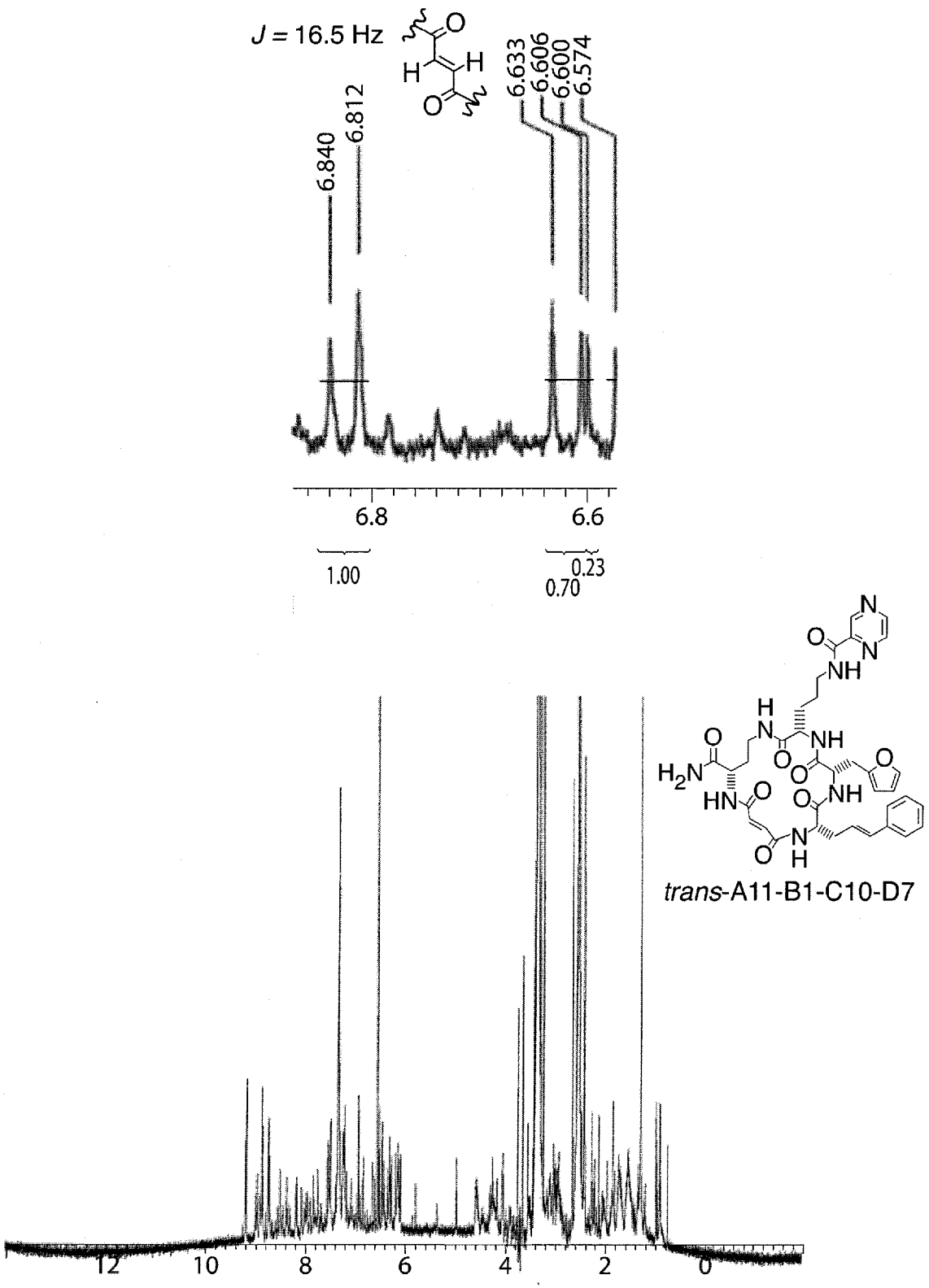

J = 16.5 Hz
6.840
6.812
6.633
6.606
6.600
6.574
6.8
6.6
1.00
0.70
0.23
trans-A11-B1-C10-D7
12
10
8
6
4
2
0

Fig. 18-4

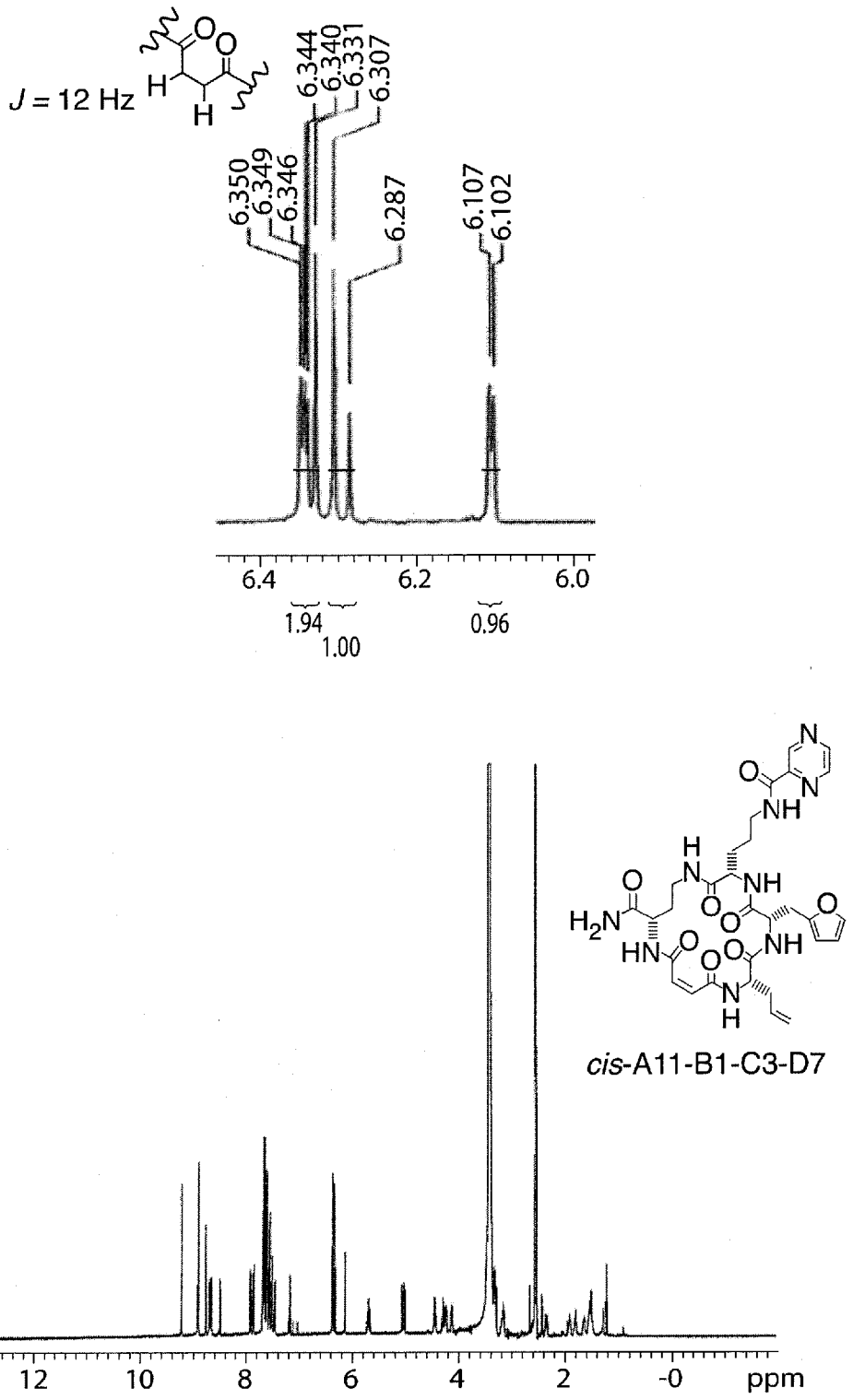

J = 12 Hz
6.344
6.340
6.331
6.307
6.350
6.349
6.346
6.287
6.107
6.102
6.4
6.2
6.0
1.94
1.00
0.96
cis-A11-B1-C3-D7
12
10
8
6
4
2
-0
ppm

Fig. 18-5

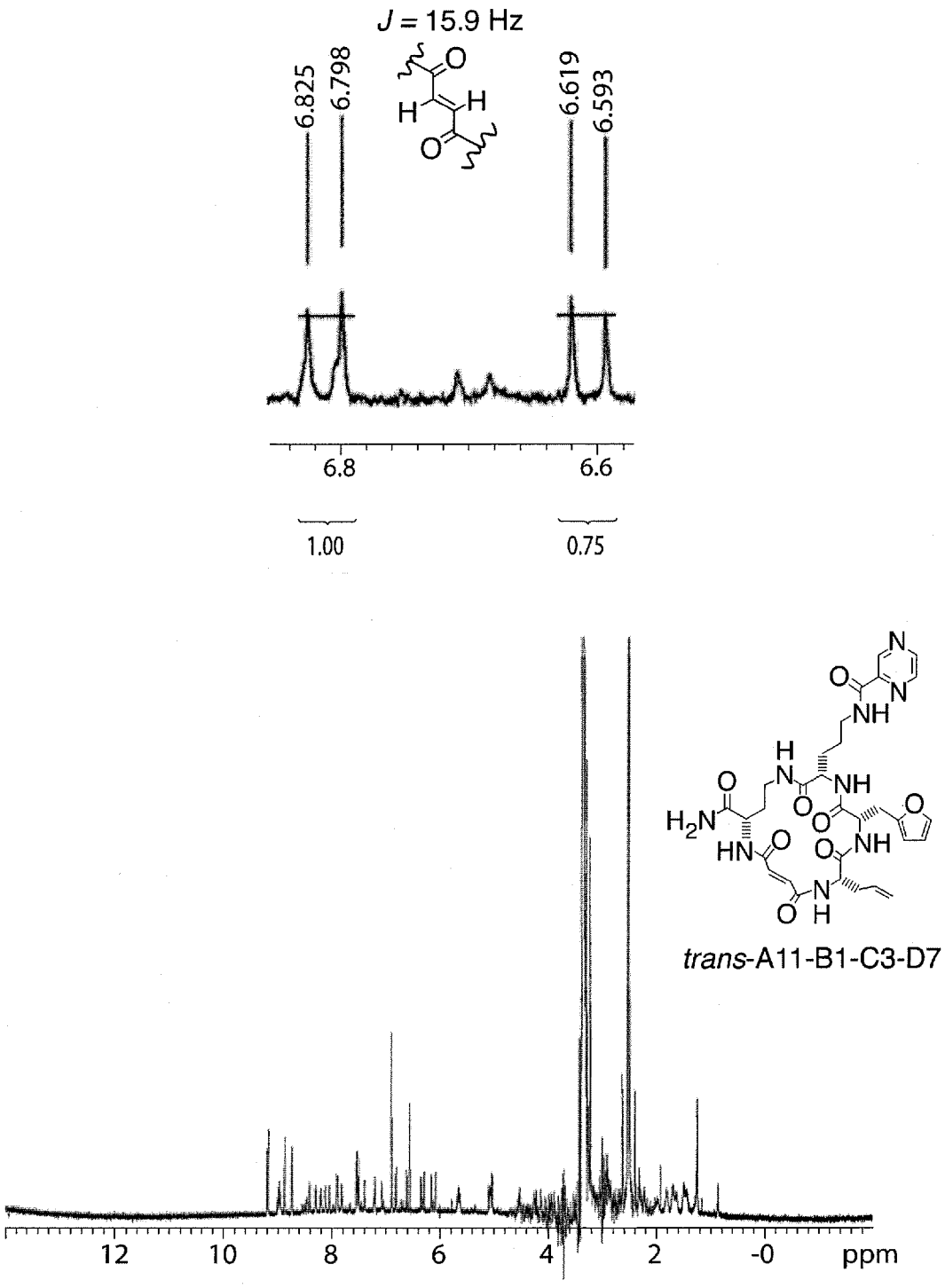

J = 15.9 Hz
6.825
6.798
6.619
6.593
6.8
6.6
1.00
0.75
trans-A11-B1-C3-D7
12
10
8
6
4
2
-0
ppm

