

COMPOUNDS AND FORMULATIONS USEFUL AS VACCINE ADJUVANTS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application Serial No. 63/503,015 filed May 18, 2023, the entire contents of which are incorporated by reference herein.

BACKGROUND OF THE INVENTION

[0002] Many vaccines are poorly immunogenic or elicit an insufficient immune response that may not provide adequate protection against a targeted infectious agent. Therefore, an adjuvant may be required in order to help generate an immune response with sufficient magnitude, quality and persistence necessary for vaccine use. An adjuvant may be used in an immunogenic composition comprising an antigen of interest to enhance the immune response against the antigen, decrease the amount of antigen necessary to provoke the desired immune response or decrease the number of injections needed in a clinical regimen to induce a durable immune response and provide protection from disease. Despite the success of adjuvants for certain compositions, there exists a need for additional adjuvants that are safe for use in vaccines and immunogenic compositions and effective at increasing the immune response against an antigen of interest.

SUMMARY OF THE INVENTION

[0003] The invention relates to compounds of Formula I, Ia, II, IIa, III, IIIa, IV and IVa and to pharmaceutically acceptable salts thereof. Further, the invention relates to formulations and compositions comprising one or more of the compounds of Formulas I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salts thereof and a pharmaceutically acceptable carrier. Still further, the invention relates to formulations and compositions comprising one or more of the compounds of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier, wherein the formulations and compositions are useful as vaccines.

[0004] The invention also relates to formulations comprising i) one or more compounds of the invention or pharmaceutically acceptable salt(s) thereof; ii) one or more emulsifiers; and iii) one or more terpenes.

[0005] The invention also relates to formulations comprising i) one or more compounds of the invention or pharmaceutically acceptable salt(s) thereof; ii) one or more sorbitan-based surfactants; and iii) one or more terpenes.

[0006] The invention also relates to formulations comprising one or more of the compounds of Formula I, Ia, II, IIa, III, IIIa, IV or IVa or pharmaceutically acceptable salt(s) thereof and further comprising sorbitan trioleate (SPAN-85); polysorbate-20 (PS-20) or polysorbate-80 (PS-80), and squalene. In some embodiments, the formulations are prepared as stable nanoemulsions (herein referred to as “SNE adjuvant formulations” or “SNEs”).

[0007] The invention also relates to compositions comprising antigens and an SNE disclosed herein. As an example, a particular SNE comprises the compound *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, otherwise referred to as “Compound A-1”. This particular SNE is referred to as Compound A-1-SNE and comprises Compound A-1, SPAN-85, PS-20 or PS-80, and squalene. As another example, a particular SNE comprises the compound *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, otherwise referred to as “Compound B-1”. This particular SNE is referred to as Compound B-1-SNE and comprises Compound B-1, SPAN-85, PS-20 or PS-80, and squalene. As another example, a particular SNE comprises the compound *N*-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)stearamide, otherwise referred to as “Compound C-4”. This particular SNE is referred to as Compound C-4-SNE and comprises Compound C-4, SPAN-85, PS-20 or PS-80, and squalene.

[0008] The invention also relates to immunogenic compositions comprising antigens and an SNE disclosed herein.

[0009] The invention also relates to methods of treating or preventing a disease in a patient in need thereof by administering an immunogenic composition of the invention to the patient.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] Figure 1 (Example 10) shows the OD640 readout following incubation with different concentrations of Compound B-1-SNE with human TLR7 (top panel) or TLR8 (bottom panel) expressing HEK-Blue™ 293 cells (InvivoGen). The Y-axis represents total Compound B-1-SNE concentration.

[0011] Figure 2 (Example 11) shows pre-immune (day 0, pooled sera), post-dose 1 (day 21), post-dose 2 (day 42) and post-dose 3 (day 70) anti-6B IgG titers after immunization of mice with formulations described in Table 7. Error bars are geometric means with 95% confidence intervals. Transformed data analyzed by one-way ANOVA with Dunnett post-test, **p* < 0.05.

[0012] Figure 3 (Example 11) shows pre-immune (day 0), post-dose 1 (day 21), post-dose 2 (day 42) post-dose 3 (day 70) anti-6B OPA titers from pooled sera after immunization of mice with formulations described in Table 7.

[0013] Figure 4 (Example 12) shows the ratio of serotype specific IgG titers in infant rhesus macaques following immunization of PCV24 with Compound B-1-SNE (150 µg dose of Compound B-1), PCV24 with Compound B-1-SNE (50 µg Compound B-1), PCV24 with Compound B-1-SNE (15 µg Compound B-1) compared to an immunization of PCV24 formulated without adjuvant at post dose 3. Serotype 6C and 15B data are included to evaluate cross reactivity.

[0014] Figure 5 (Example 12) shows the ratio of serotype specific IgG titers in infant rhesus macaques following immunization with PCV24 with Compound B-1-SNE (150 µg dose of Compound B-1), PCV24 with Compound B-1-SNE (50 µg Compound B-1), PCV24 with Compound B-1-SNE (15 µg Compound B-1) compared to an immunization of PCV24 formulated with APA at post dose 3. Serotype 6C and 15B data are included to evaluate cross reactivity.

[0015] Figure 6 (Example 12) shows the ratio of serotype specific IgG titers in infant rhesus macaques following immunization of PCV24 with Compound B-1-SNE (150 µg dose of Compound B-1), PCV24 with Compound B-1-SNE (50 µg Compound B-1), PCV24 with Compound B-1-SNE (15 µg Compound B-1) compared to an immunization of PCV20 at post dose 3.

[0016] Figure 7 (Example 13) shows the ratio of serotype specific IgG titers in infant rhesus macaques following immunization of PCV24 with Compound B-1-SNE (100 µg dose of Compound B-1), PCV24 with Compound B-1-SNE (10 µg Compound B-1), PCV24 with Compound B-1-SNE (1 µg Compound B-1), PCV24 with Compound B-1-SNE (0.1 µg Compound B-1) compared to an immunization of PCV20 at post dose 3.

[0017] Figure 8 (Example 14) shows the ratio of serotype specific IgG titers in mice following immunization of PCV26 with Compound B-1-SNE (10 µg dose of Compound B-1) compared to an immunization of PCV26 formulated with APA at post dose 2. Serotype 6C and 15B data are included to evaluate cross reactivity.

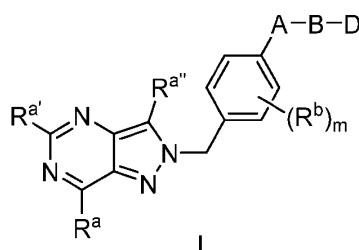
[0018] Figure 9 (Example 14) shows the IgG2a/IgG1 titer ratio of serotype specific antibodies in mice following immunization of PCV26 with Compound B-1-SNE (10 µg dose of Compound B-1) or PCV26 formulated with APA at post dose 3. Responses from three representative serotypes 18C, 19F and 24F are shown (left to right).

[0019] Figure 10A (Example 14) shows mice immunized with PCV26 with Compound B-1-SNE (10 µg dose of Compound B-1) or PCV26 formulated with APA are protected from *S. pneumoniae* 24F intra tracheal challenge. Bacteremia was quantified at 24 hours post-infection with serotype 24F bacteria and presented as log CFU/mL.

[0020] Figure 10B (Example 14) shows mice immunized with PCV26 with Compound B-1-SNE (10 µg dose of Compound B-1) or PCV26 formulated with APA are protected from *S. pneumoniae* 24F intra tracheal challenge. Survival was evaluated for approximately 240 hours post-infection with serotype 24F bacteria.

DETAILED DESCRIPTION OF THE INVENTION

[0021] The invention provides a compound having the structure set forth in Formula I:



wherein:

R^a is selected from H, -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, and -NR³R^{3'}, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, and -O(C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine; $R^{a'}$ is selected from H, -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, and -NR³R^{3'}, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, and -O(C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

$R^{a''}$ is selected from H, -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, and -NR³R^{3'}, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, and -O(C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from

the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

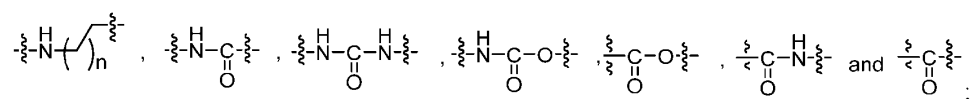
R' and R'' are independently selected from H, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, and (C₁-C₆)alkynyl, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl and (C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine, or R' and R'', together with the nitrogen to which they are attached, join together to form a (C₃-C₆)heterocycloalkyl, wherein said (C₃-C₆)heterocycloalkyl is optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

each occurrence of R^b is independently selected from H, -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, or NR'R'', wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, and -O(C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

A is a carbon- or nitrogen-linked spacer selected from (C₁-C₆)alkyl, (C₃-C₆)heterocycloalkyl, heterocycloalkyl-C(O)-R^z-, (C₁-C₄)alkyl-N(R^z)-R^z-, aryl, and heteroaryl, wherein said (C₁-C₆)alkyl, (C₃-C₆)heterocycloalkyl, aryl and heteroaryl are optionally substituted with one to six substituents, independently selected from the group consisting of -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, and NR'R'', wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, and -O(C₁-C₆)alkynyl are optionally substituted with one to six substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

each occurrence of R^z is independently H or (C₁-C₆)alkyl;

B is a functional group selected from



D is a lipid selected from (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl, wherein said (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl, and (C₆-C₂₀)alkynyl are optionally substituted with one to six

substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-

C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine; or D is $\begin{matrix} \text{Z} \\ | \\ \text{O} \\ | \\ \text{Z} \end{matrix}$;

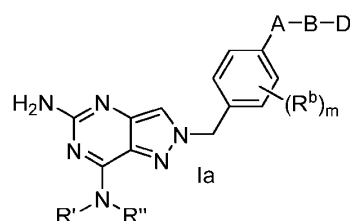
each occurrence of Z is independently selected from (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl, and (C₆-C₂₀)alkynyl, wherein said (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl, and (C₆-C₂₀)alkynyl are optionally substituted with one to six substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

m is 0, 1, 2, 3, 4 or 5; and

n is 0, 1, 2, 3, 4 or 5;

or a pharmaceutically acceptable salt thereof.