Fig. 18-6

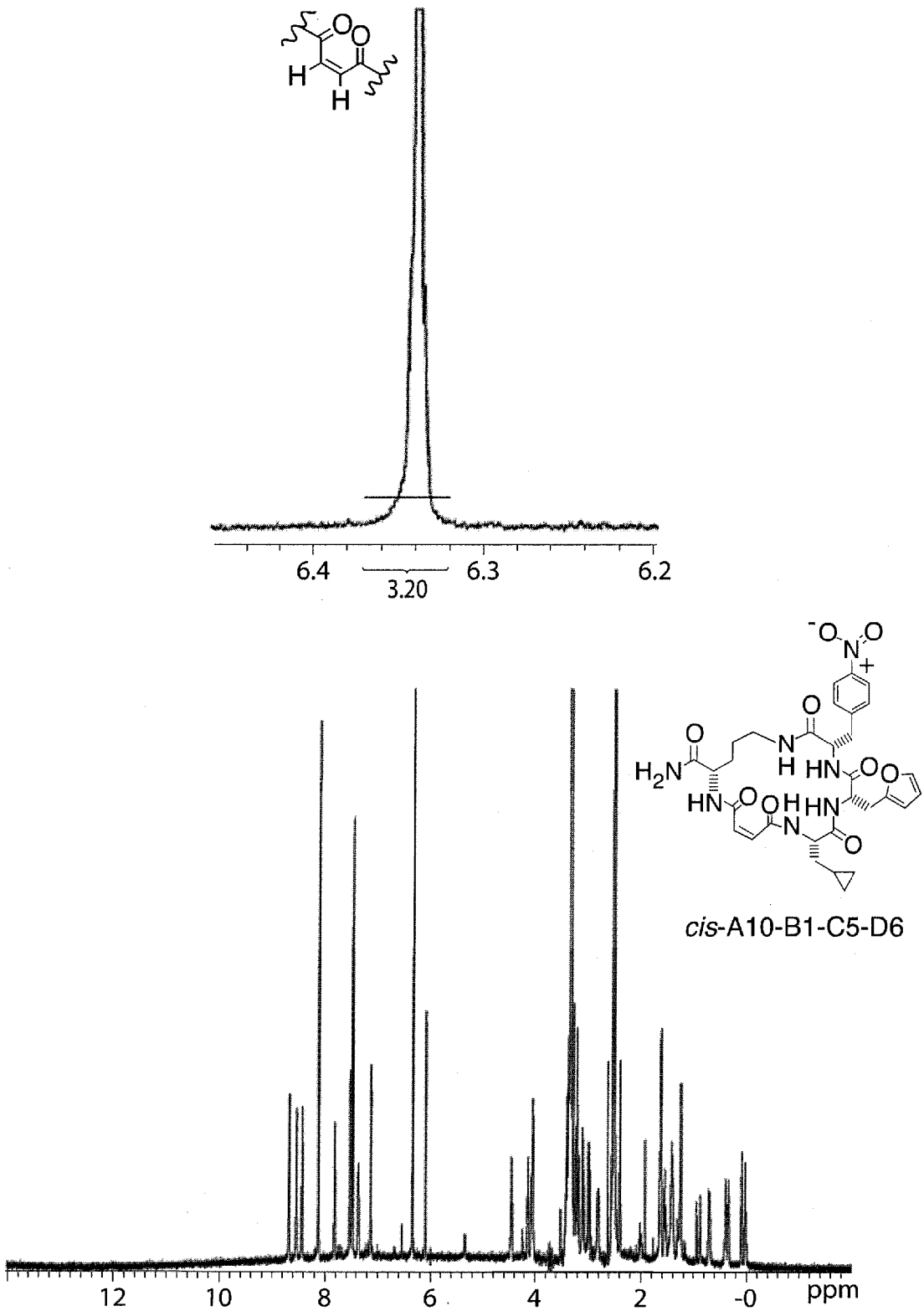


Fig. 18-7

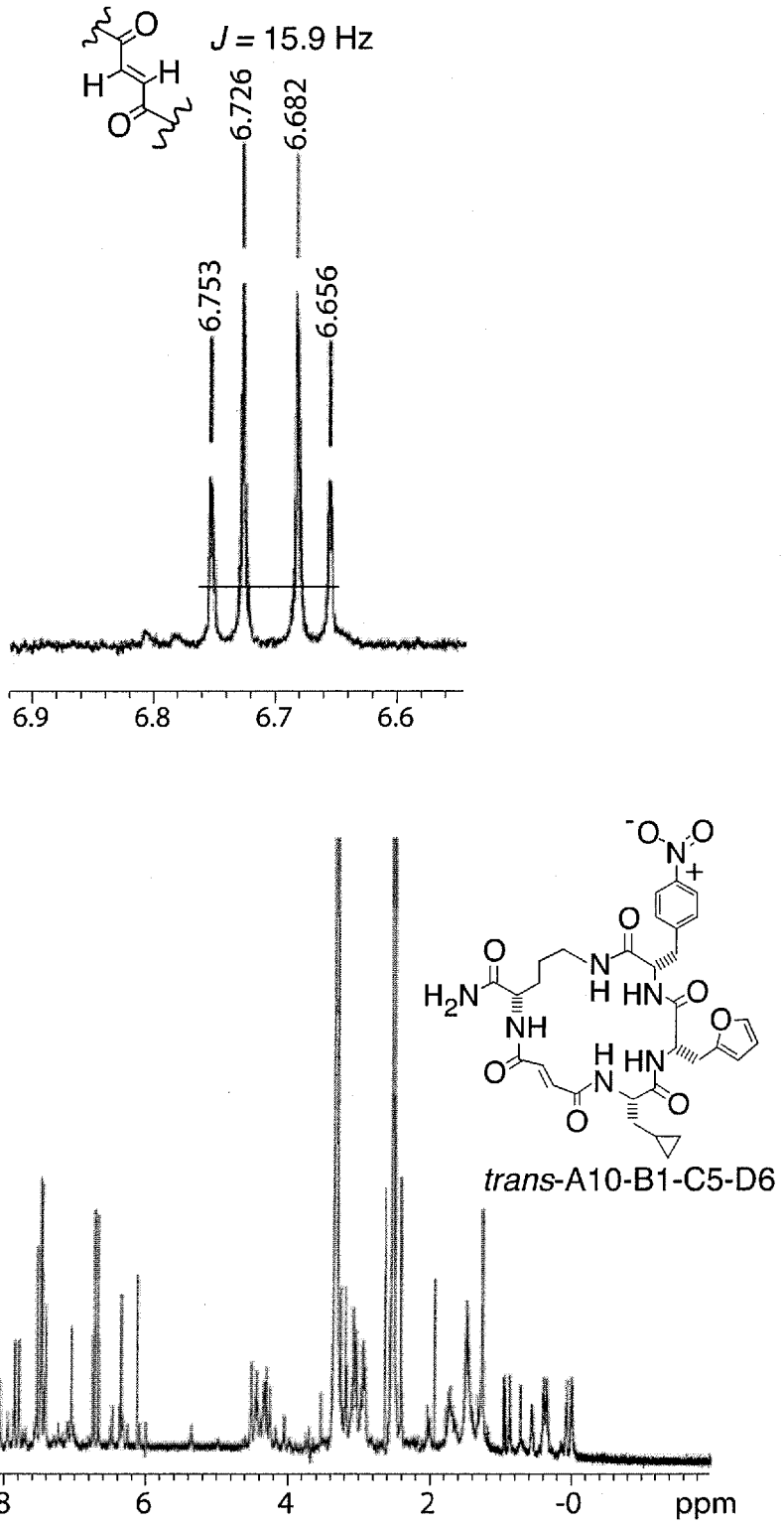

J = 15.9 Hz
6.726
6.682
6.753
6.656
6.9
6.8
6.7
6.6
trans-A10-B1-C5-D6
8
6
4
2
-0
ppm

Fig. 18-8

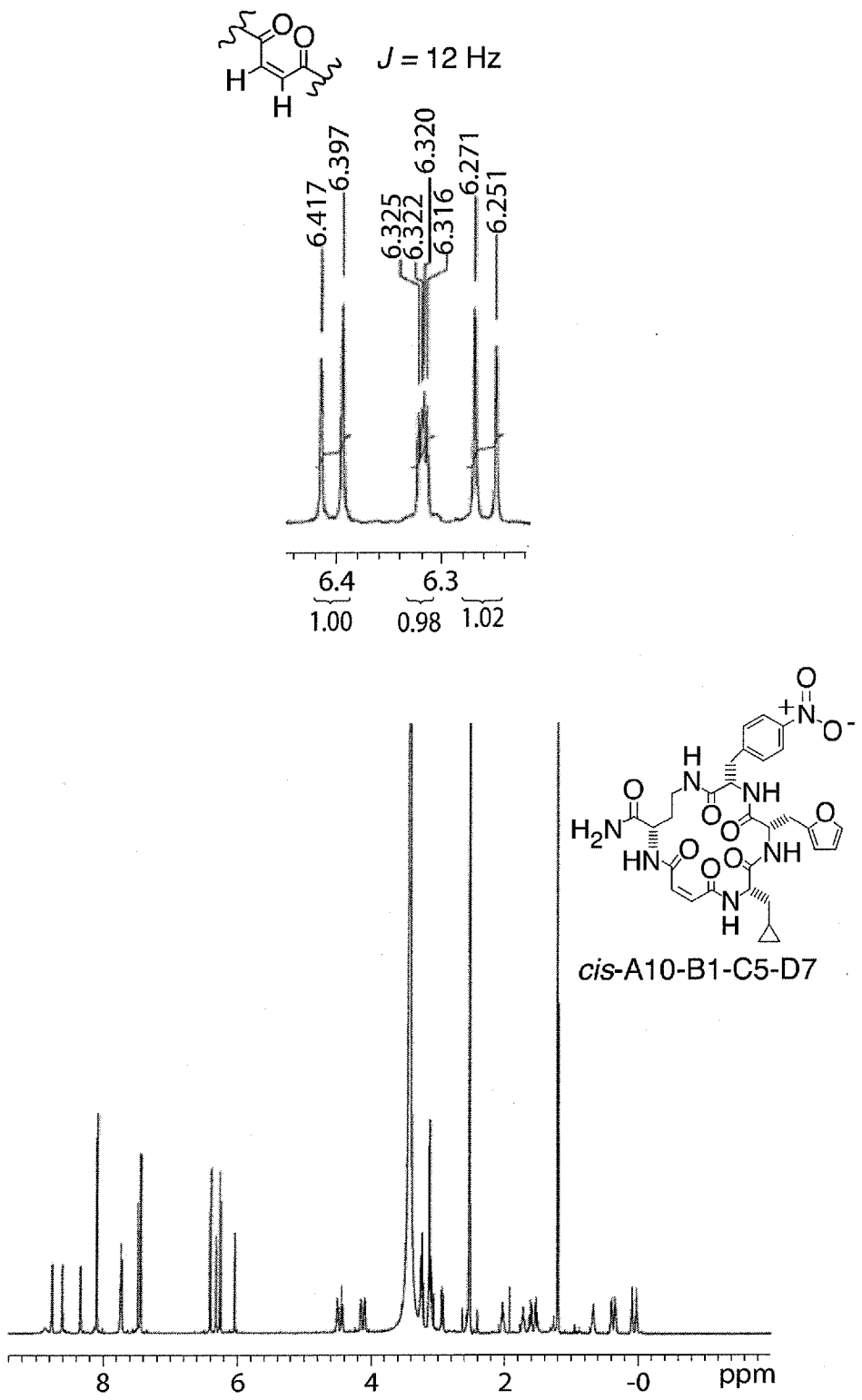

J = 12 Hz
6.417
6.397
6.325
6.322
6.320
6.316
6.271
6.251
6.4
6.3
1.00
0.98
1.02
cis-A10-B1-C5-D7
8
6
4
2
-0
ppm

Fig. 18-9

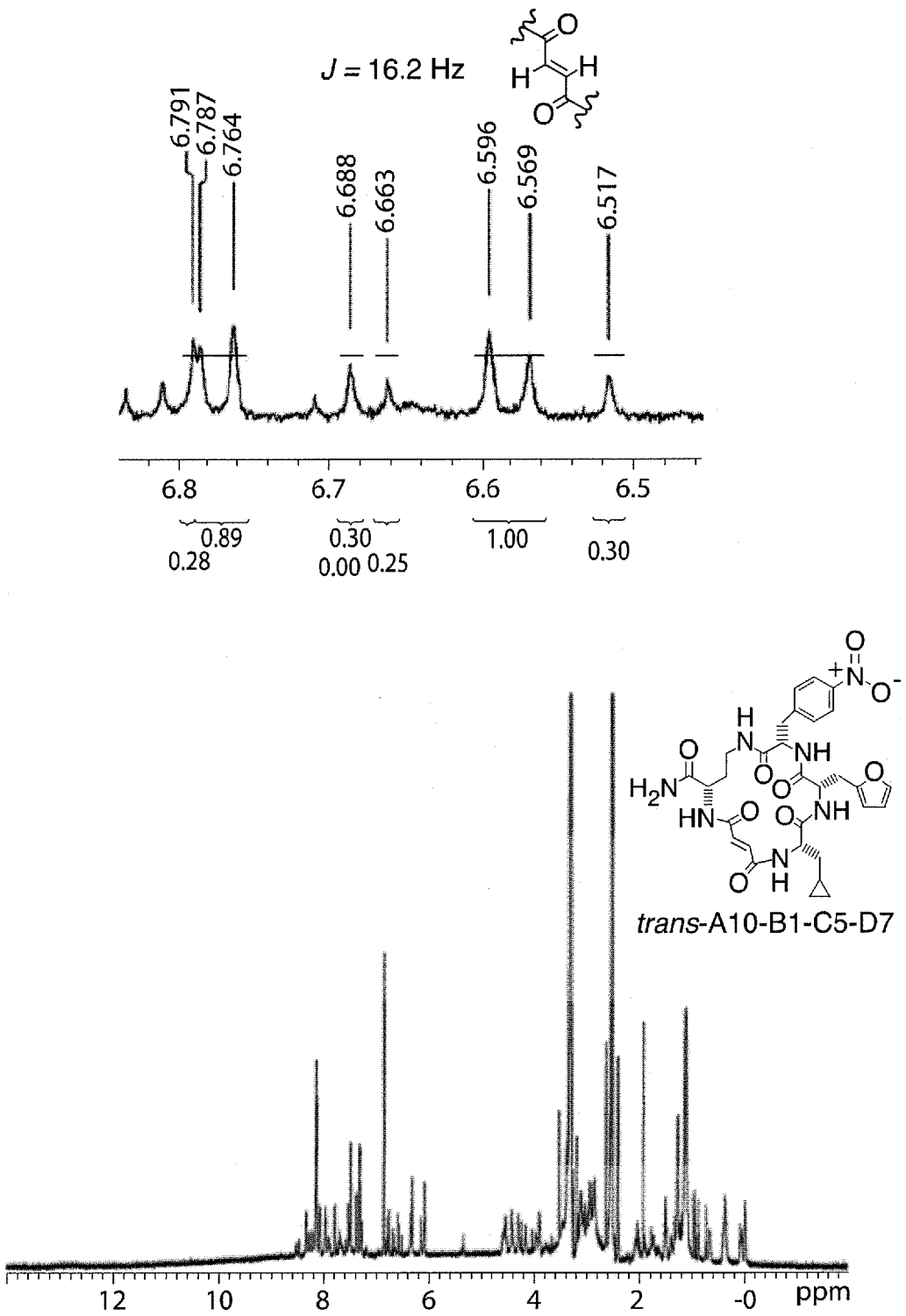

J = 16.2 Hz
6.791
6.787
6.764
6.688
6.663
6.596
6.569
6.517
6.8
6.7
6.6
6.5
0.89
0.28
0.30
0.00
0.25
1.00
0.30
trans-A10-B1-C5-D7
12
10
8
6
4
2
-0
ppm

Fig. 18-10

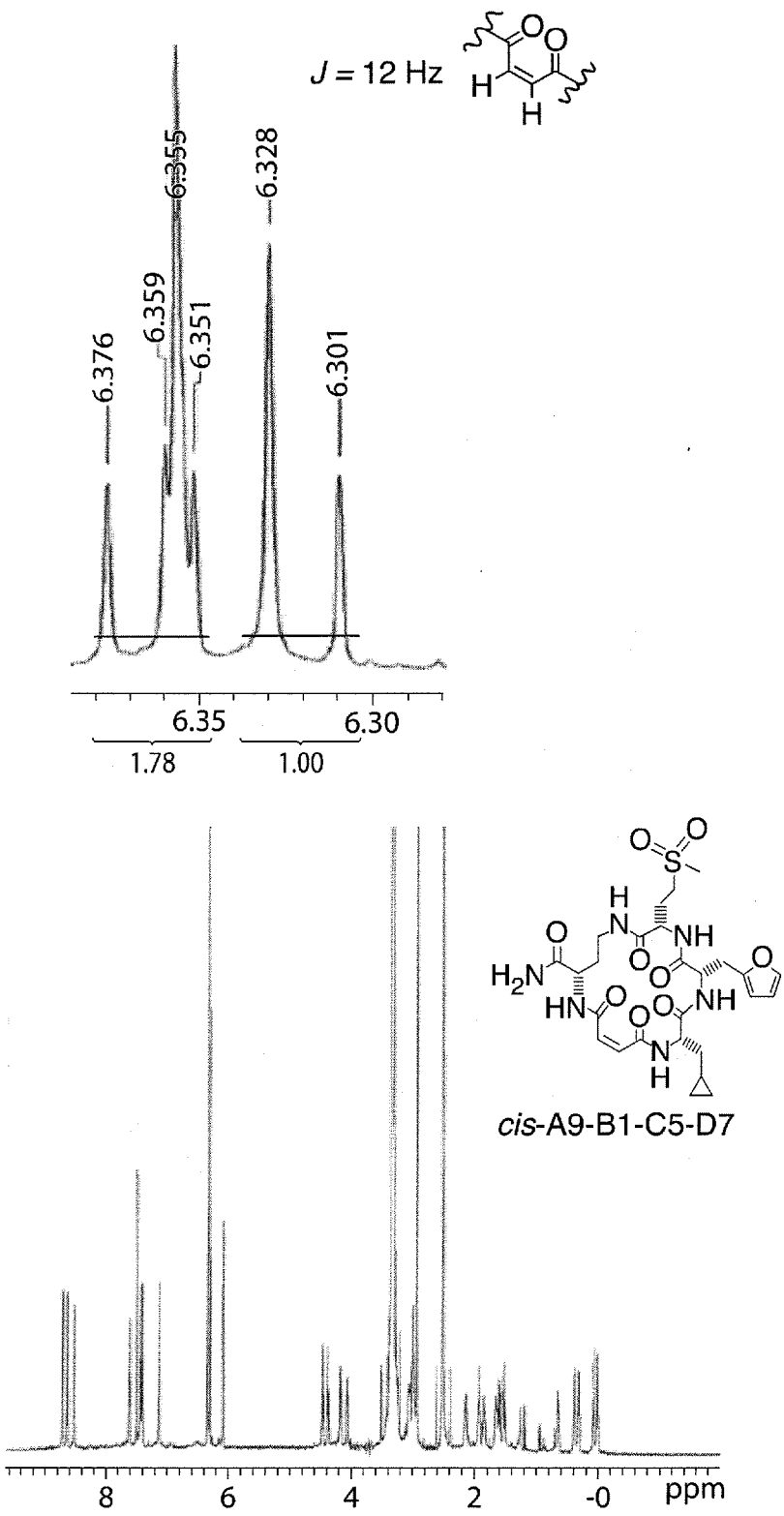

J = 12 Hz
6.376
6.359
6.355
6.351
6.328
6.301
6.35
6.30
1.78
1.00
cis-A9-B1-C5-D7
8
6
4
2
-0
ppm

Fig. 18-11

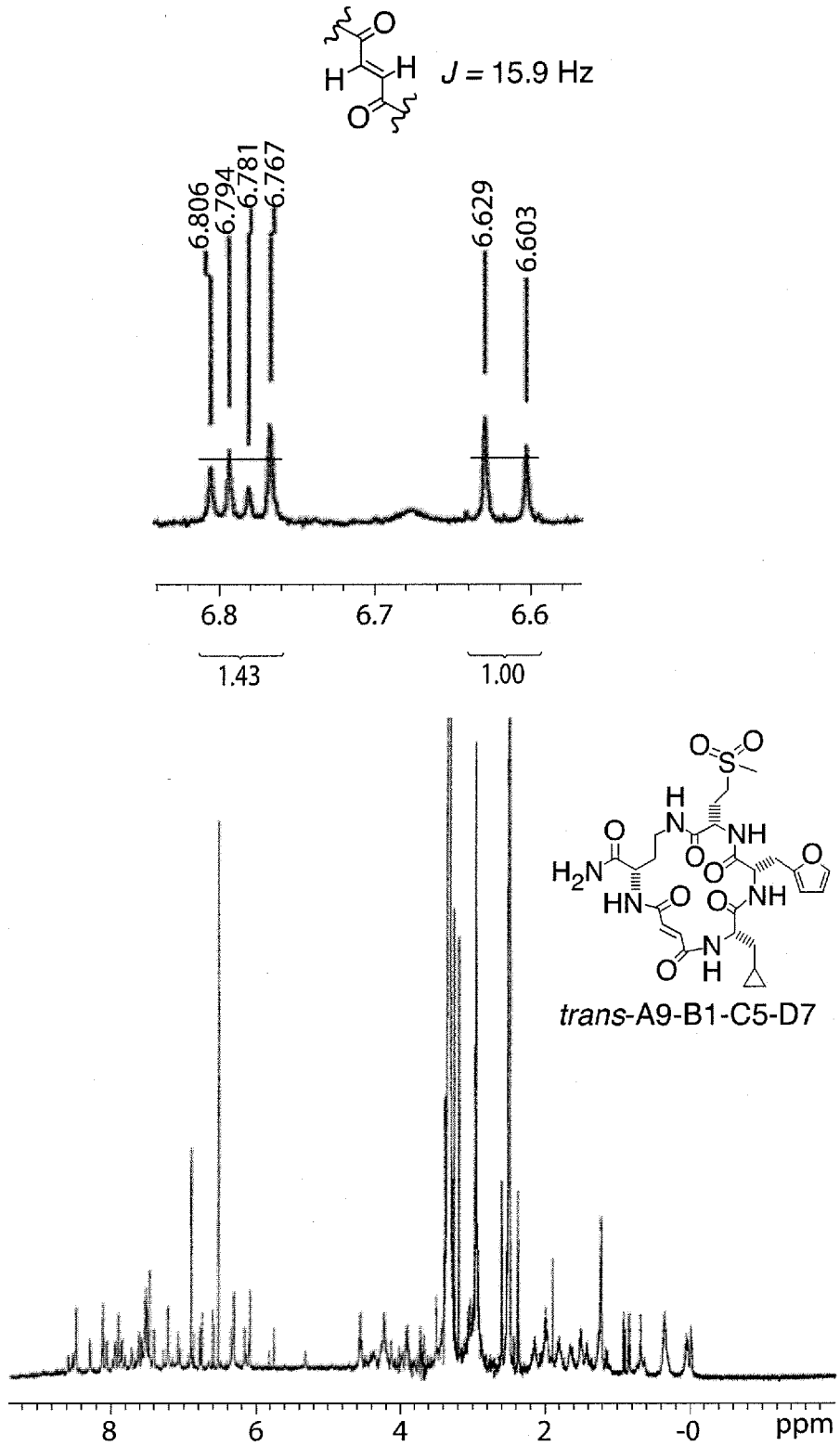

J = 15.9 Hz
6.806
6.794
6.781
6.767
6.629
6.603
6.8
6.7
6.6
1.43
1.00
trans-A9-B1-C5-D7
8
6
4
2
-0
ppm

Fig. 18-12

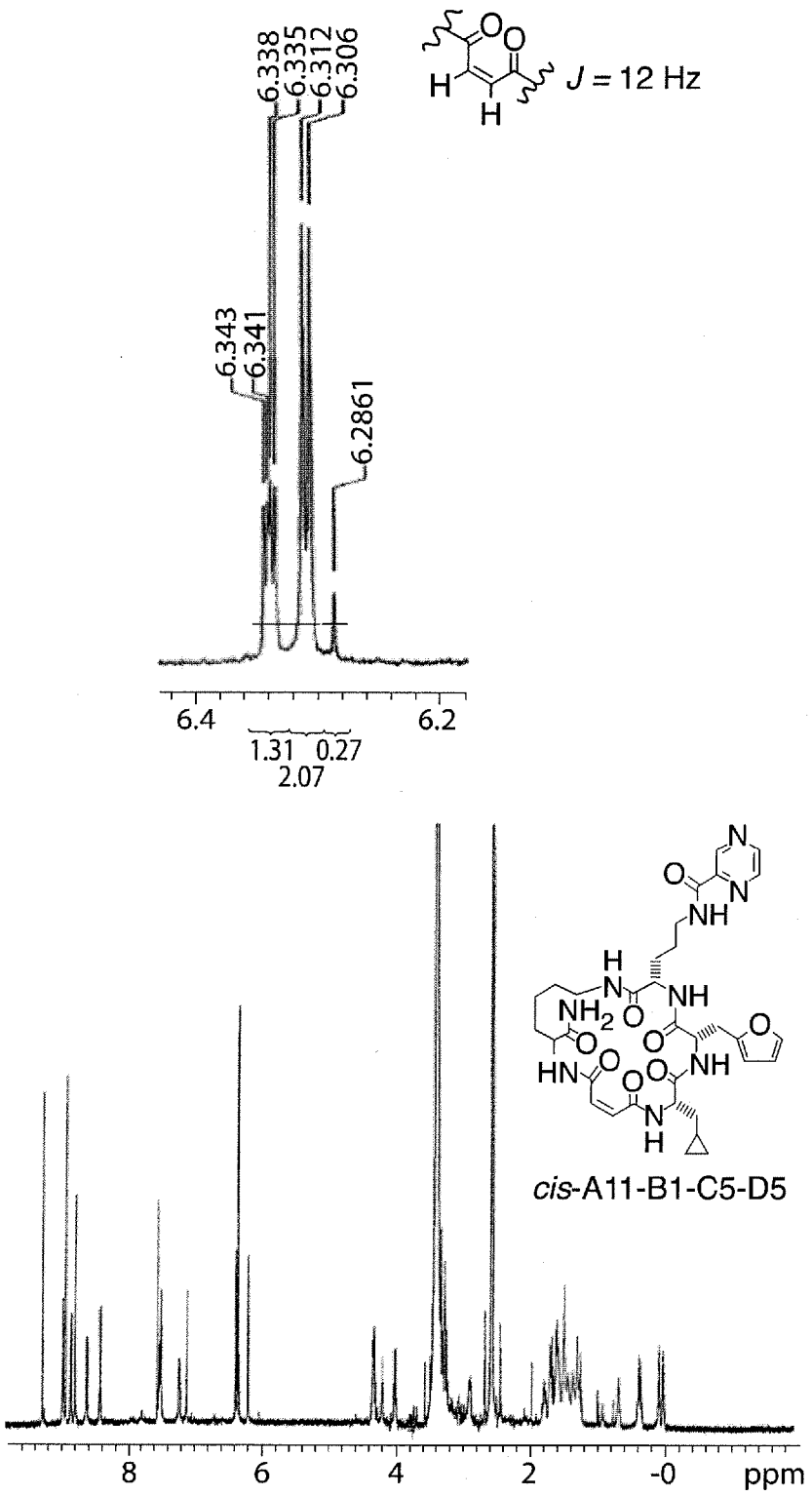

6.338
6.335
6.312
6.306
6.343
6.341
6.2861
J = 12 Hz
6.4
6.2
1.31
0.27
2.07
cis-A11-B1-C5-D5
8
6
4
2
-0
ppm

Fig. 18-13

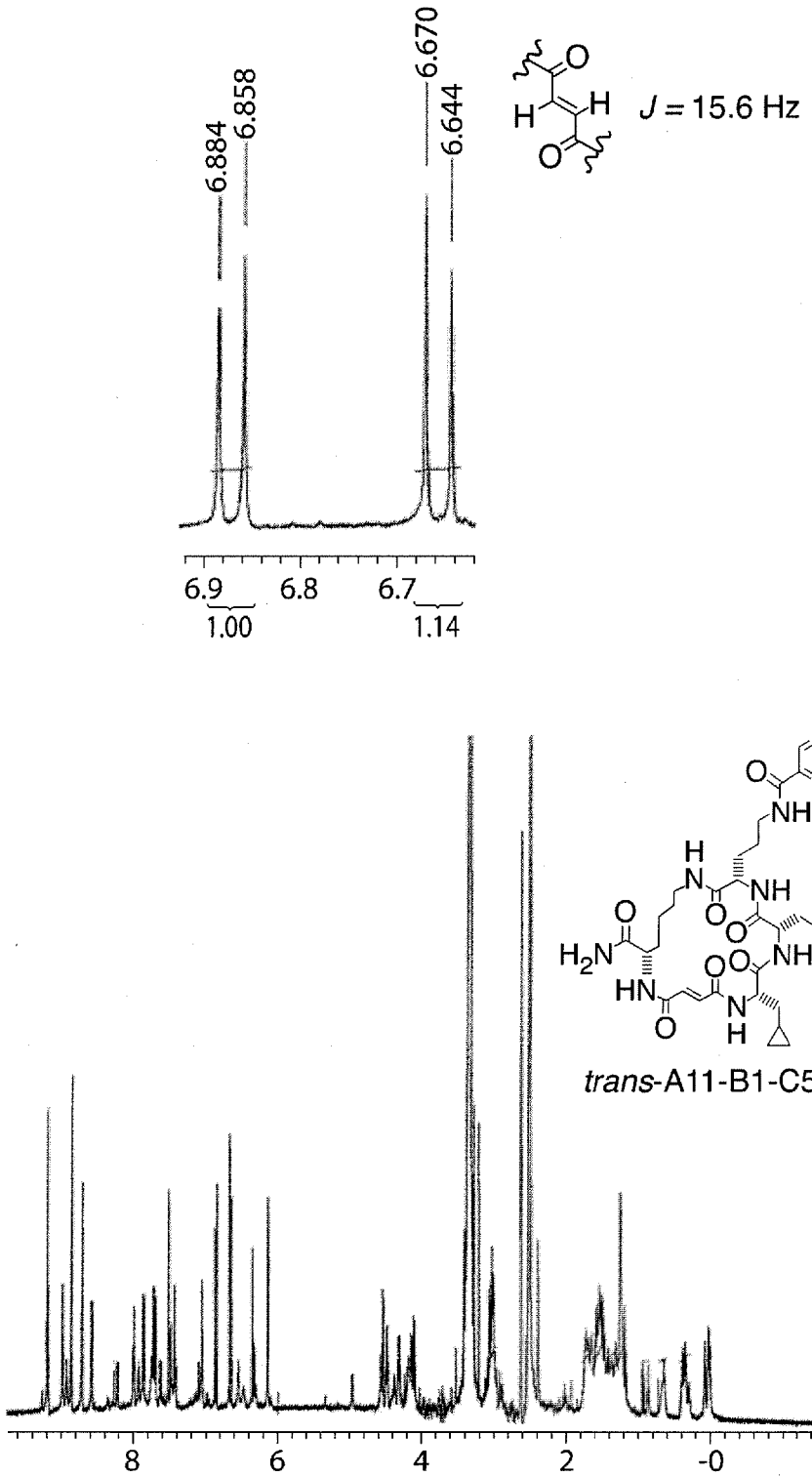

6.884
6.858
6.670
6.644
J = 15.6 Hz
6.9
6.8
6.7
1.00
1.14
trans-A11-B1-C5-D5
8
6
4
2
-0
ppm

Fig. 18-14

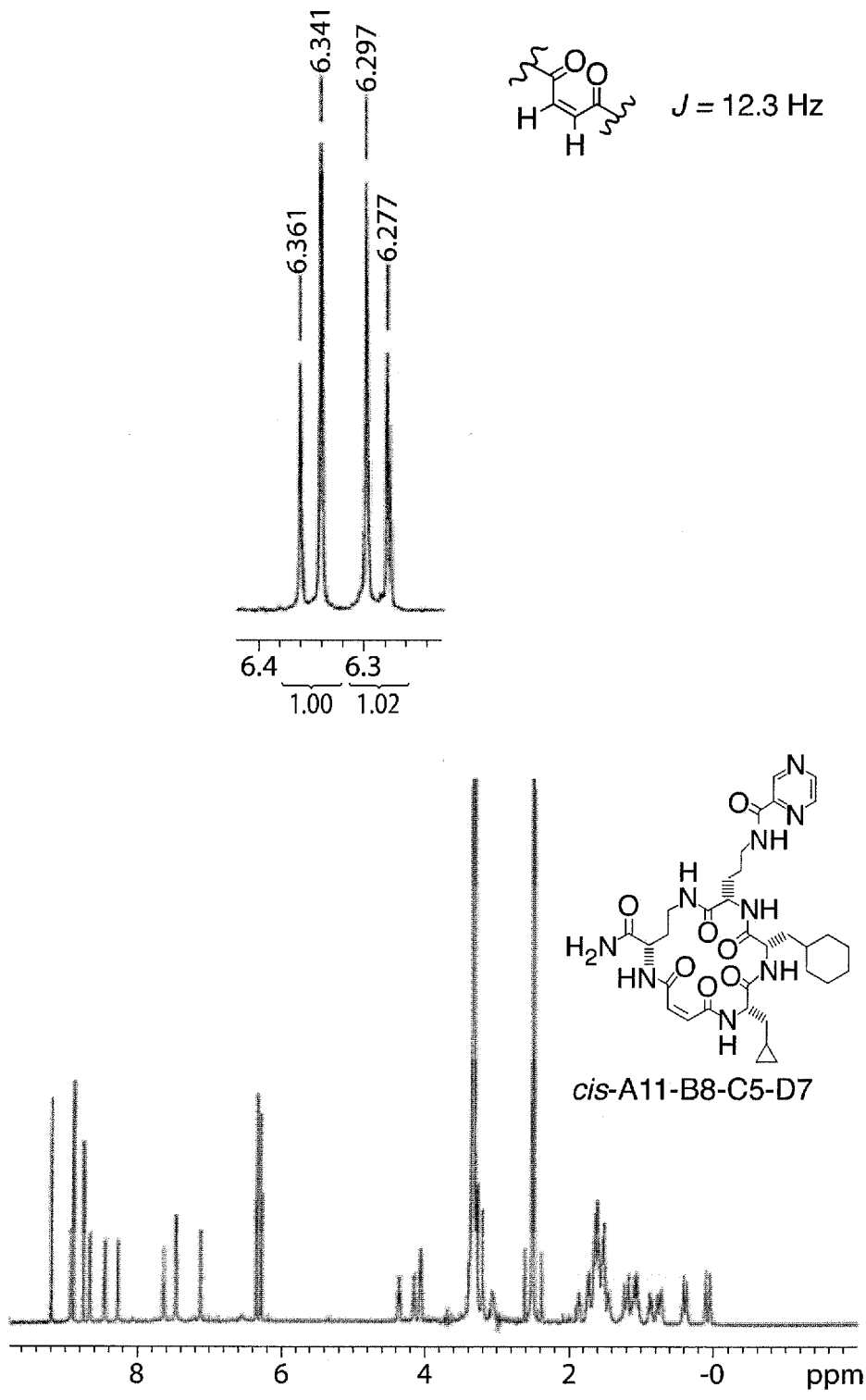

6.341
6.297
6.361
6.277
J = 12.3 Hz
6.4
6.3
1.00
1.02
cis-A11-B8-C5-D7
8
6
4
2
-0
ppm