[0022] The invention provides a compound having the structure set forth in Formula Ia:

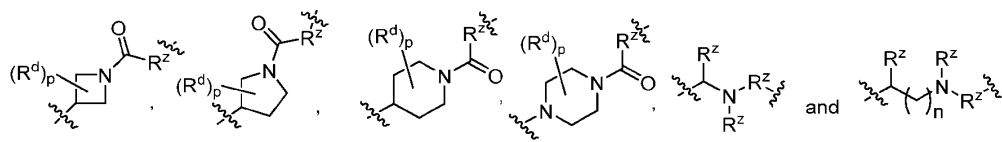


wherein:

R' and R'' are independently selected from H, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, and (C₁-C₆)alkynyl, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl and (C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine, or R' and R'', together with the nitrogen to which they are attached, join together to form a (C₃-C₆)heterocycloalkyl, wherein said (C₃-C₆)heterocycloalkyl is optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

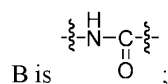
each occurrence of R^b is -O(C₁-C₄)alkyl, wherein said -O(C₁-C₄)alkyl is optionally substituted with one or two substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

A is selected from

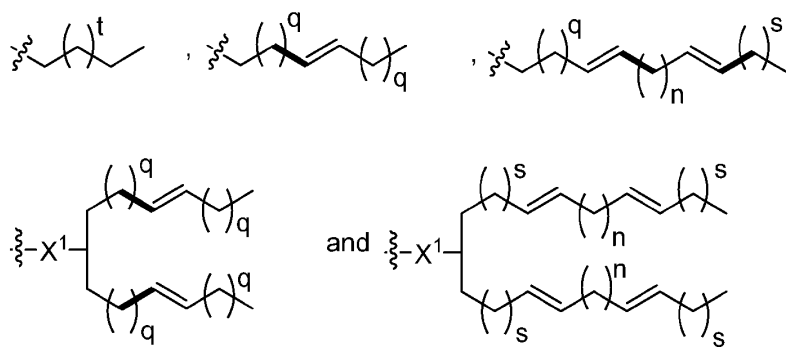


each occurrence of R^z is independently H or (C₁-C₆)alkyl;

each occurrence of R^d is independently selected from -OH, (C₁-C₄)alkyl, -O(C₁-C₄)alkyl, chlorine and fluorine;



D is a lipid chain selected from:



wherein any carbon on the lipid chain is optionally substituted with -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine,

wherein  is cis or trans stereochemistry,

X^1 is -O-, -C(R)²-, or -NR-, and

each occurrence of R is independently selected from H, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

m is 0, 1 or 2;

n is 0, 1, 2 or 3;

p is 0, 1 or 2;

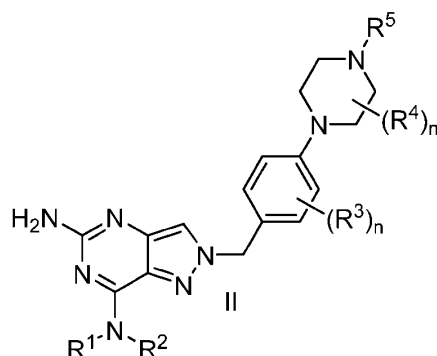
q is 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9;

s is, 1, 2, 3, 4, 5, 6, 7 or 8; and

t is 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18;

or a pharmaceutically acceptable salt thereof.

[0023] The invention provides a compound having the structure set forth in Formula II:



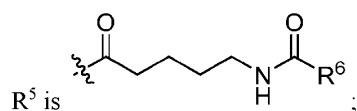
wherein:

R^1 is (C₁-C₆)alkyl, wherein said (C₁-C₆)alkyl is optionally substituted with one to four substituents selected from -OH and -O(CH₃);

R^2 is H, methyl or -O(CH₃);

each occurrence of R^3 is independently H, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl or -O(C₁-C₄)alkyl wherein said (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl or -O(C₁-C₄)alkyl are optionally substituted with one or two substituents independently selected from -OH and -O(CH₃);

each occurrence of R^4 is independently H, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl or -O(C₁-C₄)alkyl wherein said (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl or -O(C₁-C₄)alkyl are optionally substituted with one or two substituents independently selected from -OH and -O(CH₃);

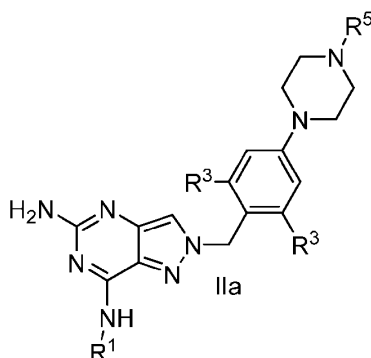


R^6 is selected from (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl, wherein said (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl, and (C₆-C₂₀)alkynyl are optionally substituted with one to six substituents independently selected from -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine; and

each occurrence of n is 4;

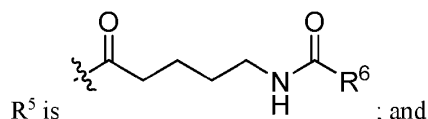
or a pharmaceutically acceptable salt thereof.

[0024] The invention provides a compound having the structure set forth in Formula IIa:



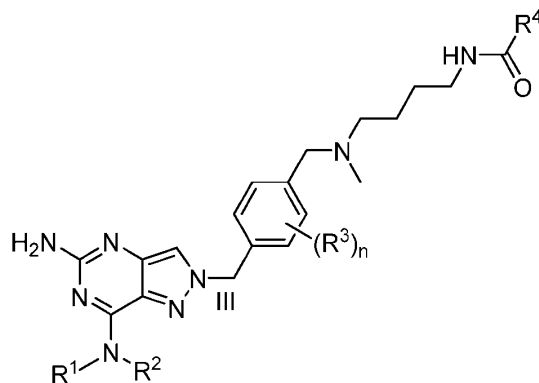
wherein:

R^1 is butyl, wherein said butyl is optionally substituted with one or two -OH;
each occurrence of R^3 is independently H or -O(CH₃); and



R^6 is selected from (C₁₀-C₂₀)alkyl, (C₁₀-C₂₀)alkenyl and (C₁₀-C₂₀)alkynyl;
or a pharmaceutically acceptable salt thereof.

[0025] The invention provides a compound having the structure set forth in Formula III:



wherein:

R^1 is (C₁-C₆)alkyl, wherein said (C₁-C₆)alkyl is optionally substituted with one to four substituents selected from -OH and -O(CH₃);

R^2 is H, methyl or -O(CH₃);

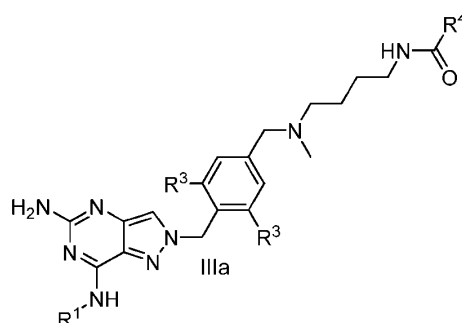
each occurrence of R^3 is independently H, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl or -O(C₁-C₄)alkyl wherein said (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl or -O(C₁-C₄)alkyl are optionally substituted with one or two substituents selected from -OH and -O(CH₃);

R^4 is selected from (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl, wherein said (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl are optionally substituted with one to six substituents selected from -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine; and

n is 4;

or a pharmaceutically acceptable salt thereof.

[0026] The invention provides a compound having the structure set forth in Formula IIIa:



wherein:

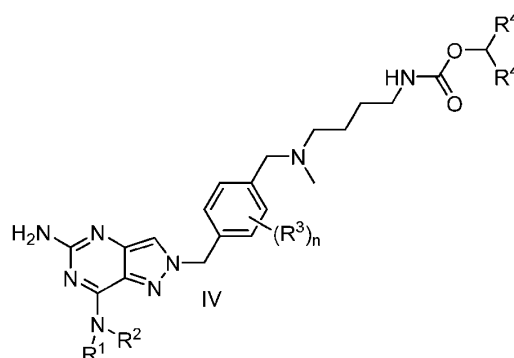
R^1 is butyl, wherein said butyl is optionally substituted with one or two -OH;

each occurrence of R^3 is independently H or -O(CH₃); and

R^4 is selected from (C₁₀-C₂₀)alkyl, (C₁₀-C₂₀)alkenyl and (C₁₀-C₂₀)alkynyl;

or a pharmaceutically acceptable salt thereof.