Fig. 18-15

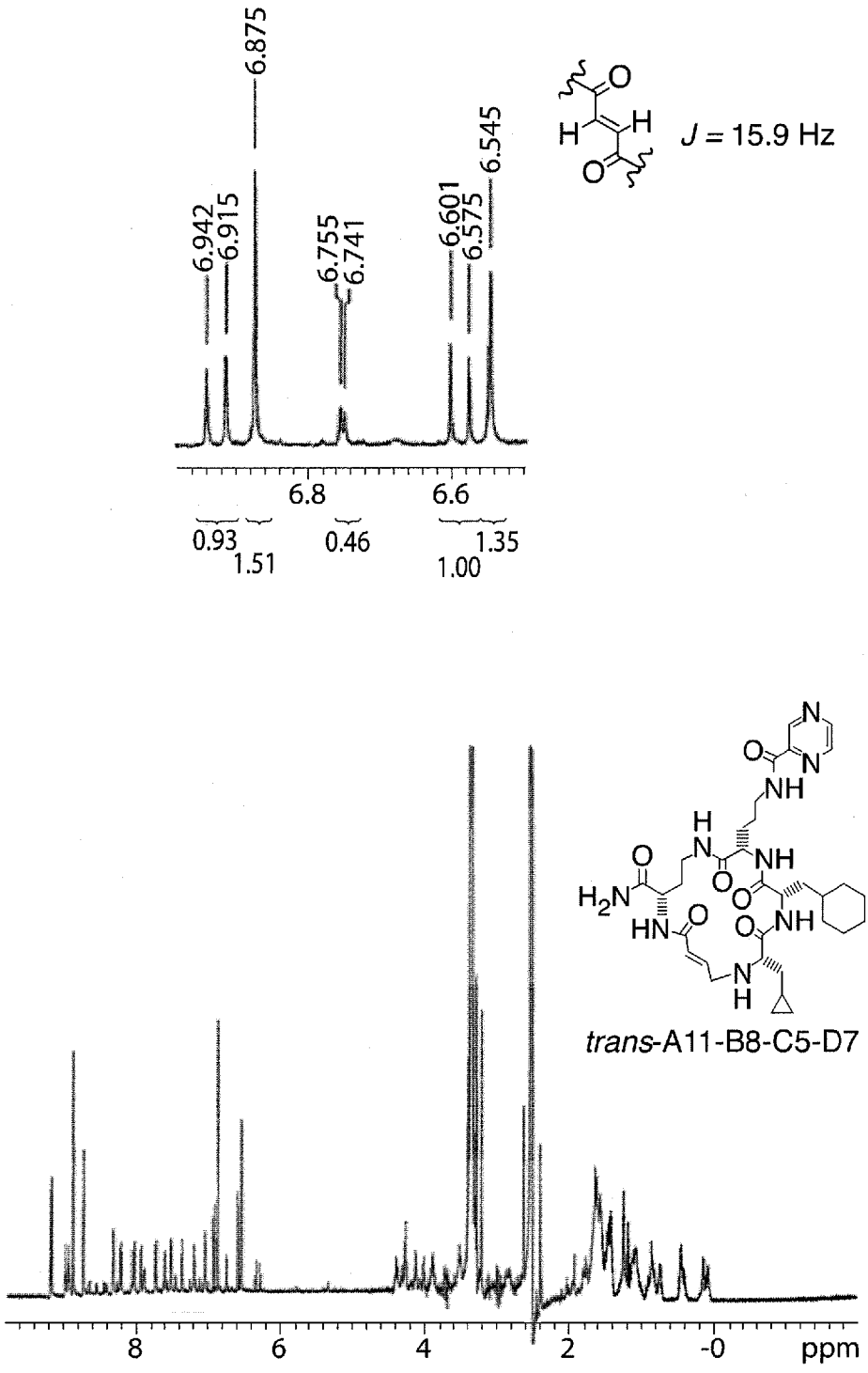

6.875
6.942
6.915
6.755
6.741
6.601
6.575
6.545
J = 15.9 Hz
6.8
6.6
0.93
1.51
0.46
1.35
1.00
trans-A11-B8-C5-D7
8
6
4
2
-0
ppm

Fig. 18-16

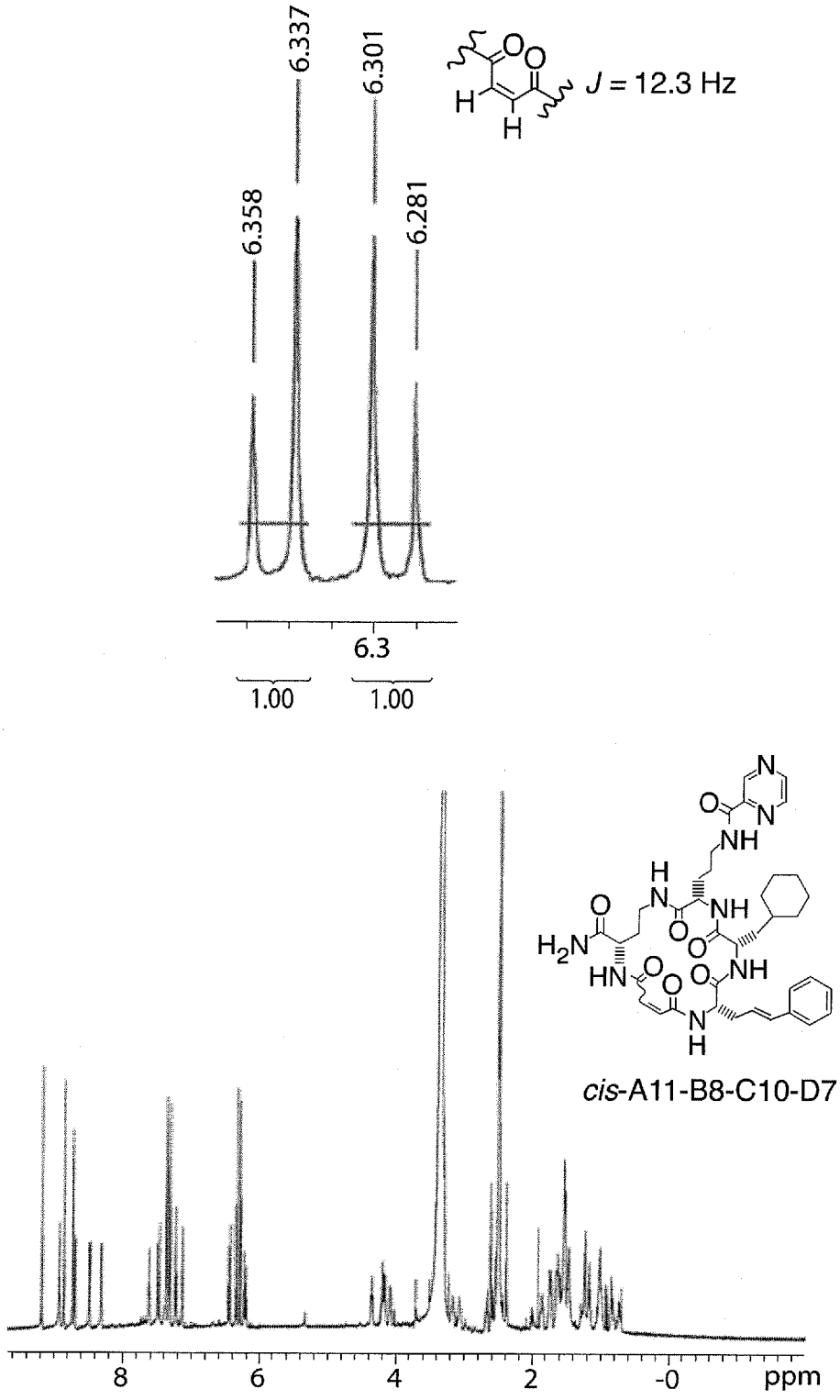

6.358
6.337
6.301
6.281
J = 12.3 Hz
6.3
1.00
1.00
cis-A11-B8-C10-D7
8
6
4
2
-0
ppm

Fig. 18-17

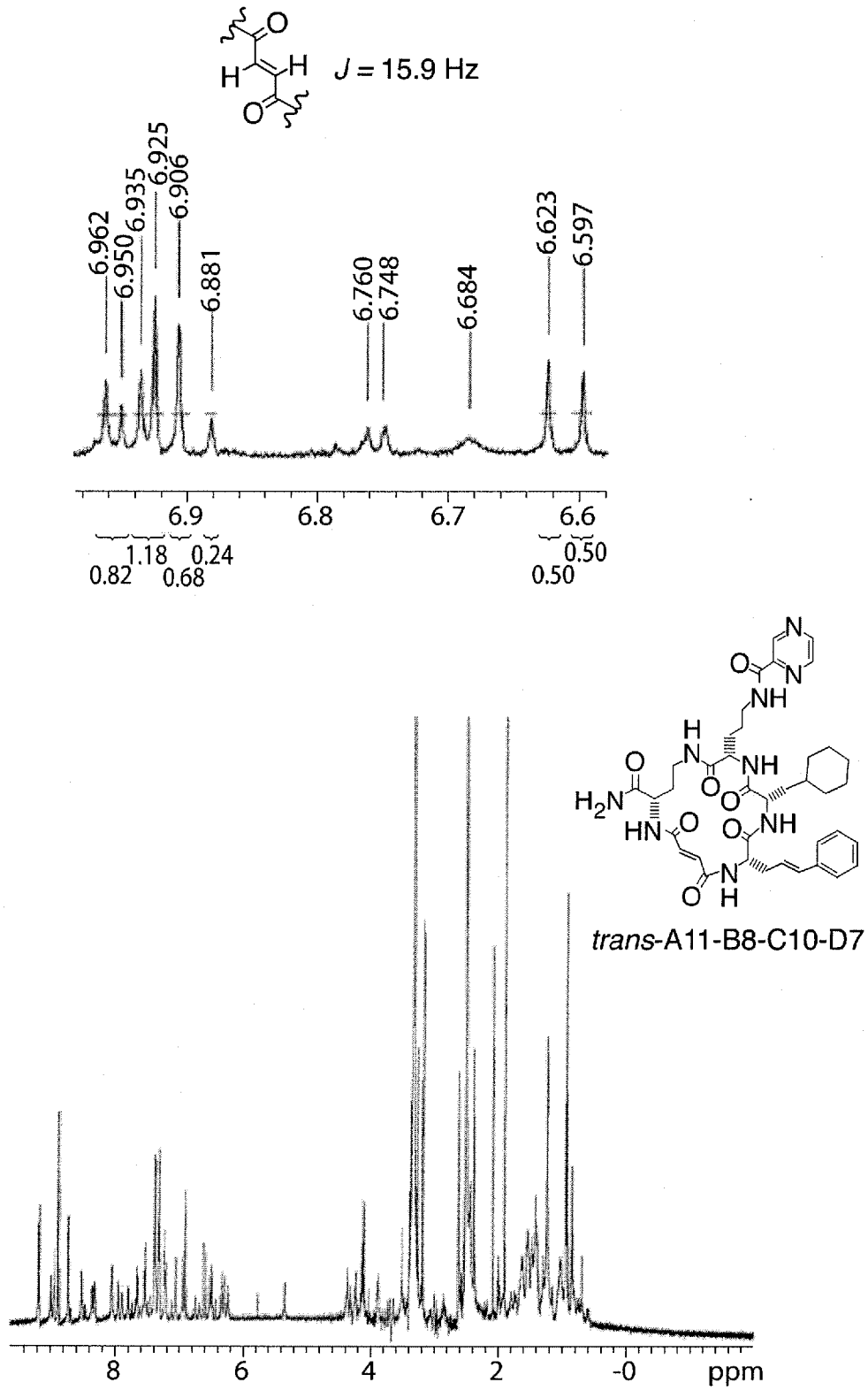

J = 15.9 Hz
6.962
6.950
6.935
6.925
6.906
6.881
6.760
6.748
6.684
6.623
6.597
6.9
6.8
6.7
6.6
0.82
1.18
0.68
0.24
0.50
0.50
trans-A11-B8-C10-D7
8
6
4
2
-0
ppm

Fig. 18-18

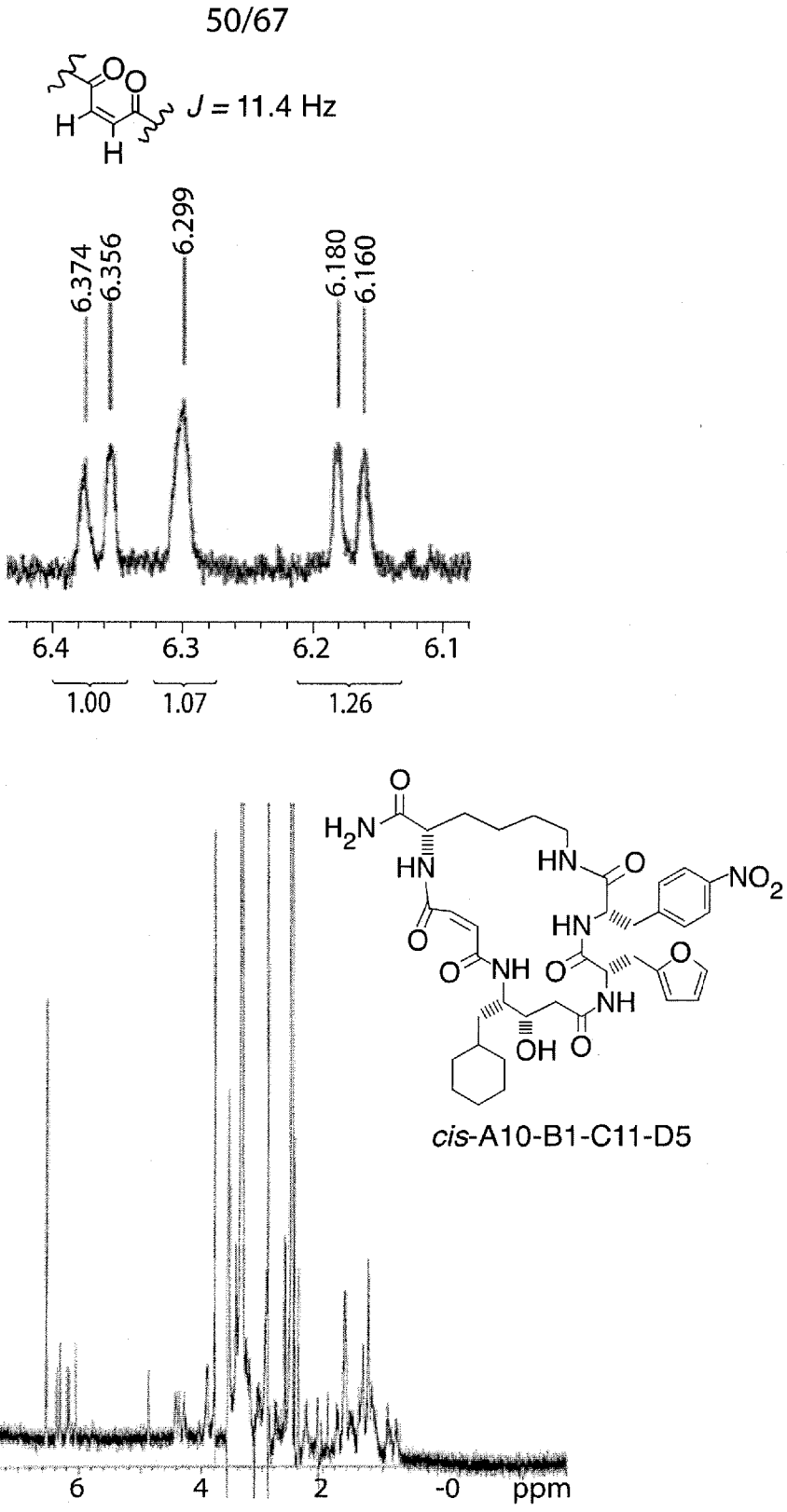

J = 11.4 Hz
6.374
6.356
6.299
6.180
6.160
6.4
6.3
6.2
6.1
1.00
1.07
1.26
cis-A10-B1-C11-D5
8
6
4
2
-0
ppm

Fig. 18-19

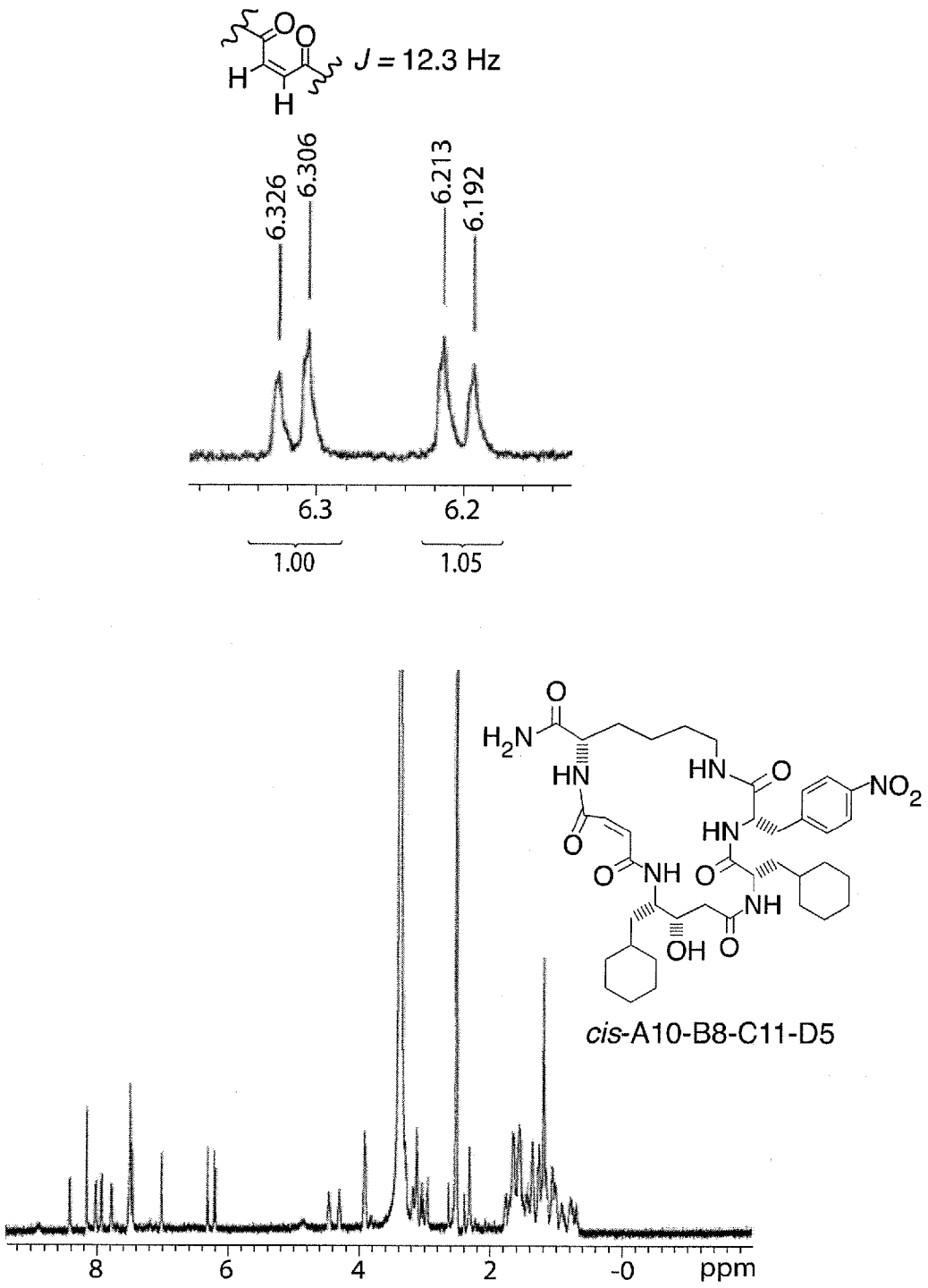

J = 12.3 Hz
6.326
6.306
6.213
6.192
6.3
6.2
1.00
1.05
cis-A10-B8-C11-D5
8
6
4
2
-0
ppm

Fig. 18-20

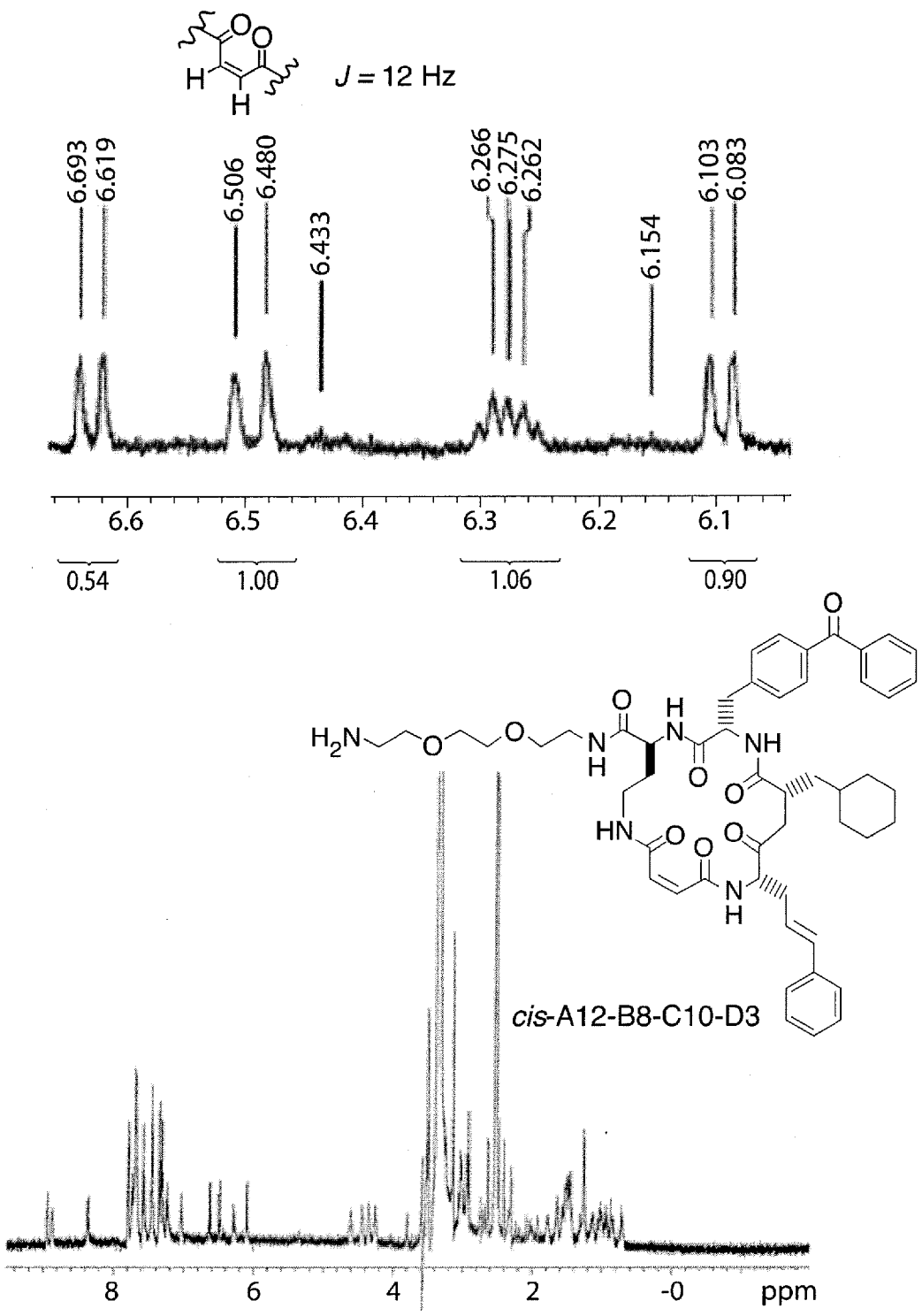

J = 12 Hz
6.693
6.619
6.506
6.480
6.433
6.266
6.275
6.262
6.154
6.103
6.083
6.6
6.5
6.4
6.3
6.2
6.1
0.54
1.00
1.06
0.90
cis-A12-B8-C10-D3
8
6
4
2
-0
ppm

Fig. 18-21

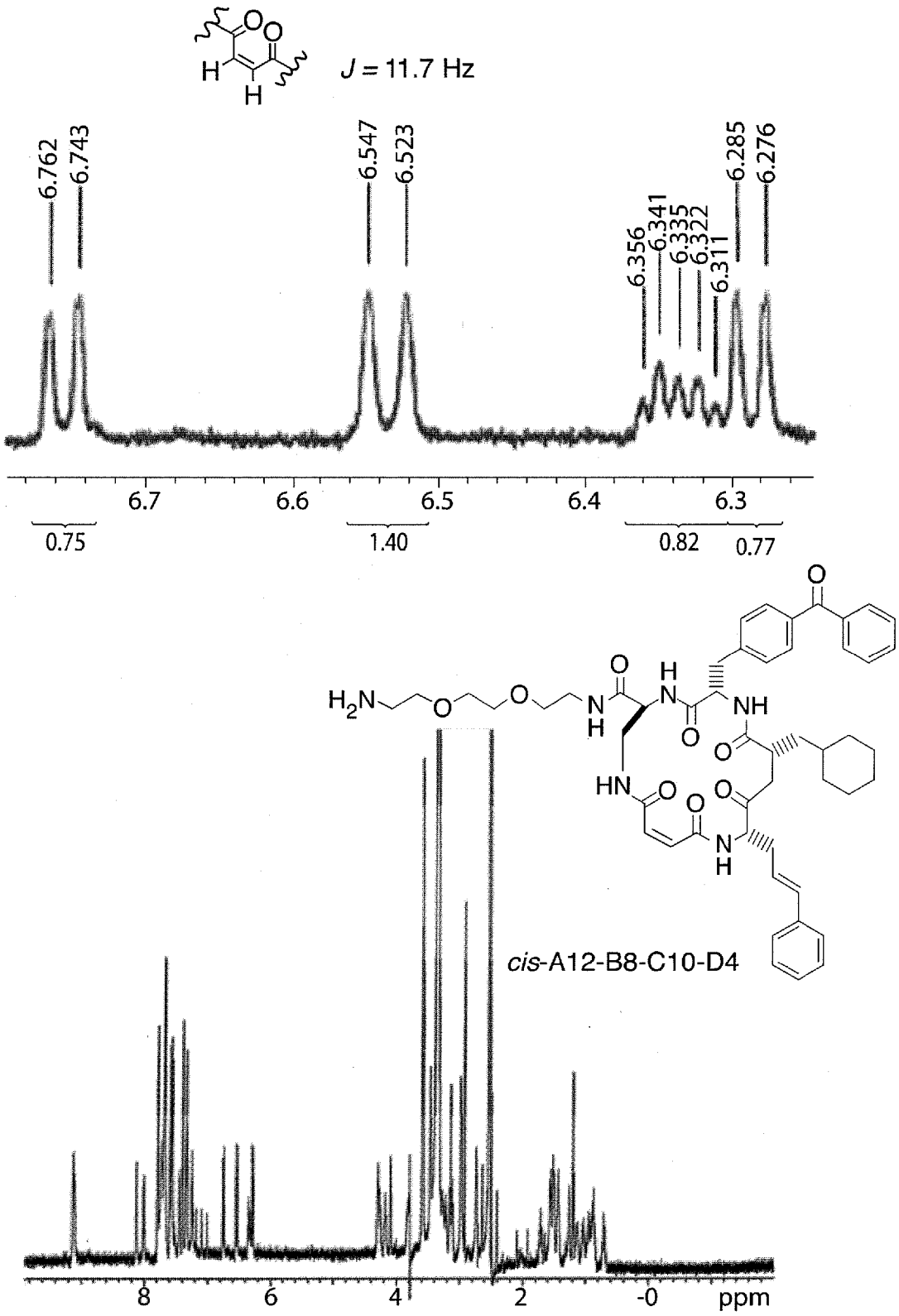

J = 11.7 Hz
6.762
6.743
6.547
6.523
6.356
6.341
6.335
6.322
6.311
6.285
6.276
6.7
6.6
6.5
6.4
6.3
0.75
1.40
0.82
0.77
cis-A12-B8-C10-D4
8
6
4
2
-0
ppm

Fig. 18-22

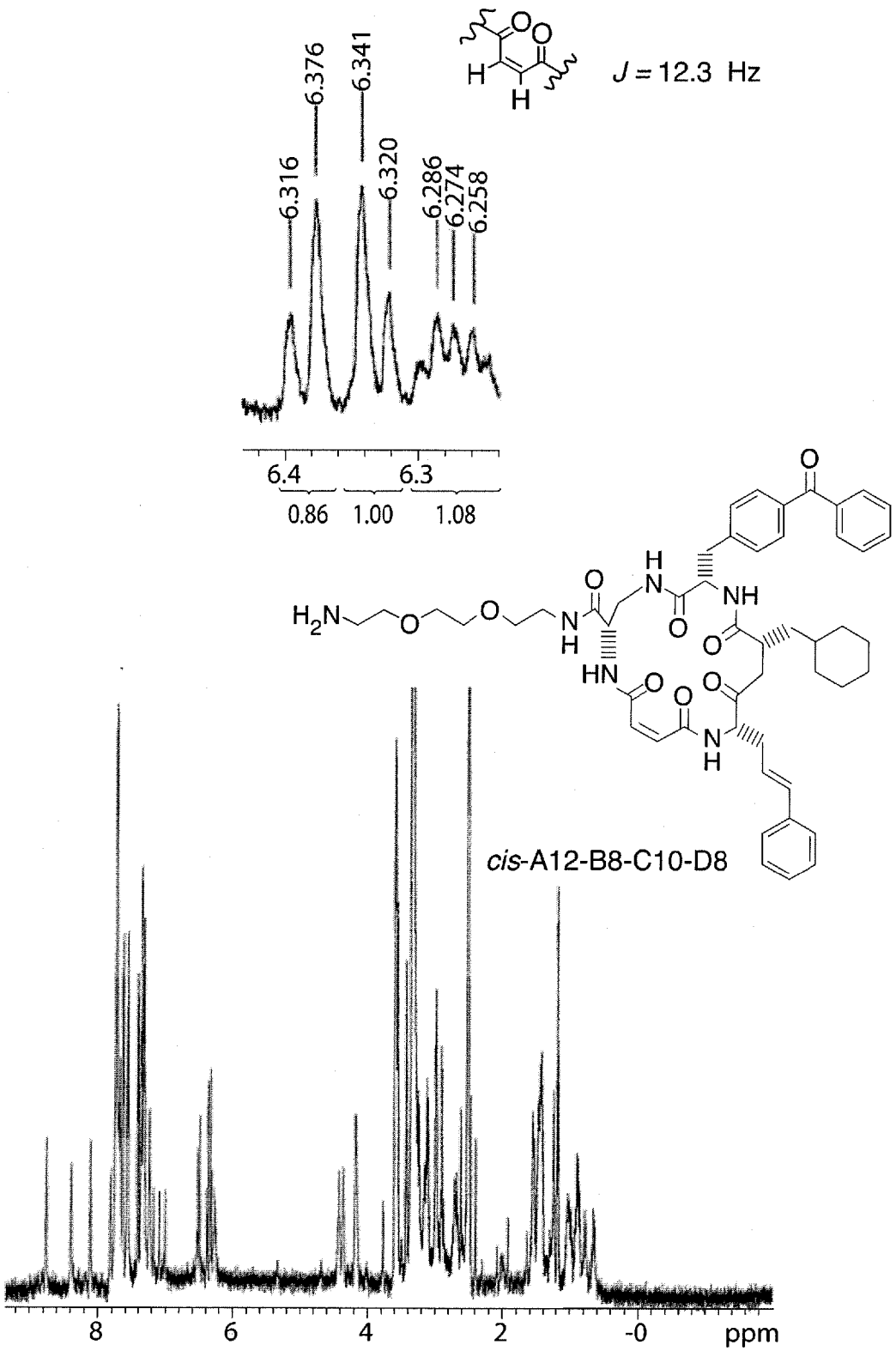

6.316
6.376
6.341
6.320
6.286
6.274
6.258
J = 12.3 Hz
6.4
6.3
0.86
1.00
1.08
cis-A12-B8-C10-D8
8
6
4
2
-0
ppm