[0027] The invention provides a compound having the structure set forth in Formula IV:



wherein:

R^1 is (C₁-C₆)alkyl, wherein said (C₁-C₆)alkyl is optionally substituted with one to four substituents selected from -OH and -O(CH₃);

R^2 is H, methyl or -O(CH₃);

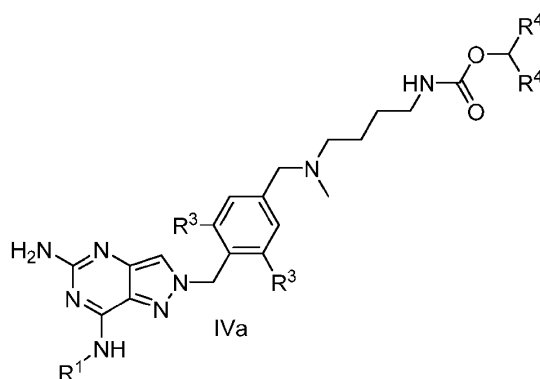
each occurrence of R^3 is independently H, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, or -O(C₁-C₄)alkyl wherein said (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, and -O(C₁-C₄)alkyl are optionally substituted with one or two substituents selected from -OH and -O(CH₃);

each occurrence of R^4 is independently selected from (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl, wherein said (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl are optionally substituted with one to six substituents selected from -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine or fluorine; and

n is 4;

or a pharmaceutically acceptable salt thereof.

[0028] The invention provides a compound having the structure set forth in Formula IVa:



wherein:

R^1 is butyl, wherein said butyl is optionally substituted with one or two -OH;

each occurrence of R^3 is independently H or -O(CH₃); and

each occurrence of R^4 is independently selected from (C₁₀-C₂₀)alkyl, (C₁₀-C₂₀)alkenyl and (C₁₀-C₂₀)alkynyl;

or a pharmaceutically acceptable salt thereof.

[0029] The invention provides the following compounds:

(*N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, also referred to as Compound A-1;

(*S*)-*N*-(5-(4-(4-((5-amino-7-((1-hydroxypentan-2-yl)amino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, also referred to as Compound A-2;

(*S*)-1-(4-(4-((5-amino-7-((1-hydroxypentan-2-yl)amino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)octadecan-1-one, also referred to as Compound A-3;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, also referred to as Compound B-1;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)tetradecanamide, also referred to as Compound B-2;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)oleamide, also referred to as Compound B-3;

(9*Z*,12*Z*)-*N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)octadeca-9,12-dienamide, also referred to as Compound B-4;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-1,4-diazepan-1-yl)-5-oxopentyl)stearamide, also referred to as Compound B-5;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperidin-1-yl)-5-oxopentyl)stearamide, also referred to as Compound B-6;

N-(5-(3-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)azetidin-1-yl)-5-oxopentyl)stearamide, also referred to as Compound B-7;

1-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-*N*-(3-stearamidopropyl)piperidine-4-carboxamide, also referred to as Compound B-8;

(1*s*,3*s*)-3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-*N*-octadecylcyclobutane-1-carboxamide, also referred to as Compound B-9;

(1*s*,3*s*)-3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-*N*-hexadecylcyclobutane-1-carboxamide, also referred to as Compound B-10;

N-(3-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-3-oxopropyl)stearamide, also referred to as Compound B-11;

N-(7-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-7-oxoheptyl)stearamide, also referred to as Compound B-12;

N-(3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)cyclobutyl)stearamide, also referred to as Compound B-13;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-4,4-dimethyl-5-oxopentyl)stearamide, also referred to as Compound B-14;

N-(6-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-methyl-6-oxohexan-2-yl)stearamide, also referred to as Compound B-15;

1-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-(octadecyloxy)pentan-1-one, also referred to as Compound B-16;

1-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-(octadecylamino)pentan-1-one, also referred to as Compound B-17;

N-(5-(4-(4-((5-amino-7-(butylamino)-3-methyl-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, also referred to as Compound B-18;

(9*Z*,12*Z*)-*N*-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)octadeca-9,12-dienamide, also referred to as Compound C-1;

N-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)tetradecanamide, also referred to as Compound C-2;

N-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)oleamide, also referred to as Compound C-3;

N-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)stearamide, also referred to as Compound C-4;

N-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)-4-oxobutyl)stearamide, also referred to as Compound C-5;

(6*Z*,9*Z*,28*Z*,31*Z*)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)carbamate, also referred to as Compound D-1;

4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-*N*-(3-stearamidopropyl)piperazine-1-carboxamide, also referred to as Compound D-2;

3-stearamidopropyl 4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazine-1-carboxylate, also referred to as Compound D-3;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-6,6,6-trifluorohexyl)stearamide, also referred to as Compound D-4;

N-(4-(4-((7-(butylamino)-5-hydroxy-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)stearamide, also referred to as Compound D-5; and

(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl (5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)carbamate, also referred to as Compound D-6;
or a pharmaceutically acceptable salt thereof.

[0030] The invention provides the following compound:

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, also referred to as Compound B-1;
or a pharmaceutically acceptable salt thereof.

[0031] The invention also provides a stable nanoemulsion comprising: (i) one or more compounds having the structure set forth in Formula I, Ia, II, IIa, III, IIIa, IV or IVa or pharmaceutically acceptable salt(s) thereof; ii) one or more emulsifiers; and iii) one or more terpenes.

[0032] The invention also provides a stable nanoemulsion comprising: (i) one or more compounds having the structure set forth in Formula I, Ia, II, IIa, III, IIIa, IV or IVa or pharmaceutically acceptable salt(s) thereof; ii) one or more sorbitan-based surfactants; and iii) one or more terpenes.

[0033] The invention also provides a stable nanoemulsion comprising: (i) *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, or pharmaceutically acceptable salt thereof; (ii) sorbitan trioleate (SPAN-85); (iii) polysorbate-20 (PS-20) or polysorbate-80 (PS-80); and (iv) squalene.

[0034] The invention also provides a stable nanoemulsion comprising: (i) at least one antigen; (ii) a compound having the structure set forth in Formula I, Ia, II, IIa, III, IIIa, IV or IVa or a pharmaceutically acceptable salt thereof; (iii) SPAN-85; (iv) PS-20 or PS-80; and (v) squalene.

[0035] The invention also provides a stable nanoemulsion comprising: (i) at least one antigen; (ii) *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, or pharmaceutically acceptable salt thereof; (iii) SPAN-85; (iv) PS-20 or PS-80; and (v) squalene.

[0036] The invention also provides a pharmaceutical composition comprising: (i) at least one antigen; (ii) a compound having the structure set forth in Formula I, Ia, II, IIa, III, IIIa, IV or IVa or a pharmaceutically acceptable salt thereof; (iii) SPAN-85; (iv) PS-20 or PS-80; (v) squalene; and (vi) a pharmaceutically acceptable carrier.

[0037] The invention also provides a pharmaceutical composition comprising: (i) at least one antigen; (ii) *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, or pharmaceutically acceptable salt

thereof; (iii) SPAN-85; (iv) PS-20 or PS-80; (v) squalene; and (vi) a pharmaceutically acceptable carrier.

[0038] The invention also provides an immunogenic composition comprising: (i) at least one antigen; (ii) a compound having the structure set forth in Formula I, Ia, II, IIa, III, IIIa, IV or IVa or a pharmaceutically acceptable salt thereof; (iii) SPAN-85; (iv) PS-20 or PS-80; (v) squalene; and (vi) a pharmaceutically acceptable carrier.

[0039] The invention also provides an immunogenic composition comprising: (i) at least one antigen; (ii) *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, or pharmaceutically acceptable salt thereof; (iii) SPAN-85; (iv) PS-20 or PS-80; (v) squalene; and (vi) a pharmaceutically acceptable carrier.

[0040] The invention also provides a single-dose vaccine composition comprising: (i) at least one antigen; (ii) a compound having the structure set forth in Formula I, Ia, II, IIa, III, IIIa, IV or IVa or a pharmaceutically acceptable salt thereof; (iii) SPAN-85; (iv) PS-20; (v) squalene; and (vi) a pharmaceutically acceptable carrier, wherein a single dose of the vaccine composition is sufficient to elicit a desired immune response against the at least one antigen.

[0041] The invention also provides a single-dose vaccine composition comprising: (i) at least one antigen; (ii) *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, or pharmaceutically acceptable salt thereof; (iii) SPAN-85; (iv) PS-20; (v) squalene; and (vi) a pharmaceutically acceptable carrier, wherein a single dose of the vaccine composition is sufficient to elicit a desired immune response against the at least one antigen.

[0042] The invention also provides a method of treating or preventing diseases in a patient comprising administering to the patient a pharmaceutical composition comprising: (i) at least one antigen; (ii) a compound having the structure set forth in Formula I, Ia, II, IIa, III, IIIa, IV or IVa or a pharmaceutically acceptable salt thereof; (iii) SPAN-85; (iv) PS-20; (v) squalene; and (vi) a pharmaceutically acceptable carrier.

[0043] The invention also provides a method of treating or preventing diseases in a patient comprising administering to the patient a pharmaceutical composition comprising: (i) at least one antigen; (ii) *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, or pharmaceutically acceptable salt thereof; (iii) SPAN-85; (iv) PS-20; (v) squalene; and (vi) a pharmaceutically acceptable carrier.

[0044] In some embodiments of the methods disclosed herein, the patient is a human.

[0045] In some embodiments of the methods disclosed herein, the patient is a non-human animal.

[0046] In some embodiments of the i) stable nanoemulsions, ii) pharmaceutical compositions, iii) immunogenic compositions, and iv) single-dose vaccine compositions above, the concentration of compound (i.e., (a) a compound having the structure set forth in Formula I, Ia, II, IIa, III, IIIa, IV or IVa or a pharmaceutically acceptable salt thereof or (b) *N*-(5-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, or pharmaceutically acceptable salt thereof) is 0.01 µg/mL to 1000 µg/mL or 0.1 µg/mL to 100 µg/mL or 80 µg/mL or 16 µg/mL or 4 µg/mL.

[0047] In some embodiments of the i) stable nanoemulsions, ii) pharmaceutical compositions, iii) immunogenic compositions, and iv) single-dose vaccine compositions above, the concentration of SPAN-85 is 0.001 mg/mL to 100 mg/mL or 0.01 mg/mL to 50 mg/mL or 0.1 mg/mL to 10 mg/mL or 0.001 mg/mL to 30 mg/mL or 0.2 mg/mL to 10 mg/mL.

[0048] In some embodiments of the i) stable nanoemulsions, ii) pharmaceutical compositions, iii) immunogenic compositions, and iv) single-dose vaccine compositions above, the concentration of PS-20 or PS-80 0.001 mg/mL to 100 mg/mL or 0.01 mg/mL to 50 mg/mL or 0.1 mg/mL to 10 mg/mL or 0.001 mg/mL to 30 mg/mL or 0.2 mg/mL to 10 mg/mL.

[0049] In some embodiments of the i) stable nanoemulsions, ii) pharmaceutical compositions, iii) immunogenic compositions, and iv) single-dose vaccine compositions above, the concentration of squalene is 0.01 mg/mL to 100 mg/mL or 0.02 mg/mL to 20 mg/mL or 1 mg/mL to 20 mg/mL or 0.03 mg/mL to 30 mg/mL or 0.5 mg/mL to 20 mg/mL.

[0050] In some embodiments of the i) stable nanoemulsions, ii) pharmaceutical compositions, iii) immunogenic compositions, and iv) single-dose vaccine compositions above, the concentration of compound is 80 µg/mL, the concentration of SPAN-85 is 4.8 mg/mL, the concentration of PS-20 or PS-80 is 4.8 mg/mL, and the concentration of squalene is 16 mg/mL.

[0051] In some embodiments of the i) stable nanoemulsions, ii) pharmaceutical compositions, iii) immunogenic compositions, and iv) single-dose vaccine compositions above, the concentration of compound is 16 µg/mL, the concentration of SPAN-85 is 4.8 mg/mL, the concentration of PS-20 or PS-80 is 4.8 mg/mL, and the concentration of squalene is 16 mg/mL.

[0052] In some embodiments of the i) stable nanoemulsions, ii) pharmaceutical compositions, iii) immunogenic compositions, and iv) single-dose vaccine compositions above, the concentration of compound is 4 µg/mL, the concentration of SPAN-85 is 4.8 mg/mL, the concentration of PS-20 or PS-80 is 4.8 mg/mL, and the concentration of squalene is 16 mg/mL.

[0053] In some embodiments of the i) stable nanoemulsions, ii) pharmaceutical compositions, iii) immunogenic compositions, and iv) single-dose vaccine compositions above, the nanoemulsions and/or the compositions further comprise L-met and EDTA.

DEFINITIONS

The following abbreviations are used herein:

[Ni(dtbbpy)(H ₂ O) ₄]Cl ₂	tetraaquo[4,4'-bis(1,1-dimethylethyl)-2,2'-bipyridine]nickel(II) dichloride
AcOH	acetic acid
Anh	Anhydrous
APA	aluminum phosphate adjuvant
Aq	Aqueous
Bn	Benzyl
Boc or BOC	<i>tert</i> -butoxycarbonyl
BOP	(benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate)
Bs	broad singlet
BSI	bloodstream infection
BTMG	2- <i>tert</i> -butyl-1,1,3,3-tetramethylguanidine
Bz	Benzoyl
calc'd	Calculated
Cbz	Benzylloxycarbonyl
CD ₃ OD	deuterated methanol
CDCl ₃	deuterated chloroform
CDI	carbonyl diimidazole
Celite	diatomaceous earth
CH ₃ CN	Acetonitrile
CMBP	(cyanomethylene)tributylphosphorane
CRAB	carbapenem-resistant <i>Acinetobacter baumannii</i>
Cs ₂ CO ₃	cesium carbonate
D	Doublet
DBa	Dibenzylideneacetone
DBAB	di- <i>tert</i> -butyl azodicarboxylate
DBU	1,8-diazabicyclo(5.4.0)undec-7-ene
DCE	Dichloroethane
DCM	Dichloromethane
DIEA or DIPEA	<i>N,N</i> -diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	<i>N,N</i> -dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
Dppf	1,1'-bis(diphenylphosphino)ferrocene
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
ESI	electrospray ionization

Et	Ethyl
Et ₂ O	diethyl ether
Et ₃ N	Triethylamine
EtOAc	ethyl acetate
EtOH	Ethanol
FL	Compound
G	Gram
H	Hour
HAP	hospital-acquired pneumonia
HATU	1-[bis(dimethylamino)methylene]-1 <i>H</i> -1,2,3-triazolo[4,5- b]pyridinium 3-oxide hexafluorophosphate
HCl	hydrochloric acid
Hex	Hexanes
HMDS	hexamethyldisilazane
HPLC	high-performance liquid chromatography
HPSEC	high performance size exclusion chromatography
IBX	2-iodoxybenzoic acid
IM	inner membrane
Int	Intermediate
IPA	Isopropanol
iPr	Isopropyl
Ir[df(CF ₃)ppy] ₂ (dtbbpy)PF ₆	[4,4'-bis(1,1-dimethylethyl)-2,2'-bipyridine- <i>N</i> 1, <i>N</i> 1']bis[3,5- difluoro-2-[5-(trifluoromethyl)-2-pyridinyl- <i>N</i>]phenyl- C]Iridium(III) hexafluorophosphate
K ₂ CO ₃	potassium carbonate
LC	liquid chromatography
LC/MS	liquid chromatography mass spectrometry
LED	light emitting diode
LOS	Lipooligosaccharide
LPS	Lipopolysaccharide
M	Multiplet
MDR	multi-drug resistant
Me	Methyl
Me ₂ S	dimethyl sulfide
MeCN	Acetonitrile
MeOH	Methanol
Mg	Milligrams
MgSO ₄	magnesium sulfate
Min	Minutes
mL	Milliliters
Mmol	Millimoles
MP-(OAc) ₃ BH	MP-triacetoxyborohydride
MS	mass spectrometry

Mw	molecular weight
Na ₂ SO ₄	sodium sulfate
NaBH(OAc) ₃	sodium triacetoxyborohydride
NaBH ₄	sodium borohydride
NaN ₃	sodium azide
NaOH	sodium hydroxide
NaOMe	sodium methoxide
NaOtBu	sodium <i>tert</i> -butoxide
n-Bu	n-butyl
NCS	<i>N</i> -chlorosuccinimide
NH ₄ OH	ammonium hydroxide
NMI	<i>N</i> -methylimidazole
NMR	nuclear magnetic resonance spectroscopy
NMWCO	nominal molecular weight cut off
OAc	Acetate
OM	outer membrane
PCV	pneumococcal conjugate vaccine
Pd	Palladium
PD1	post dose 1
PD2	post dose 2
PD3	post dose 3
Pet.	Petroleum
Ph	Phenyl
PMe ₃	Trimethylphosphine
PnPs	pneumococcal polysaccharide
PPh ₃	Triphenylphosphine
Ppm	parts per million
prep-TLC	preparative thin layer chromatography
Ps	Polysaccharide
PS-20	polysorbate-20
PS-80	polysorbate-80
Psi	pounds per square inch
Py	Pyridine
Q	Quartet
RT or rt	room temperature
RuPhos	2-dicyclohexylphosphino-2',6'-diisopropoxybiphenyl
RuPhos Pd G2	chloro(2-dicyclohexylphosphino-2',6'-diisopropoxy-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II)
S	Singlet
Sat	Saturated
SFC	supercritical fluid chromatography

SNE	stable nanoemulsion
SPAN-85	sorbitan-trioleate
ST	Serotype
T	Triplet
TBAF	<i>tert</i> -butyl ammonium fluoride
TBDPS	<i>tert</i> -butyldiphenylsilyl
TBDPSCl	<i>tert</i> -butyldiphenylsilyl chloride
TBS or TBDMS	<i>tert</i> -butyldimethylsilyl
TBSCl	<i>tert</i> -butyldimethylsilyl chloride
t-Bu	<i>tert</i> -butyl
TCFH	chloro- <i>N,N,N',N'</i> -tetramethylformamidine hexafluorophosphate
TEA	Triethylamine
TFA	trifluoroacetic acid
THF	Tetrahydrofuran
TLC	thin layer chromatography
TMS	Trimethylsilyl
TMSBr	trimethylsilyl bromide
TMSCl	Chlorotrimethylsilane
TMSN ₃	trimethylsilyl azide
Tris	tris(hydroxymethyl)aminomethane
UPLC	ultra-performance liquid chromatography
w/v	weight per volume
μL	Microliters

[0054] As used throughout the specification and in the appended claims, the singular forms “a,” “an,” and “the” include the plural reference unless the context clearly dictates otherwise.