Fig. 18-23

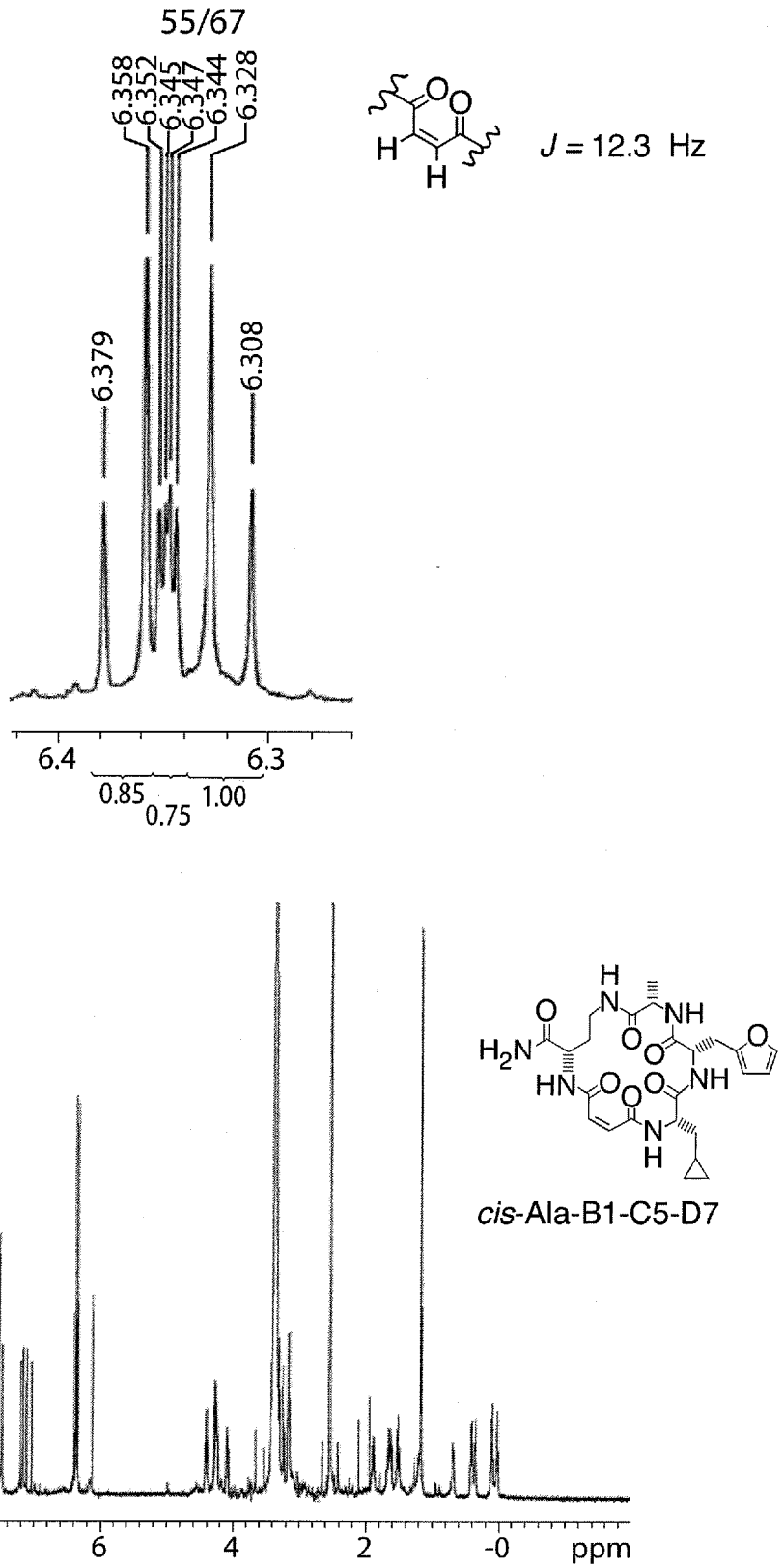

55/67
6.358
6.352
6.345
6.347
6.344
6.328
6.379
6.308
6.4
6.3
0.85
0.75
1.00
J = 12.3 Hz
cis-Ala-B1-C5-D7
8
6
4
2
-0
ppm

Fig. 18-24

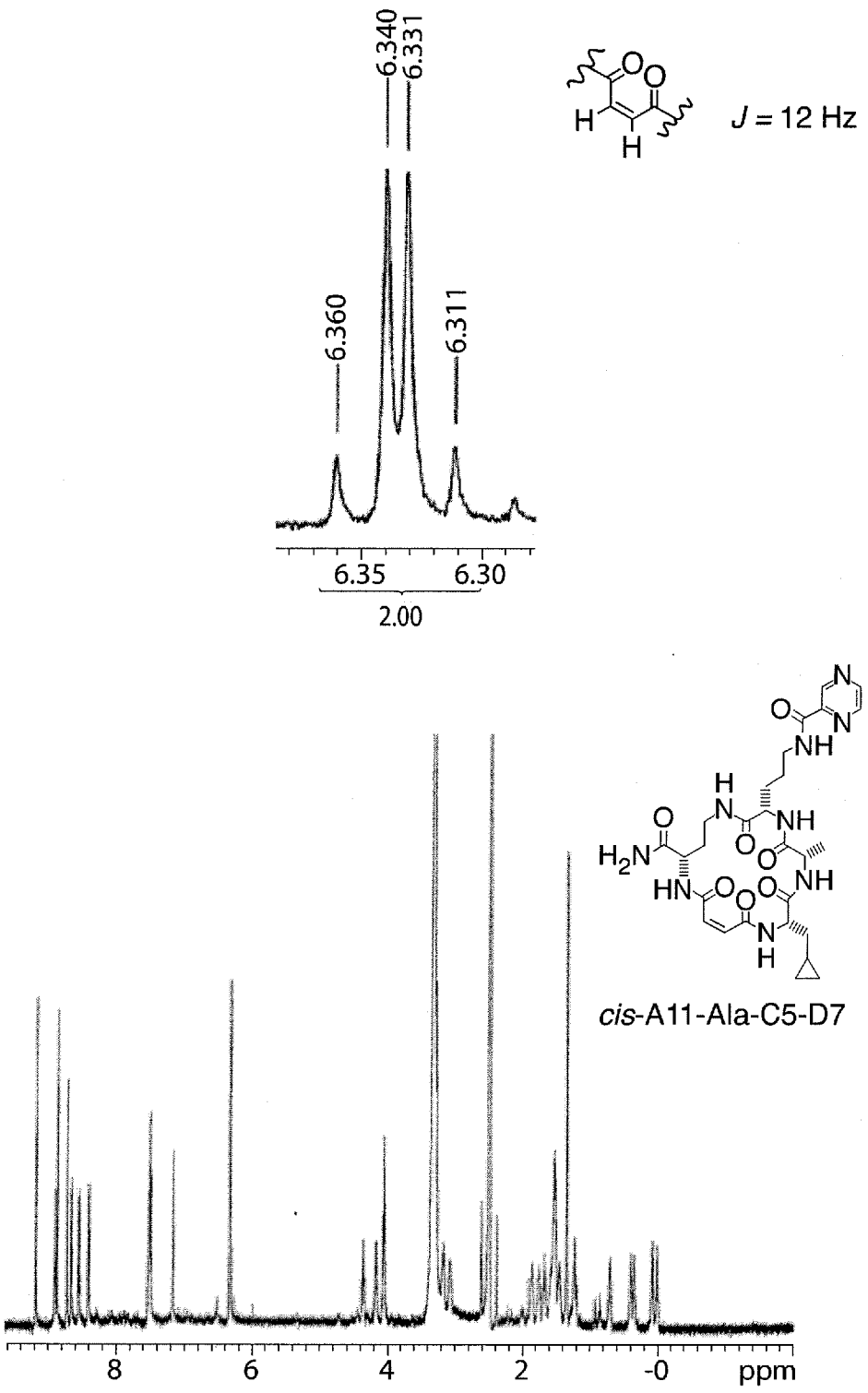

6.340
6.331
6.360
6.311
6.35
6.30
2.00
J = 12 Hz
cis-A11-Ala-C5-D7
8
6
4
2
-0
ppm

Fig. 18-25

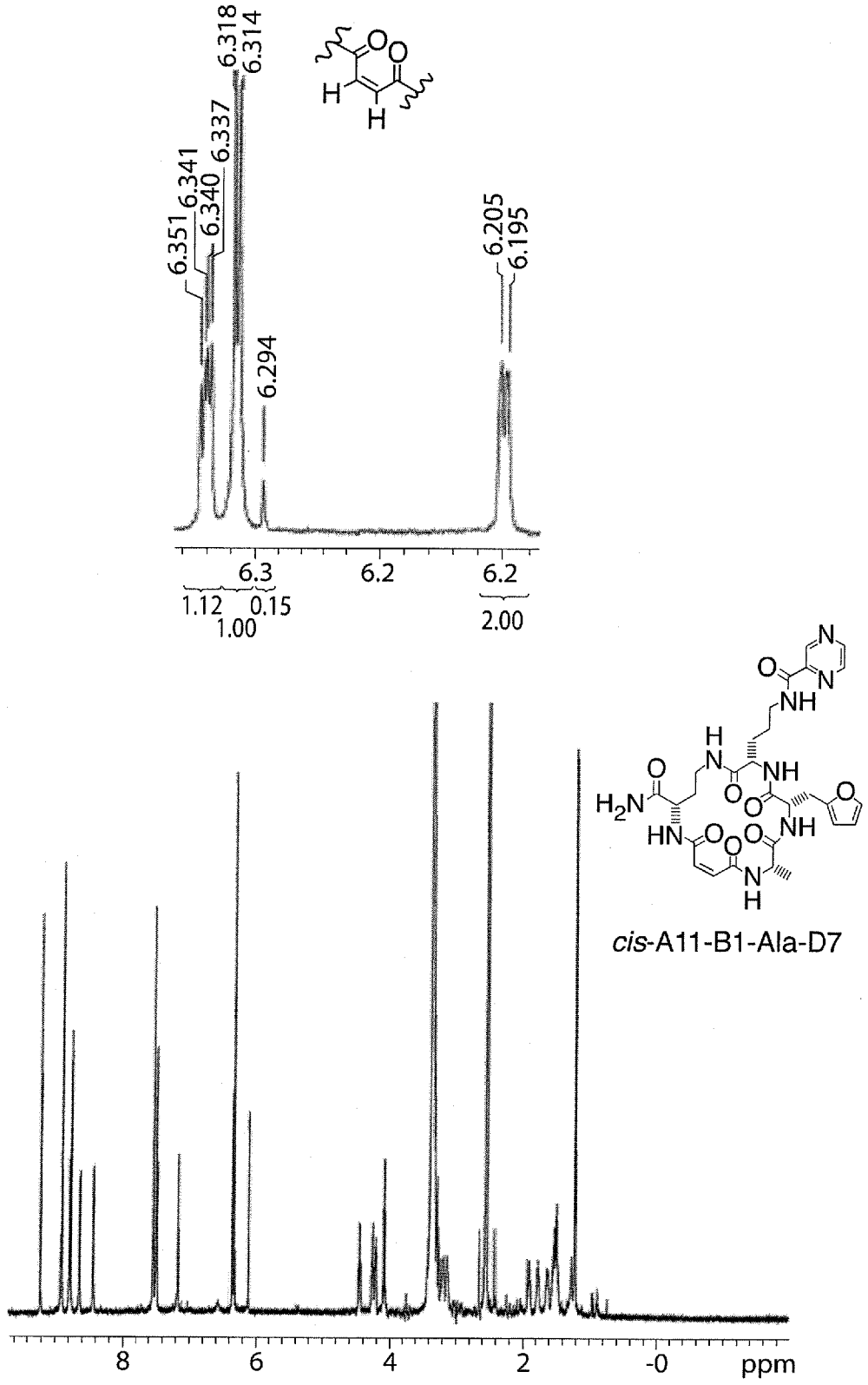
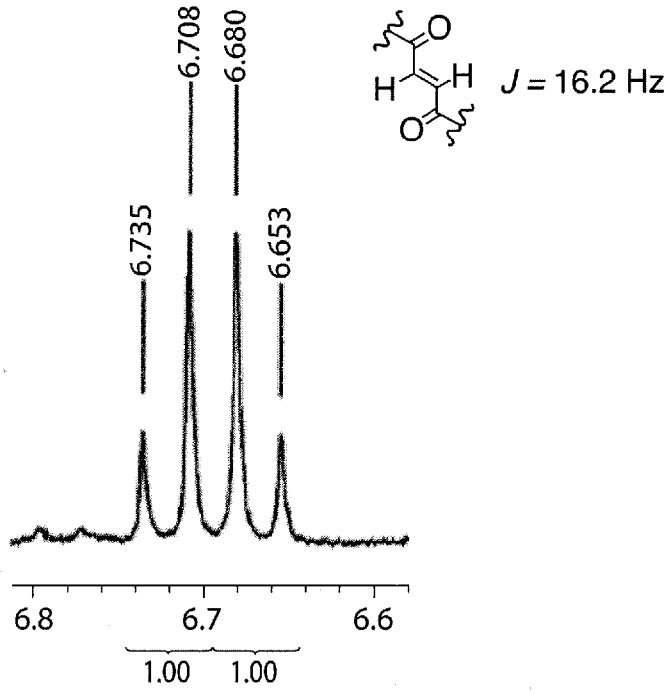

6.318
6.314
6.337
6.341
6.340
6.351
6.294
6.205
6.195
6.3
6.2
6.2
1.12
0.15
1.00
2.00
cis-A11-B1-Ala-D7
8
6
4
2
-0
ppm