[0055] The term “about,” when used herein in reference to a value, refers to a value that is the same as or, in context, is similar to the referenced value. In general, those skilled in the art, familiar with the context, will appreciate the absolute amount and/or relative degree of difference encompassed by “about” in that context. For example, in some embodiments, the term “about” can encompass a range of values that are within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less of the referenced value.

[0056] An “adjuvant,” as defined herein, refers to a compound or compound formulation or composition that serves to enhance the immunogenicity of a composition of the invention. An adjuvant may i) enhance an immune response to an antigen (for example, a pneumococcal polysaccharide) that is weakly immunogenic when administered alone, e.g., inducing no or weak antibody titers or cell-mediated immune response, ii) increase antibody titers to the antigen,

and/or iii) lower the dose of the antigen effective to achieve an immune response in the individual.

[0057] As used herein, the term “administration” refers to the act of providing an active agent, composition, or formulation to a subject. Exemplary routes of administration to the human body can be through the eyes (ophthalmic), mouth (oral), skin (transdermal), nose (nasal), lungs (inhalant), rectal, vaginal, oral mucosa (buccal), ear, by injection (e.g., intravenously (IV), subcutaneously, intratumorally, intraperitoneally, intramuscular (IM), intradermal (ID) etc.) and the like.

[0058] As used herein, an “agent” refers to a particle, compound, molecule, or entity of any chemical class.

[0059] As used herein, the term “alkyl” refers to a straight chain, cyclic or branched saturated aliphatic hydrocarbon having the specified number of carbon atoms. A numerical range, which refers to the chain length in total, may be given. For example, C₁-C₆ alkyl has a chain length of 1 to 6 atoms. An alkyl group can be optionally substituted with one or more “substituents” which may be the same or different and are as defined herein below. Unless otherwise indicated, an alkyl group is unsubstituted.

[0060] As used herein, the term “alkenyl” means a straight chain, cyclic or branched unsaturated aliphatic hydrocarbon having the specified number of carbon atoms including but not limited to diene, triene and tetraene unsaturated aliphatic hydrocarbons. An alkenyl group can be optionally substituted with one or more “substituents” which may be the same or different and are as defined herein below. Unless otherwise indicated, an alkenyl group is unsubstituted.

[0061] As used herein, the term “alkynyl” means a straight chain, cyclic or branched unsaturated aliphatic hydrocarbon having the specified number of carbon atoms including but not limited to diene, triene and tetraene unsaturated aliphatic hydrocarbons. An alkynyl group can be optionally substituted with one or more “substituents” which may be the same or different and are as defined herein below. Unless otherwise indicated, an alkynyl group is unsubstituted.

[0062] As used herein, the term “antigen” refers to any antigen that can generate one or more immune responses. The antigen may be a protein, peptide or polypeptide. In certain embodiments, the antigen is a lipid or a carbohydrate. In certain embodiments, the antigen is a polysaccharide. In certain embodiments the antigen is a pneumococcal polysaccharide. In certain embodiments the polysaccharide is an *S. pneumoniae* polysaccharide. The antigen may be one that generates a humoral and/or CTL immune response.

[0063] As used herein, the term “aryl” refers to a carbocycle aromatic monocyclic or bicyclic ring system comprising from about 6 to about 14 carbon atoms. In one embodiment, an aryl

group contains from about 6 to about 10 carbon atoms. An aryl group can be optionally substituted with one or more "ring system substituents" which may be the same or different and are as defined herein below. Non-limiting examples of aryl groups include phenyl and naphthyl. In one embodiment, an aryl group is phenyl. Unless otherwise indicated, an aryl group is unsubstituted.

[0064] As used herein, the term "composition" refers to a formulation containing an active pharmaceutical or biological ingredient (for example, a pneumococcal polysaccharide-carrier protein conjugate and a compound), along with one or more additional components. The term "composition" is used interchangeably with "pharmaceutical composition" and "formulation". The compositions can be liquid or solid (e.g., lyophilized). Additional components that may be included as appropriate include pharmaceutically acceptable excipients, additives, diluents, buffers, sugars, amino acids, chelating agents, surfactants, polyols, bulking agents, stabilizers, lyo-protectants, solubilizers, emulsifiers, salts, adjuvants, tonicity enhancing agents, delivery vehicles, and anti-microbial preservatives. Compositions are nontoxic to recipients at the dosages and concentrations employed.

[0065] As used herein the term "comprises" when used with the composition of the invention refers to the inclusion of any other components, such as adjuvants and excipients, or the addition of one or more polysaccharide-carrier protein conjugates that are not specifically enumerated.

[0066] As used herein, the term "consisting of" and variations such as "consist of" when used with a multivalent polysaccharide mixture or a multivalent polysaccharide-carrier protein conjugate mixture refers to a mixture having those particular *S. pneumoniae* polysaccharides or *S. pneumoniae* polysaccharide-carrier protein conjugates and no other *S. pneumoniae* polysaccharides or *S. pneumoniae* polysaccharide-carrier protein conjugates from a different serotype.

[0067] As used herein, the term "consists essentially of" and variations such as "consist essentially of" or "consisting essentially of," indicate the inclusion of any recited elements or group of elements, and the optional inclusion of other elements, of similar or different nature than the recited elements, which do not materially change the basic or novel properties of the specified dosage regimen, method, or composition.

[0068] As used herein, the term "de-O-acetylated-15B" or "de-O-acetyl-15B" or "de-O-Ac-15B" refers to a de-O-acetylated serotype 15B wherein the O-acetyl content is less than 10% per repeating unit. In another embodiment the O-acetyl content is less than 5% per repeating unit. In another embodiment the O-acetyl content is less than about 1% per repeating unit. In another embodiment the O-acetyl content is less than 1% per repeating unit. In another embodiment the

O-acetyl content is less than 0.5% per repeating unit. In another embodiment the O-acetyl content is less than 0.1% per repeating unit. In another embodiment the O-acetyl content is 0% per repeating unit. Processes for de-O-acetylation are known in the art, for example as described in Rajam et al., *Clinical and Vaccine Immunology*, 2007, 14(9):1223-1227.

[0069] As used herein, the term “dose” means a quantity of an agent, API (active pharmaceutical ingredient), formulation, composition, pharmaceutical composition or immunogenic composition taken or recommended to be taken at a particular time.

[0070] As used herein, the term “heteroaryl” refers to an aromatic monocyclic or multicyclic ring system comprising about 5 to about 14 ring atoms, wherein from 1 to 4 of the ring atoms is independently O, N or S and the remaining ring atoms are carbon atoms. In one embodiment, a heteroaryl group has 5 to 10 ring atoms. In another embodiment, a heteroaryl group is monocyclic and has 5 or 6 ring atoms. In another embodiment, a heteroaryl group is bicyclic. A heteroaryl group can be optionally substituted by one or more “ring system substituents” which may be the same or different and are as defined herein below. A heteroaryl group is joined via a ring carbon atom, and any nitrogen atom of a heteroaryl can be optionally oxidized to the corresponding N-oxide. In one embodiment, a heteroaryl group is a 5-membered heteroaryl. In another embodiment, a heteroaryl group is a 6-membered heteroaryl. In another embodiment, a heteroaryl group comprises a 5- to 6-membered heteroaryl group fused to a benzene ring. Unless otherwise indicated, a heteroaryl group is unsubstituted.

[0071] As used herein, the term “heterocycloalkyl” means a saturated or partly unsaturated non-aromatic monocyclic, bicyclic (including spirocyclic) or bridged carbocyclic ring or ring system comprising 3 to about 11 ring atoms, containing at least one ring heteroatom selected from N, S and O and the remainder of the ring atoms are carbon atoms. A heterocycloalkyl group can be joined via a ring carbon, or ring nitrogen atom, unless specified otherwise. The heterocycloalkyl ring may be substituted on the ring carbons and/or the ring nitrogen(s). In one embodiment, a heterocycloalkyl group is monocyclic and has from about 3 to about 7 ring atoms. In another embodiment, a heterocycloalkyl group is monocyclic has from about 4 to about 7 ring atoms. In other embodiments, the heterocycloalkyl group is bicyclic and has 7-10 ring atoms, 8-10 ring atoms, or 9 or 10 ring atoms. In still another embodiment, a heterocycloalkyl group is monocyclic and has 5 or 6 ring atoms. In one embodiment, a heterocycloalkyl group is monocyclic. In another embodiment, a heterocycloalkyl group is bicyclic. The heterocycloalkyl groups may be substituted. In some embodiments, the heterocycloalkyl groups have 1-2 heteroatoms selected from nitrogen, sulfur and oxygen atoms in the ring. In some embodiments, the heterocycloalkyl groups have 1 heteroatom selected from nitrogen, sulfur and oxygen atoms

in the ring. In some embodiments, the heteroatoms are selected from O, S, S(O), S(O)₂, and —NH—, —N(alkyl)–. Non-limiting examples include ethers, thioethers, amines, hydroxymethyl, 3-hydroxypropyl, 1,2-dihydroxyethyl, 2-methoxyethyl, 2-aminoethyl, 2-dimethylaminoethyl, and the like an aliphatic group containing a heteroatom.

[0072] As used herein, the term “immunogenic” or “immunogenicity” refers to the ability of an antigen (for example, an *S. pneumoniae* polysaccharide) to provoke an immune response in a subject. The term “immunogenic composition” refers to the ability of an agent, API, formulation, composition or pharmaceutical composition to provoke an immune response in a subject.

[0073] As used herein, the phrase “in need of treatment” include those previously exposed to or infected with a disease-causing agent, those who were previously vaccinated against a disease-causing agent, as well as those prone to have an infection or any person in which a reduction in the likelihood of infection is desired, e.g., the immunocompromised, the elderly, children, adults, or healthy individuals.

[0074] As used herein, the phrase “indicated for the prevention of disease” means that a vaccine or composition is approved by one or more regulatory authorities, such as the US Food and Drug Administration, for the prophylaxis of one or more diseases caused by an infectious agent or antigen. Infectious agents or antigens include HIV, HPV, CMV, VZV, EBV, adenovirus, coronavirus and influenza, among other viruses. Diseases include bacterial diseases such as tuberculosis and pneumococcal pneumoniae, and others.

[0075] As used herein, the term “multiple-dose” refers to a vaccine composition, or pharmaceutical composition, or an immunogenic composition that requires more than one dose or administration or injection of the components therein in a clinical regimen to induce a durable immune response and provide protection from a disease or to decrease the likelihood of infection with an infectious agent. One of skill in the art would understand how to determine a durable immune response, e.g., by measuring antibody titers over a specified period of time.

[0076] As used herein, a “patient” (alternatively referred to herein as a “subject”) refers to a mammal capable of being infected with a disease-causing agent. In some embodiments, the patient is a human. A patient can be treated prophylactically or therapeutically. Prophylactic treatment provides sufficient protective immunity to reduce the likelihood or severity of an infection or the effects thereof. Therapeutic treatment can be performed to reduce the severity or prevent recurrence of an infection or the clinical effects thereof. Prophylactic treatment can be performed using a composition or vaccine or immunogenic composition of the invention, as described herein. The compositions or vaccines or immunogenic compositions of the invention

can be administered to the general population or to those persons at an increased risk infection, e.g., the elderly, or those who live with or care for the elderly.

[0077] As used herein, the term “PCV1” refers to a 1-valent pneumococcal conjugate vaccine or composition comprising one *S. pneumoniae* polysaccharide-carrier protein conjugate, comprising capsular polysaccharide from a *S. pneumoniae* serotype conjugated to a carrier protein. In specific embodiments, the carrier protein is CRM197.

[0078] As used herein, the term “PCV24” refers to a 24-valent pneumococcal conjugate vaccine or composition comprising twenty-three *S. pneumoniae* polysaccharide-carrier protein conjugates, each comprising capsular polysaccharide from a *S. pneumoniae* serotype conjugated to a carrier protein, wherein the serotypes of *S. pneumoniae* are: 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 18C, 19A, 19F, 22F, 23B, 24F, 31, 33F and 35B, and at least one of the following serogroup 15 serotypes: 15B, 15C or de-O-acetylated-15B. In a particular embodiment, the serogroup 15 serotype is serotype 15C or de-O-acetylated 15B. In another embodiment, the serogroup 15 serotype is serotype de-O-acetylated 15B. In specific embodiments, the carrier protein of one or more of the *S. pneumoniae* polysaccharide-carrier protein conjugates is CRM197. In further embodiments, the carrier protein of each of the *S. pneumoniae* polysaccharide-carrier protein conjugates is CRM197.

[0079] As used herein, the term “PCV26” refers to a 26-valent pneumococcal conjugate vaccine or composition comprising twenty-five *S. pneumoniae* polysaccharide-carrier protein conjugates, each comprising capsular polysaccharide from a *S. pneumoniae* serotype conjugated to a carrier protein, wherein the serotypes of *S. pneumoniae* are: 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 16F, 18C, 19A, 19F, 22F, 23A, 23B, 24F, 31, 33F and 35B, and at least one of the following serogroup 15 serotypes: 15B, 15C or de-O-acetylated-15B. In a particular embodiment, the serogroup 15 serotype is serotype 15C or de-O-acetylated 15B. In another embodiment, the serogroup 15 serotype is serotype de-O-acetylated 15B. In specific embodiments, the carrier protein of one or more of the *S. pneumoniae* polysaccharide-carrier protein conjugates is CRM197. In further embodiments, the carrier protein of each of the *S. pneumoniae* polysaccharide-carrier protein conjugates is CRM197.

[0080] As used herein, the term “pharmaceutically acceptable”, with respect to a carrier, diluent or excipient of a pharmaceutical composition, indicates that a carrier, diluent, or excipient must be compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

[0081] As used herein, the term “pharmaceutical composition” refers to a composition containing an active pharmaceutical or biological ingredient, along with one or more additional

components, e.g., a composition in which an active agent is formulated together with one or more pharmaceutically acceptable carriers. As used herein, the terms “pharmaceutical formulation” and “formulation” are used interchangeably with “pharmaceutical composition.” In some embodiments, the active agent is present in a unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. The pharmaceutical compositions or formulations can be liquid or solid (e.g., lyophilized). Additional components that may be included as appropriate include pharmaceutically acceptable excipients, additives, diluents, buffers, sugars, amino acids, chelating agents, surfactants, polyols, bulking agents, stabilizers, lyo-protectants, solubilizers, emulsifiers, salts, adjuvants, tonicity enhancing agents, delivery vehicles, and anti-microbial preservatives. The pharmaceutical compositions or formulations are nontoxic to recipients at the dosages and concentrations employed. In some embodiments, a pharmaceutical composition can be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, e.g., those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces. In some embodiments, the term formulation refers to a single-dose of vaccine, which can be included in any volume suitable for injection.

[0082] As used herein, the terms “pneumococcal conjugate” or “pneumococcal polysaccharide-carrier protein conjugate” refer to an *S. pneumoniae* polysaccharide-carrier protein conjugate.

[0083] As used herein, the term “pneumococcal conjugate vaccine” (or “PCV”) is a pharmaceutical preparation or composition comprising pneumococcal polysaccharide-carrier protein conjugate(s) that provide active immunity to disease or pathological conditions caused by serotype(s) of *S. pneumoniae*.

[0084] As used herein, the term, “ring system substituent” refers to a substituent group attached to an aromatic or non-aromatic ring system which, for example, replaces an available hydrogen on the ring system. Ring system substituents may be the same, or different, and are each independently selected. Examples of ring system substituents include alkyl, alkenyl, alkynyl, aryl, heteroaryl, -OH, hydroxyalkyl, haloalkyl, -O-alkyl, -O-haloalkyl, -alkylene-O-alkyl, -O-

aryl, -O-alkylene-aryl, acyl, -C(O)-aryl, halo, -NO₂, -CN, -SF₅, -C(O)OH, -C(O)O-alkyl, -C(O)O-aryl, -C(O)O-alkylene-aryl, -S(O)-alkyl, -S(O)₂-alkyl, -S(O)-aryl, -S(O)₂-aryl, -S(O)-heteroaryl, -S(O)₂-heteroaryl, -S-alkyl, -S-aryl, -S-heteroaryl, -S-alkylene-aryl, -S-alkylene-heteroaryl, -S(O)₂-alkylene-aryl, -S(O)₂-alkylene-heteroaryl, cycloalkyl, heterocycloalkyl, -O-C(O)-alkyl, -O-C(O)-aryl, and -O-C(O)-cycloalkyl. A further example of ring system substituents include (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine.

[0085] As used herein, the term “single-dose” refers to a vaccine composition that only requires one administration or injection in a clinical regimen to induce a durable immune response and provide protection from a disease (for example, a pneumococcal disease). One of skill in the art would understand how to determine a durable immune response, e.g., by measuring antibody titers over a specified period of time.

[0086] As used herein, the term, “substituent” or “optional substituent” or “optionally substituted” refers to a substituent group attached to an alkyl group, or an alkenyl group, or an alkynyl group which, for example, replaces an available hydrogen on the group. Substituents may be the same, or different, and are each independently selected. Examples of substituents include alkyl, alkenyl, alkynyl, aryl, heteroaryl, -OH, hydroxyalkyl, haloalkyl, -O-alkyl, -O-haloalkyl, -alkylene-O-alkyl, -O-aryl, -O-alkylene-aryl, acyl, -C(O)-aryl, halo, -NO₂, -CN, -SF₅, -C(O)OH, -C(O)O-alkyl, -C(O)O-aryl, -C(O)O-alkylene-aryl, -S(O)-alkyl, -S(O)₂-alkyl, -S(O)-aryl, -S(O)₂-aryl, -S(O)-heteroaryl, -S(O)₂-heteroaryl, -S-alkyl, -S-aryl, -S-heteroaryl, -S-alkylene-aryl, -S-alkylene-heteroaryl, -S(O)₂-alkylene-aryl, -S(O)₂-alkylene-heteroaryl, cycloalkyl, heterocycloalkyl, -O-C(O)-alkyl, -O-C(O)-aryl, and -O-C(O)-cycloalkyl. A further example of substituents include (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine.

[0087] As used herein, the term “therapeutically effective amount” refers to an amount of an active ingredient (antigen) sufficient to produce the desired therapeutic effect in a human or animal, e.g., the amount necessary to elicit an immune response, treat, cure, prevent, or inhibit development and progression of a disease or the symptoms thereof and/or the amount necessary to ameliorate symptoms or cause regression of a disease. Therapeutically effective amount may vary depending on the structure and potency of the active ingredient and the contemplated mode of administration. One of skill in the art can readily determine a therapeutically effective amount of a given active ingredient in a vaccine.