Fig. 18-26


6.708
6.680
6.735
6.653
J = 16.2 Hz
6.8
6.7
6.6
1.00
1.00

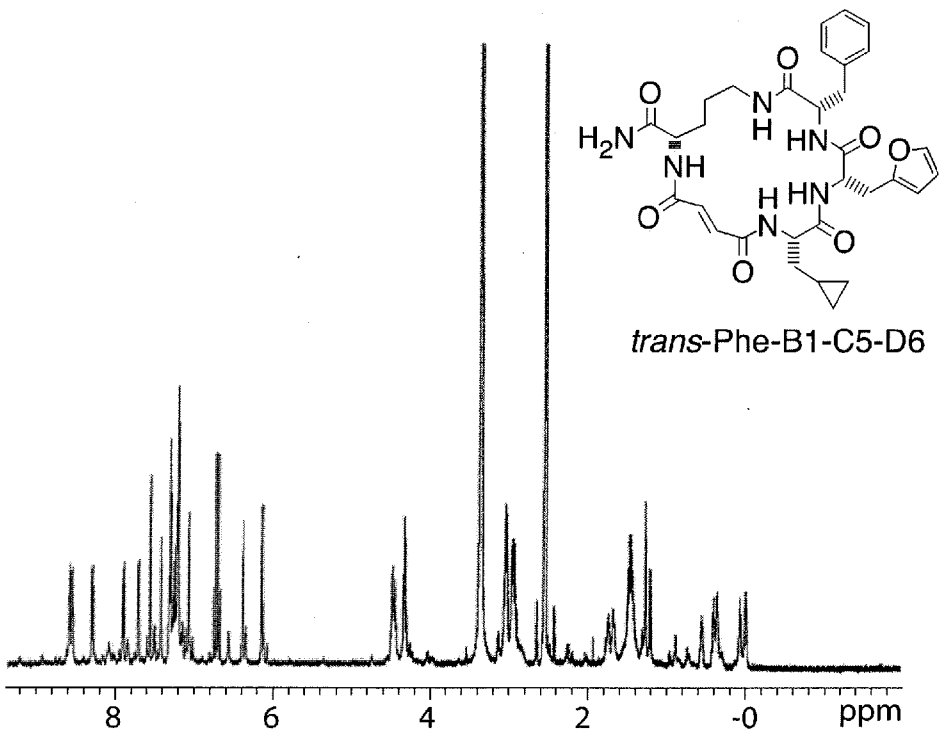

trans-Phe-B1-C5-D6
8
6
4
2
-0
ppm

Fig. 18-27

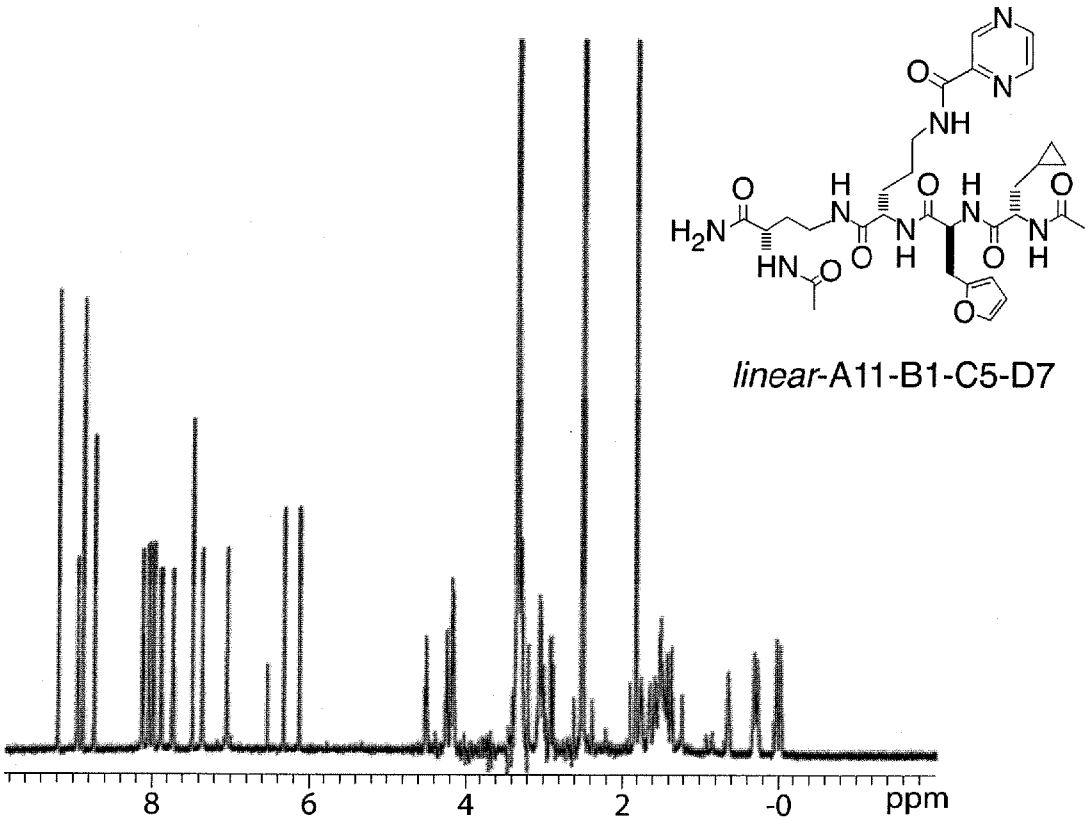


Fig. 18-28

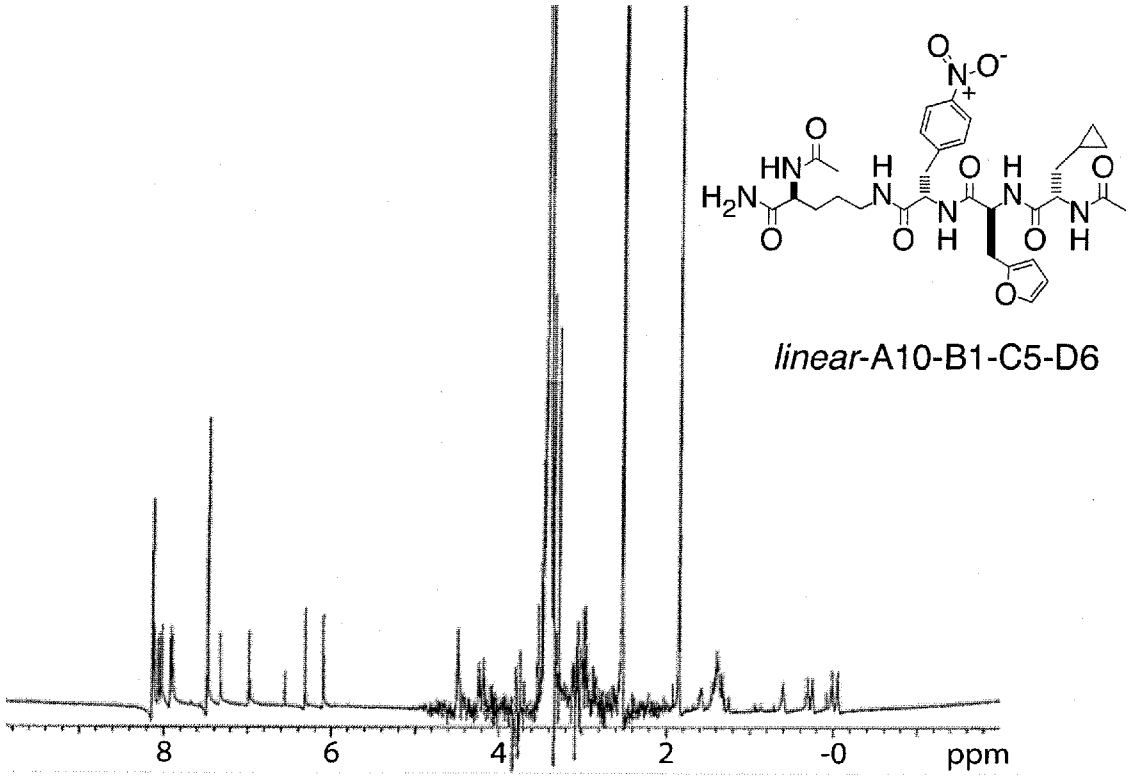


Fig. 18-29

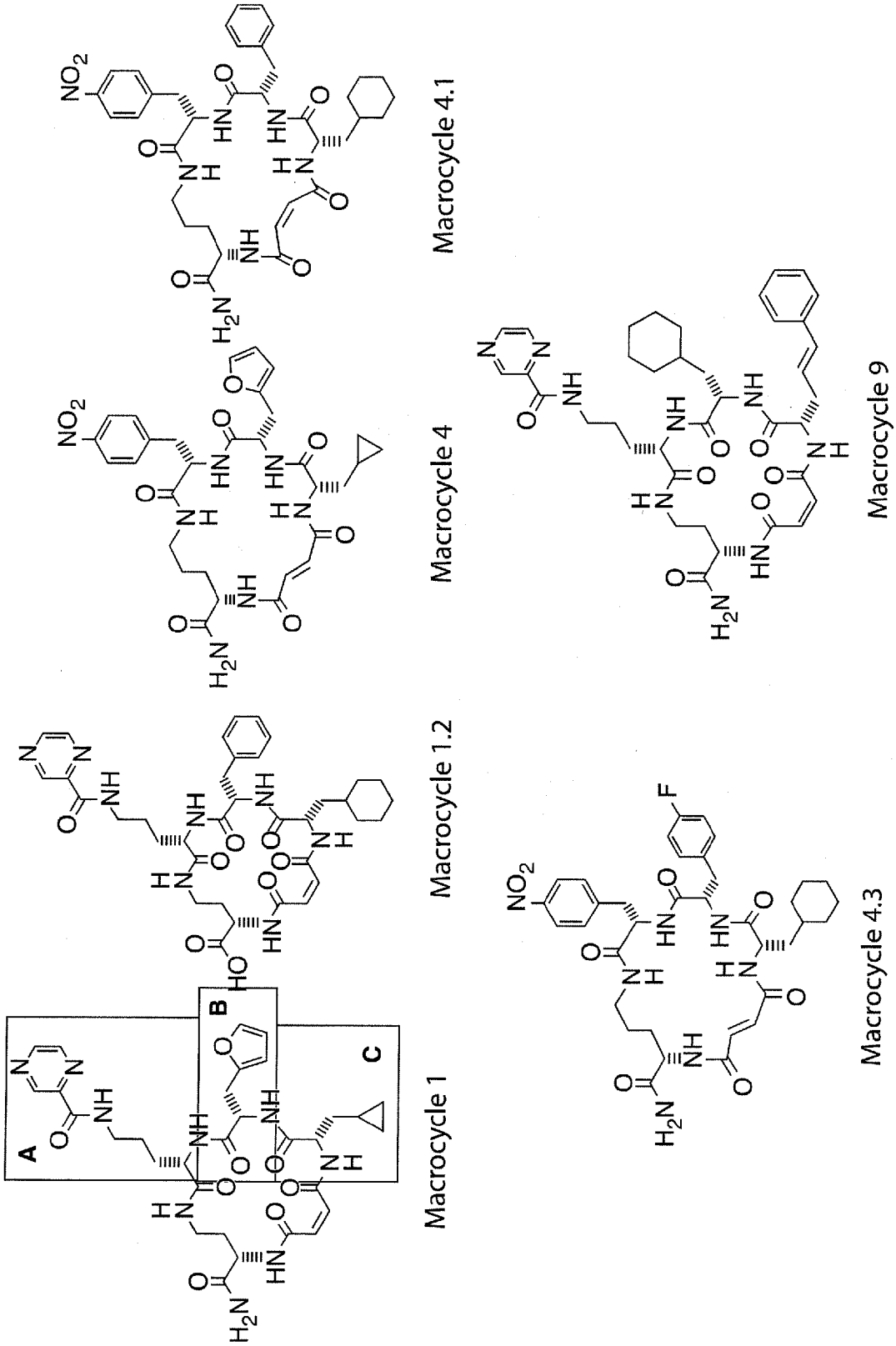

Macrocycle 4.1
Macrocycle 4
Macrocycle 9
Macrocycle 1.2
Macrocycle 4.3
Macrocycle 1
A
B
C

Fig. 19-1

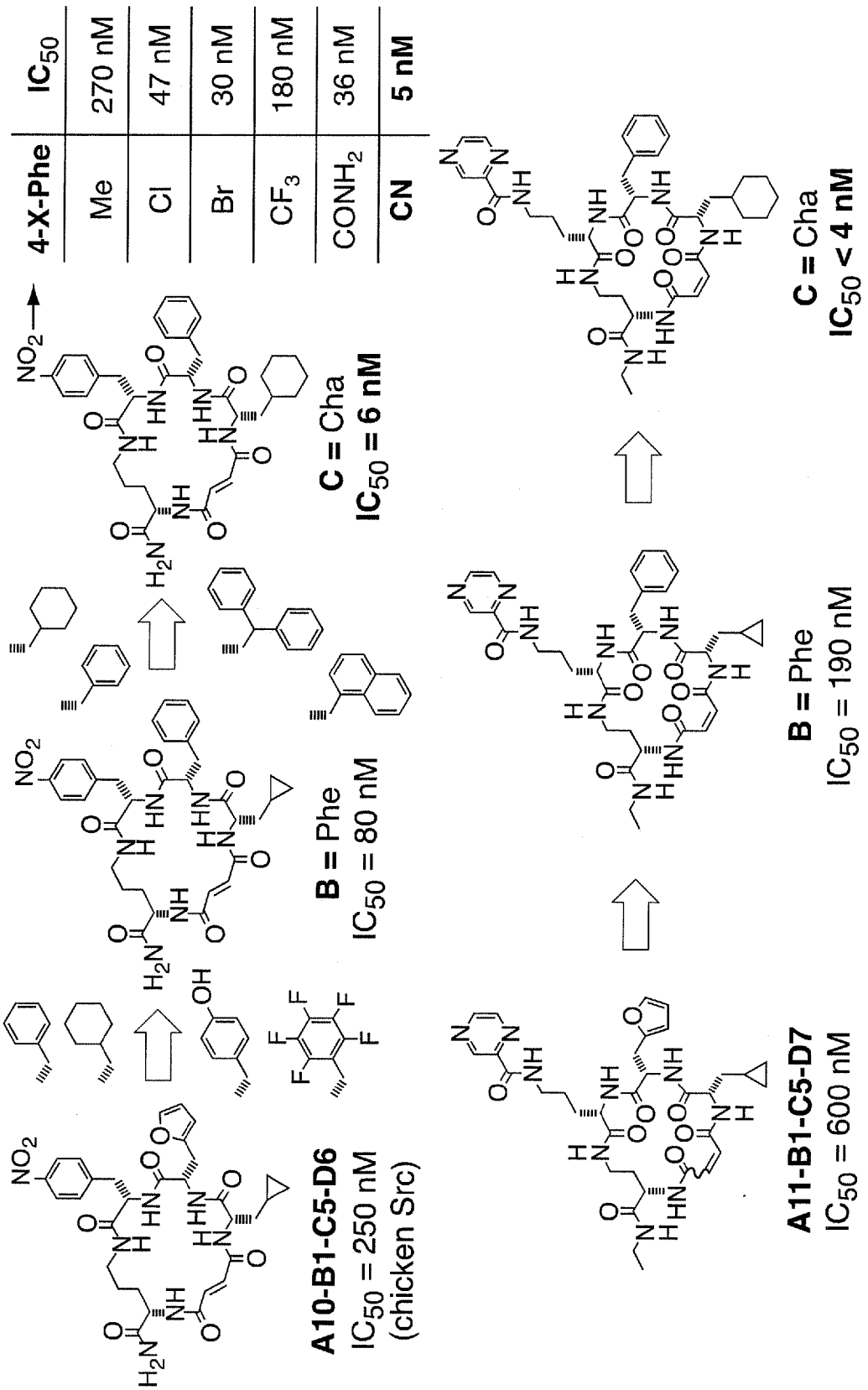

4-X-Phe
IC50
Me
270 nM
Cl
47 nM
Br
30 nM
CF3
180 nM
CONH2
36 nM
CN
5 nM
NO2
A10-B1-C5-D6
IC50 = 250 nM
(chicken Src)
B = Phe
IC50 = 80 nM
C = Cha
IC50 = 6 nM
A11-B1-C5-D7
IC50 = 600 nM
B = Phe
IC50 = 190 nM
C = Cha
IC50 < 4 nM