[0088] As used herein, the term “valent” refers to the presence of a specified number of polysaccharides or polysaccharide-carrier protein conjugates in a composition.

[0089] As used herein, the term “vaccine” or “vaccine composition” refers to a biological preparation used to stimulate the production of antibodies and provide immunity against an infectious disease.

[0090] An “SNE”, for example, refers to a composition comprising a compound of the instant invention, SPAN-85, PS-20 and squalene; wherein the composition is in the form of a nanoemulsion. In some embodiments, an SNE comprises 0.01 µg/mL to 1000 µg/mL compound, 0.001 mg/mL to 60 mg/mL SPAN-85, 0.001 mg/mL to 60 mg/mL PS-20 and 0.01 mg/mL to 200 mg/mL squalene. In further embodiments, an SNE comprises 100 µg/mL or 80 µg/mL compound, 0.01 mg/mL to 10 mg/mL SPAN-85, 0.01 mg/mL to 10 mg/mL PS-20 and 0.03 mg/mL to 30 mg/mL squalene. In still further embodiments, an SNE comprises 80 µg/mL or 16 µg/mL or 4 µg/mL or 0.5 µg/mL compound, 0.2 mg/mL to 10 mg/mL SPAN-85, 0.2 mg/mL to 10 mg/mL PS-20 and 0.5 mg/mL to 20 mg/mL squalene.

[0091] A “Compound B-1-SNE”, as an example, refers to a composition comprising Compound B-1, SPAN-85, PS-20 and squalene; wherein the composition is in the form of a nanoemulsion. In some embodiments, a Compound B-1 SNE comprises 0.01 µg/mL to 1000 µg/mL Compound B-1, 0.001 mg/mL to 60 mg/mL SPAN-85, 0.001 mg/mL to 60 mg/mL PS-20 and 0.01 mg/mL to 200 mg/mL squalene. In further embodiments, a Compound B-1 SNE comprises 100 µg/mL or 80 µg/mL Compound B-1, 0.01 mg/mL to 10 mg/mL SPAN-85, 0.01 mg/mL to 10 mg/mL PS-20 and 0.03 mg/mL to 30 mg/mL squalene. In still further embodiments, a Compound B-1 SNE comprises 80 µg/mL or 16 µg/mL or 4 µg/mL or 0.5 µg/mL Compound B-1, 0.2 mg/mL to 10 mg/mL SPAN-85, 0.2 mg/mL to 10 mg/mL PS-20 and 0.5 mg/mL to 20 mg/mL squalene.

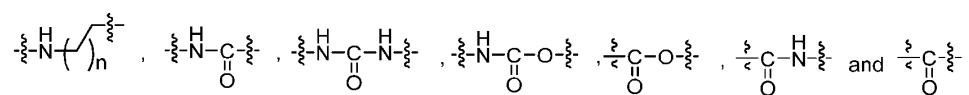
[0092] A “nanoemulsion”, also known as a nanometric-sized emulsion, is a fine oil-in-water (o/w) dispersion of two immiscible fluids. Nanoemulsions are a colloidal particulate system in the submicron size range acting as carriers of drug molecules. Their size varies from 10 to 1,000 nm. These carriers are solid spheres and their surface is amorphous and lipophilic.

[0093] As used herein, the term “carbon- or nitrogen-linked spacer” refers to any chemistry that links a carbon (-C) or a nitrogen (-N) to the benzyl group of the Formulas of the invention (see attachment of variable “A” in Formulas I and Ia). Examples of carbon or nitrogen linked spacers are C₁-C₆ alkyl, heterocycloalkyl, aryl, and heteroaryl, wherein said alkyl, heterocycloalkyl, aryl and heteroaryl are optionally substituted.

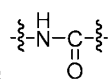
[0094] Unless otherwise noted, when describing a linker throughout this disclosure, the first available bond on a linker group connects to the portion of the compound flanking the linker group to the left and the terminal available bond of the linker group connects to the terminal

portion of the compound flanking the linker group to the right. For example, if the linker (L) is $-\text{CH}_2\text{-CF}_2-$, the definition of L includes only $\text{A}-\text{CH}_2\text{-CF}_2-\text{B}$ and does not include $\text{A}-\text{CF}_2\text{-CH}_2-\text{B}$.

[0095] As used herein, the term “functional group” refers to any chemistry that links the carbon or nitrogen linked spacer to the lipid group. An example of a functional group is:



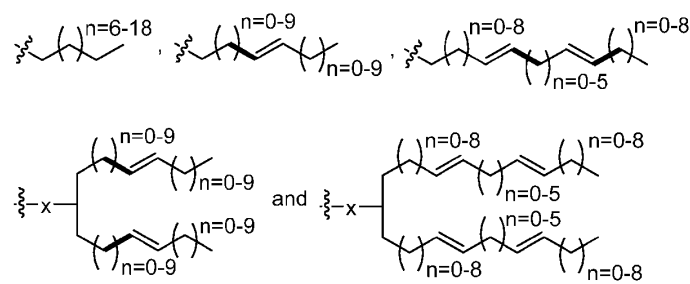
wherein n is 0, 1, 2, 3, 4, or 5.



[0096] A further example of a functional group is

[0097] As used herein, the term “lipid” refers to any chemistry that is insoluble in water but soluble in organic solvents. Examples of lipids are represented by variable D (see Formulas I and Ia) and are selected from (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl, wherein said alkyl, alkenyl, and alkynyl are optionally substituted.

[0098] A further example of a lipid is



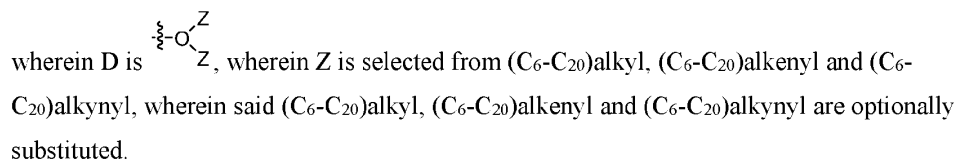
wherein any carbon on the lipid chain is optionally substituted,

 is cis or trans stereochemistry,

X is $\mathbf{O}$, $\mathbf{C}(\mathbf{R})^2$, or $\mathbf{NR}$, and

R is independently selected from H, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, OH, O(C₁-C₄)alkyl, O(C₁-C₄)alkenyl, O(C₁-C₄)alkynyl, chlorine and fluorine.

[0099] A further example of a lipid is represented by variable D (see Formulas I and Ia)



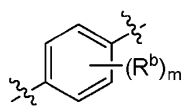
[0100] A compound of the invention that has one or more asymmetric centers can occur as a mixture of the stereoisomers or a substantially pure individual diastereomer or enantiomer unless

expressly depicted otherwise. The invention encompasses all stereoisomeric forms of the compounds of Formula I, Ia, II, IIa, III, IIIa, IV and IVa. Unless a specific stereochemistry is indicated, the invention is meant to include all such isomeric forms of these compounds. Centers of asymmetry that are present in the compounds of Formula I, Ia, II, IIa, III, IIIa, IV and IVa can all independently of one another have (R) configuration or (S) configuration. When bonds to the chiral carbon are depicted as straight lines in the structural Formulas of the invention, it is understood that both the (R) and (S) configurations of the chiral carbon, and hence both enantiomers and mixtures thereof, are embraced within the Formula. Similarly, when a compound name is recited without a chiral designation for a chiral carbon, it is understood that both the (R) and (S) configurations of the chiral carbon, and hence individual enantiomers, diastereomers and mixtures thereof, are embraced by the name. The production of specific stereoisomers or mixtures thereof may be identified in the Examples where such stereoisomers or mixtures were obtained, but this in no way limits the inclusion of all stereoisomers and mixtures thereof from being within the scope of this invention.

[0101] When any variable (e.g., n, R^b, etc.) occurs more than one time in any constituent or in Formula I, Ia, II, IIa, III, IIIa, IV, and IVa, its definition on each occurrence is independent of its definition at every other occurrence. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

[0102] A "stable" compound is a compound which can be prepared and isolated and whose structure and properties remain or can be caused to remain essentially unchanged for a period of time sufficient to allow use of the compound for the purposes described herein (e.g., use as an adjuvant in an immunogenic composition to be administered to a subject). The compounds of the present invention are limited to stable compounds embraced by Formulas I, Ia, II, IIa, III, IIIa, IV, and IVa.

[0103] A wavy line ~~~~~, as used herein, indicates a point of attachment to the rest of the

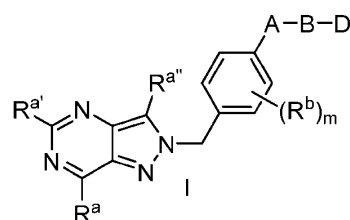
compound. Lines drawn into a ring system, for example: , indicate that the bond may be attached to any of the substitutable ring atoms.