Fig. 19-2

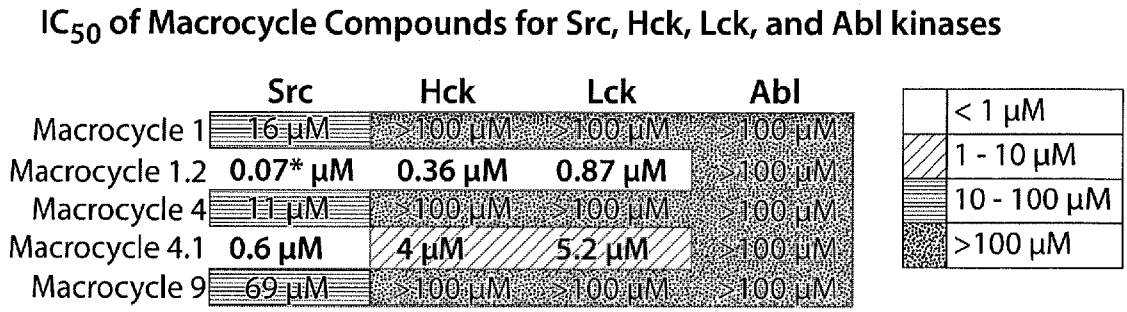


Fig. 20A

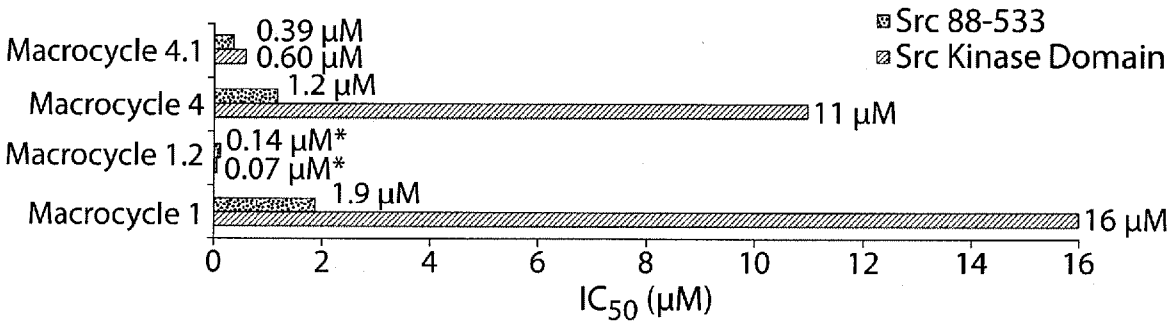


Fig. 20B

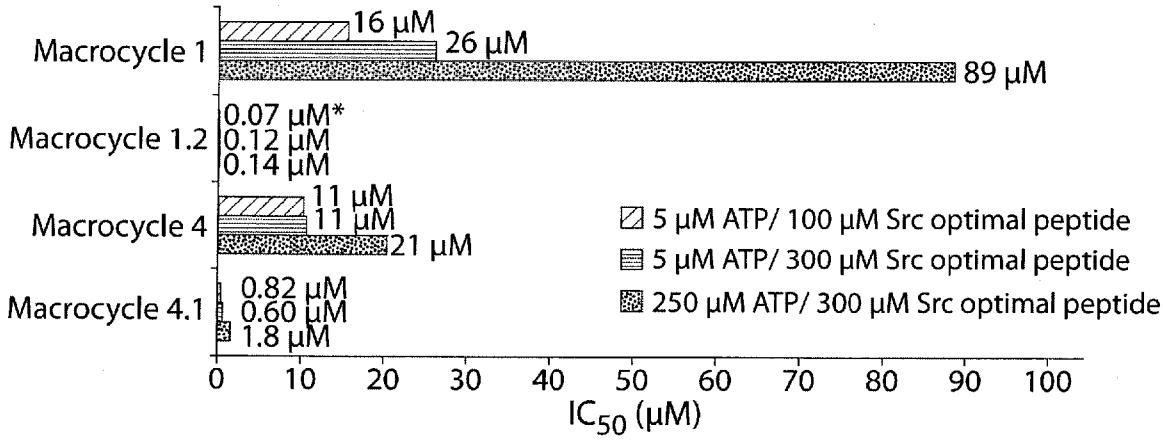


Fig. 20C

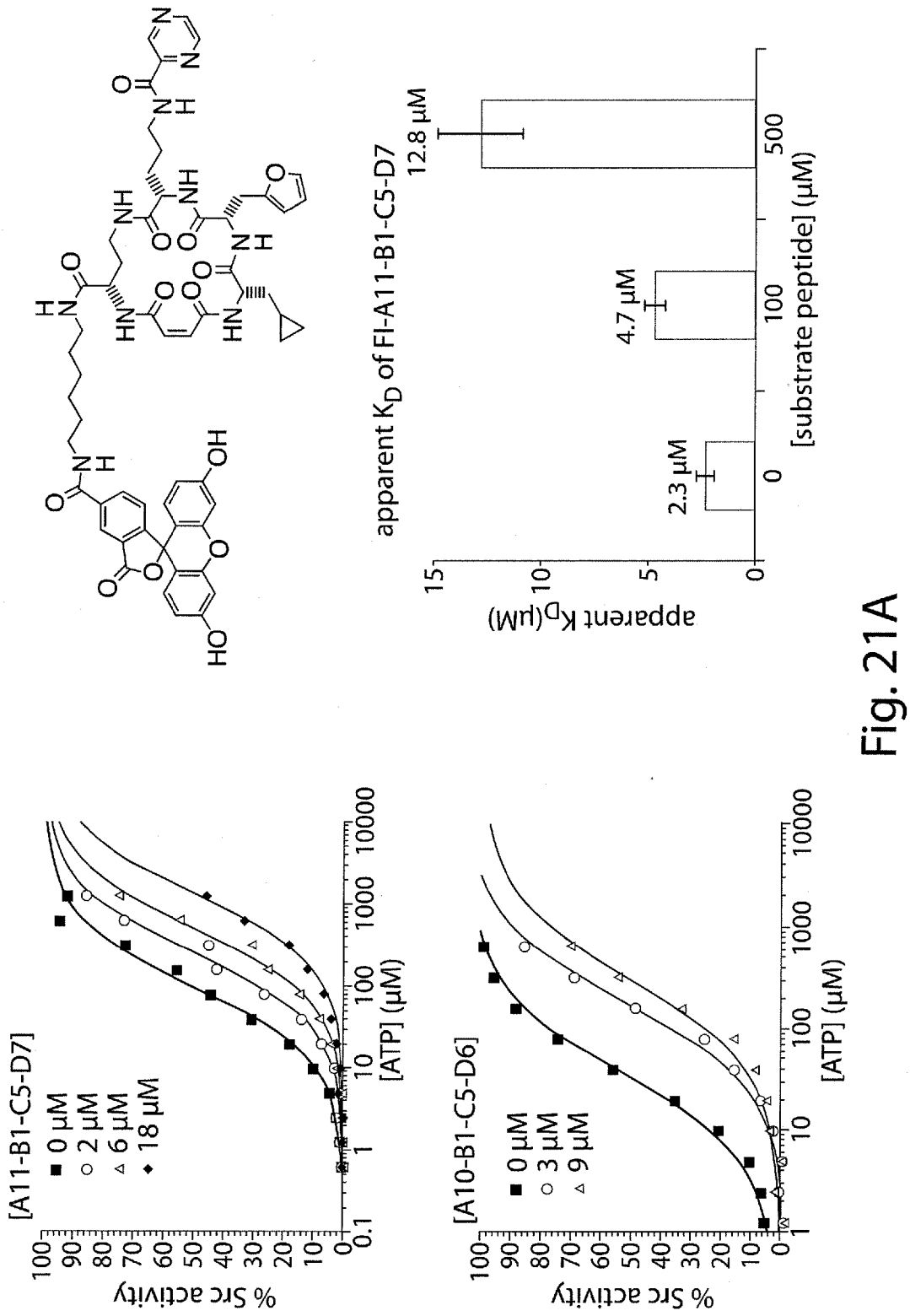

apparent K_D of FI-A11-B1-C5-D7
12.8 µM
4.7 µM
2.3 µM
apparent K_D(µM)
[substrate peptide] (µM)
[A11-B1-C5-D7]
0 µM
2 µM
6 µM
18 µM
% Src activity
[ATP] (µM)
[A10-B1-C5-D6]
0 µM
3 µM
9 µM
% Src activity
[ATP] (µM)

Fig. 21A

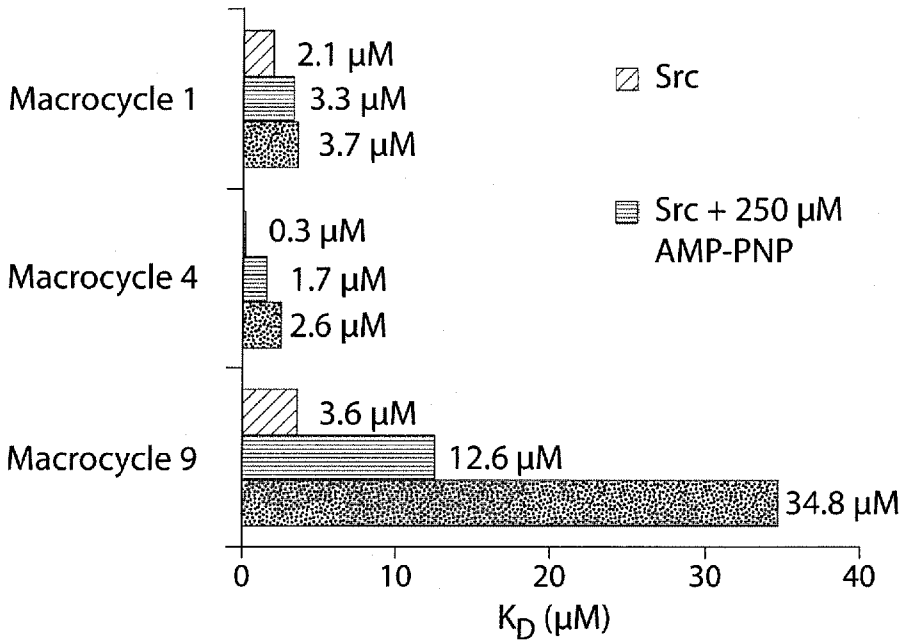

Macrocycle 1
2.1 µM
3.3 µM
3.7 µM
Macrocycle 4
0.3 µM
1.7 µM
2.6 µM
Macrocycle 9
3.6 µM
12.6 µM
34.8 µM
Src
Src + 250 µM AMP-PNP
0
10
20
30
40
K_D (µM)

Fig. 21B

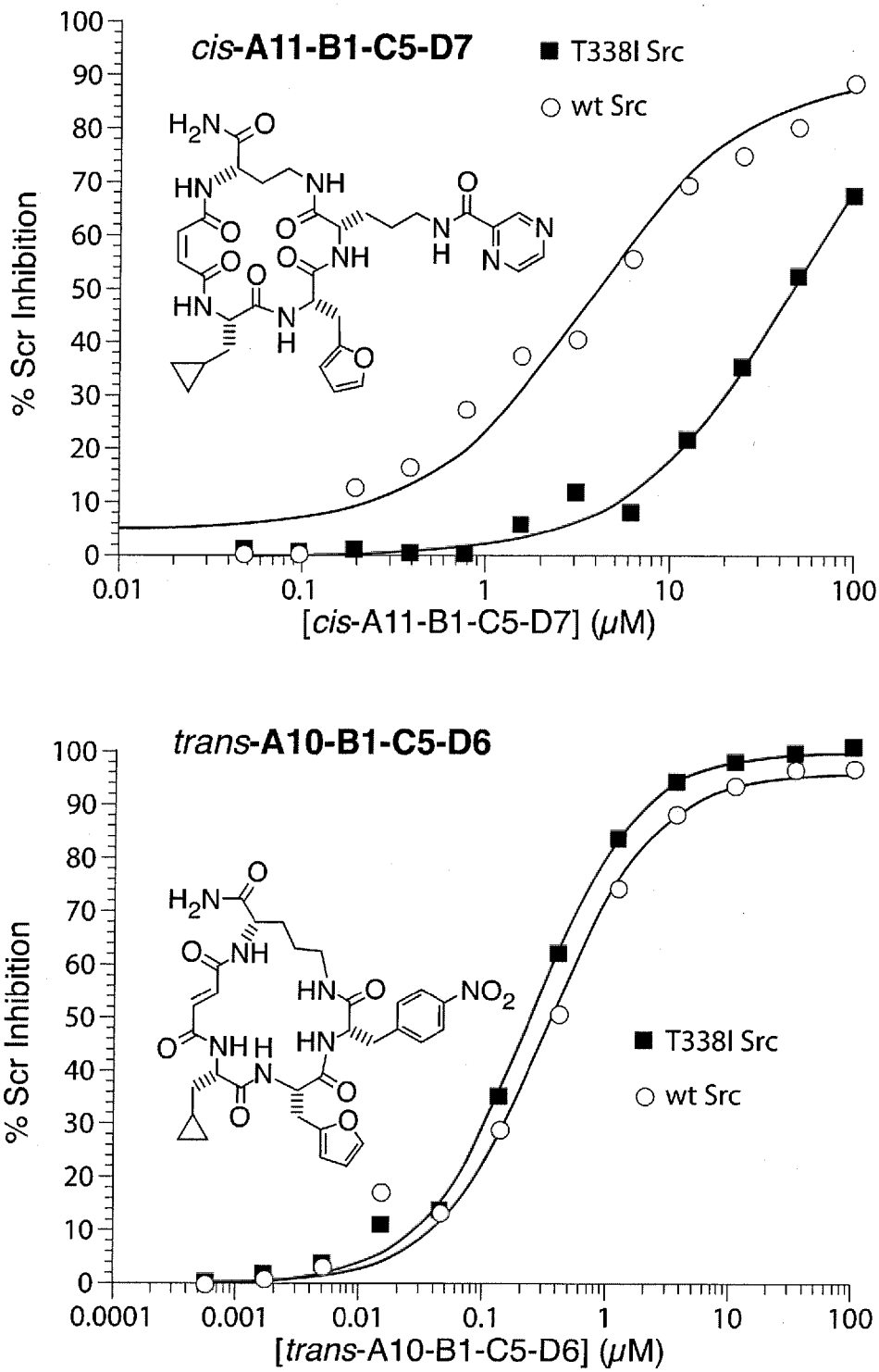

cis-A11-B1-C5-D7
T338I Src
wt Src
% Scr Inhibition
[cis-A11-B1-C5-D7] (μM)
trans-A10-B1-C5-D6
T338I Src
wt Src
% Scr Inhibition
[trans-A10-B1-C5-D6] (μM)

Fig. 22

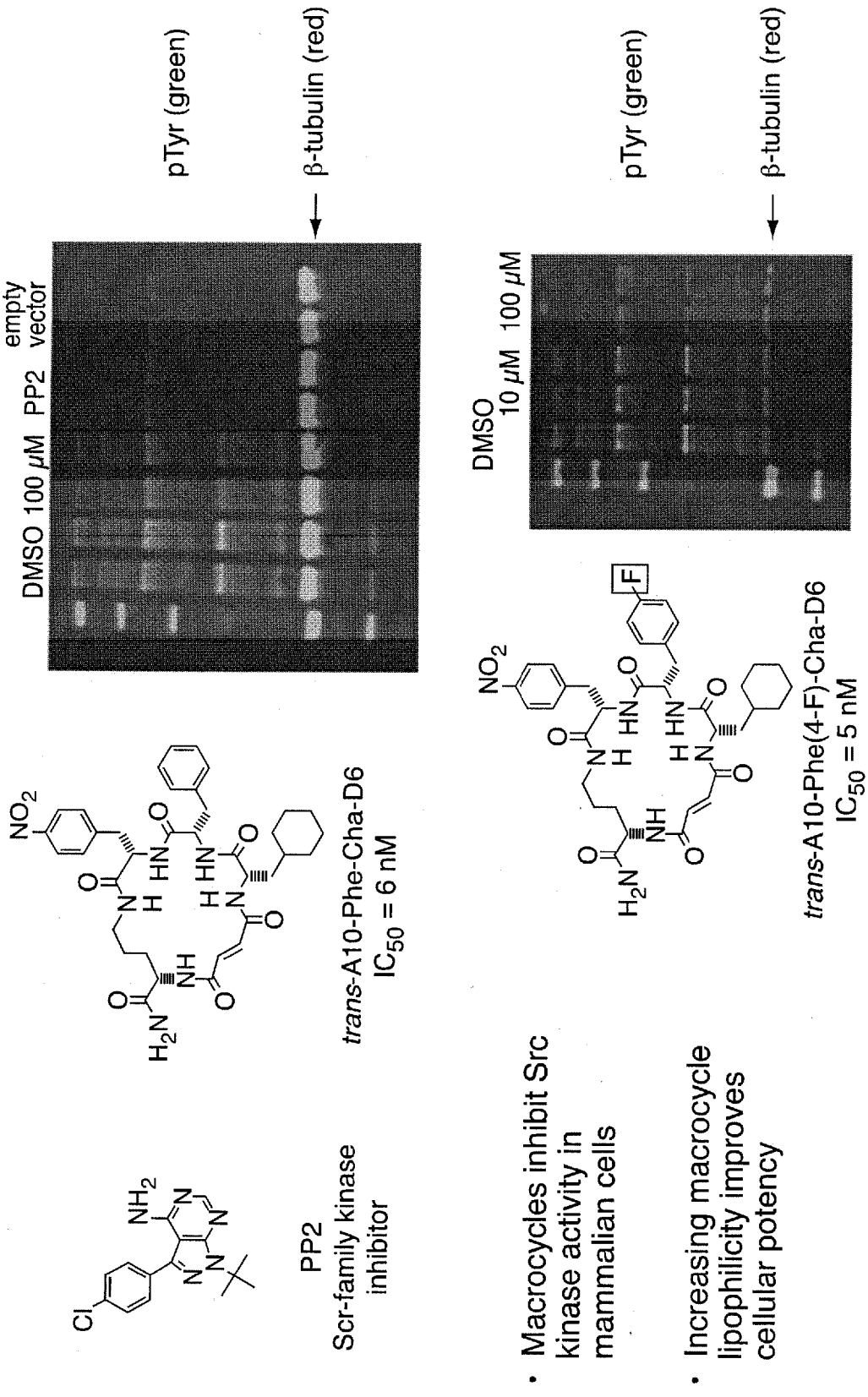

pTyr (green)
β-tubulin (red)
empty vector
DMSO
PP2 100 μM
pTyr (green)
β-tubulin (red)
DMSO
10 μM
100 μM
PP2
Scr-family kinase inhibitor
trans-A10-Phe-Cha-D6
IC50 = 6 nM
trans-A10-Phe(4-F)-Cha-D6
IC50 = 5 nM
• Macrocycles inhibit Src kinase activity in mammalian cells
• Increasing macrocycle lipophilicity improves cellular potency

Fig. 23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/45966

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/12; A61K 38/06; A61K 38/08 (2011.01)
USPC - 514/21.1; 514/21.9; 514/21.8
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)
IPC(8): A61K 38/12; A61K 38/06; A61K 38/08 (2011.01)
USPC: 514/21.1; 514/21.9; 514/21.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8): A61K 38/12; 38/00; 38/04; 38/06 (2011.01)
USPC: 530/317; 530/323; 530/332; 530/329; 530/330; 530/331

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST;PGPB, USPT, EPAB, JPAB, SureChem, PubChem, ScienceDirect: Macrocycles, cyclic peptide, Src, Akt3, MAPKAPK2, p38a, Pim1 kinases

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2008/0242598 A1 (FAIRLIE et al.) 2 October 2008 (02.10.2008) para [0046]-[0047], [0050]-[0052]	1-17, 24-158 and 160-167
A	US 2008/0139456 A1 (BURKE et al.) 12 June 2008 (12.06.2008) para [0009], [0047]	1-17, 24-158 and 160-167
A	US 2002/0068301 A1 (LAI et al.) 6 June 2002 (06.06.2002) para [0043], [0062]	1-17, 24-158 and 160-167
A	US 2006/0025566 A1 (HOVEYDA et al.) 2 February 2006 (02.02.2006) para [0030]	1-17, 24-158 and 160-167
A	US 2010/0105601 A2 (BRADY et al.) 29 April 2010 (29.04.2010) para [0031],[0034]	1-17, 24-158 and 160-167
T	PubChem CID 46938796. 2010-11-15. [Retrieved from the Internet 02 December 2011:<http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=46938796&loc=ec_rcs>]	1-17, 24-158 and 160-167

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 December 2011 (02.12.2002)	16 DEC 2011
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/45966

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 18-23 and 159
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.