Compounds

[0104] The compounds of the invention are useful in the adjuvant formulations described herein. These adjuvant formulations are useful to boost the immunological response of a vaccine formulation. For example, the adjuvant formulations described herein are useful to boost the immunological response of an adenovirus vaccine, an anthrax vaccine, a cholera vaccine, a

dengue vaccine, a diphtheria vaccine, a hepatitis vaccine, a *Haemophilus influenzae* type b (Hib) vaccine, a human papillomavirus (HPV) vaccine, a seasonal influenza (Flu) vaccine, a Japanese encephalitis vaccine, a measles vaccine, a meningococcal vaccine, a mumps vaccine, a pertussis vaccine, a pneumococcal vaccine, a polio vaccine, a rabies vaccine, a rotavirus vaccine, a rubella vaccine, a shingles vaccine, a smallpox vaccine, a tetanus vaccine, a tuberculosis vaccine, a typhoid fever vaccine, a varicella vaccine and a yellow fever vaccine. For example, the adjuvant formulations described herein are useful to boost the immunological response of a pneumococcal composition or a pneumococcal conjugate composition or a pneumococcal vaccine (PV) or a pneumococcal conjugate vaccine (PCV).

[0105] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I:



wherein:

R^a is selected from H, -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, and -NR[']R^{''}, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, and -O(C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

R^{a'} is selected from H, -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, and -NR[']R^{''}, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, and -O(C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

R^{a''} is selected from H, -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, and -NR[']R^{''}, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, and -O(C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from

the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

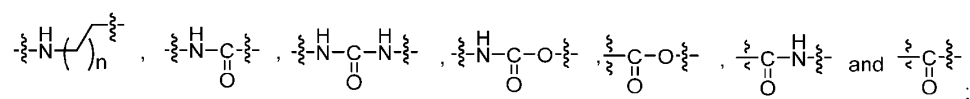
R' and R'' are independently selected from H, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, and (C₁-C₆)alkynyl, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl and (C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine, or R' and R'', together with the nitrogen to which they are attached, join together to form a (C₃-C₆)heterocycloalkyl, wherein said (C₃-C₆)heterocycloalkyl is optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

each occurrence of R^b is independently selected from H, -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, or NR^aR^a, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, and -O(C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

[0106] A is a carbon- or nitrogen-linked spacer selected from (C₁-C₆)alkyl, heterocycloalkyl, heterocycloalkyl-C(O)-R^Z, (C₁-C₄)alkyl-N(R^Z)-R^Z, aryl, and heteroaryl, wherein said (C₁-C₆)alkyl, heterocycloalkyl, aryl and heteroaryl are optionally substituted with one to six substituents, independently selected from the group consisting of -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, and NR^{R'}R^{R''}, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, and -O(C₁-C₆)alkynyl are optionally substituted with one to six substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

each occurrence of R^z is independently H or (C₁-C₆)alkyl;

B is a functional group selected from



D is a lipid selected from (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl, wherein said (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl, and (C₆-C₂₀)alkynyl are optionally substituted with one to six

substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-

C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine; or D is $\frac{1}{2}-\text{O}-\begin{matrix} \text{Z} \\ \diagup \\ \text{Z} \end{matrix}$;

each occurrence of Z is independently selected from (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl, and (C₆-C₂₀)alkynyl, wherein said (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl, and (C₆-C₂₀)alkynyl are optionally substituted with one to six substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

m is 0, 1, 2, 3, 4 or 5; and

n is 0, 1, 2, 3, 4 or 5.

[0107] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein m is 0, 1, 2, 3, 4 or 5.

[0108] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein m is 0, 1, 2, 3 or 4.

[0109] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein m is 0, 1, 2 or 3.

[0110] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein m is 0, 1 or 2.

[0111] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein m is 2.

[0112] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein m is 1.

[0113] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein m is 0.

[0114] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein n is 0, 1, 2, 3, 4 or 5.

[0115] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein n is 0, 1, 2, 3 or 4.

[0116] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein n is 0, 1, 2 or 3.

[0117] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein n is 0, 1 or 2.

[0118] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein n is 2.

[0119] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein n is 1.

[0120] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein n is 0.

[0121] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R' and R'' are independently H, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, or (C₁-C₆)alkynyl, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl and (C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine.

[0122] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R' and R'' are independently H, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, or (C₁-C₆)alkynyl, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl and (C₁-C₆)alkynyl are optionally substituted with one to four -OH or -O(CH₃), independently selected.

[0123] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R' and R'' are independently H, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, or (C₁-C₄)alkynyl, wherein said (C₁-C₄)alkyl, (C₁-C₄)alkenyl and (C₁-C₄)alkynyl are optionally substituted with one or two -OH or -O(CH₃), independently selected.

[0124] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R' and R'' are independently H and (C₁-C₄)alkyl, wherein said (C₁-C₄)alkyl is optionally substituted with one or two -OH or -O(CH₃), independently selected.

[0125] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R' and R'' are independently H and (C₁-C₄)alkyl.

[0126] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R' and R'', together with the nitrogen to which they are attached, join together to form a (C₃-C₆)heterocycloalkyl, wherein said (C₃-C₆)heterocycloalkyl is optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine.

[0127] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^a is H, -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, or NR'R'', wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl and -O(C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine.

[0128] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^a is H, -OH, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine, fluorine, or NR^{'R''}, wherein said (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl and -O(C₁-C₄)alkynyl are optionally substituted with one to two substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine.

[0129] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^a is H, -OH, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine, fluorine, or NR^{'R''}.

[0130] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^a is NR^{'R''}.

[0131] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^a is H and $R^{''}$ is pentyl.

[0132] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^a is H and $R^{''}$ is butyl.

[0133] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^a is H and $R^{''}$ is propyl.

[0134] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^a is H and $R^{''}$ is ethyl.

[0135] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^a is H and $R^{''}$ is methyl.

[0136] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^a is H and $R^{''}$ is H.

[0137] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^a is H, -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, or NR^{'R''}, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl and -O(C₁-C₆)alkynyl are optionally substituted with one to two substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine.

[0138] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^a is H, -OH, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine, fluorine, or NR^{'R''}, wherein said (C₁-

C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl and -O(C₁-C₄)alkynyl are optionally substituted with one to two substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine.

[0139] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^a is H, -OH, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine, fluorine, or NR^{'R''}.

[0140] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^a is NR^{'R''}.

[0141] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R['] is H and R^{''} is pentyl.

[0142] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R['] is H and R^{''} is butyl.

[0143] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R['] is H and R^{''} is propyl.

[0144] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R['] is H and R^{''} is ethyl.

[0145] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R['] is H and R^{''} is methyl.

[0146] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R['] is H and R^{''} is H.

[0147] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^{a''} is H, -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, or NR^{'R''}, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl and -O(C₁-C₆)alkynyl are optionally substituted with one to two substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine.

[0148] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^{a'''} is H, -OH, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine, fluorine, or NR^{'R''}, wherein said (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl and -O(C₁-C₄)alkynyl are optionally substituted with one to two substituents, independently selected from

the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine.

[0149] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^{a''} is H, -OH, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine, fluorine, or NR[']R^{''}.

[0150] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^{a''} is NR[']R^{''}.

[0151] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R['] is H and R^{''} is pentyl.

[0152] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R['] is H and R^{''} is butyl.

[0153] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R['] is H and R^{''} is propyl.

[0154] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R['] is H and R^{''} is ethyl.

[0155] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R['] is H and R^{''} is methyl.

[0156] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R['] is H and R^{''} is H.

[0157] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^{a''} is H.

[0158] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^b is H, -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, or NR[']R^{''}, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl and -O(C₁-C₆)alkynyl are optionally substituted with one to two substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine.

[0159] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^b is H, -OH, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine, fluorine, or NR[']R^{''}, wherein said (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl and -O(C₁-C₄)alkynyl are optionally substituted with one to two substituents, independently selected from

the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine.

[0160] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein R^b is independently H, -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, or NR[']R^{''}.

[0161] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein each occurrence of R^b is independently H, -OH, (C₁-C₄)alkyl, -O(C₁-C₄)alkyl, chlorine, fluorine, or NH₂.

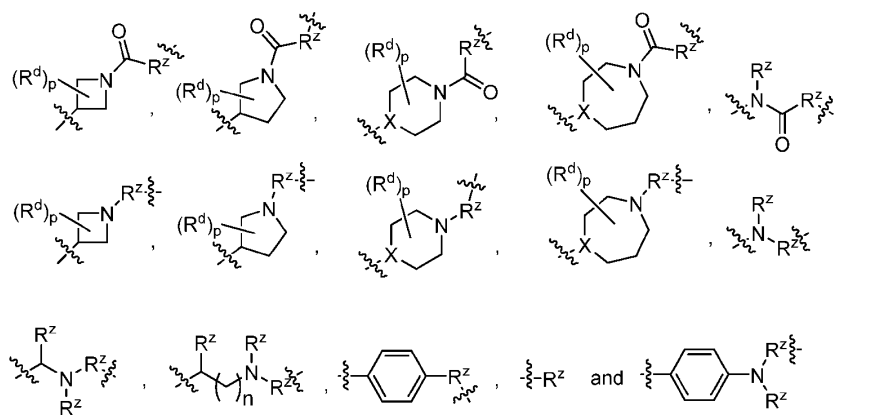
[0162] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein each occurrence of R^b is independently H and O(CH₃).

[0163] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein A is a (C₁-C₆)alkyl or heterocycloalkyl, wherein said alkyl or heterocycloalkyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, oxo, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, and NR[']R^{''}, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, and -O(C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine.

[0164] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein A is a (C₁-C₄)alkyl or heterocycloalkyl, wherein said alkyl or heterocycloalkyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, oxo, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine, fluorine, and NR[']R^{''}, wherein said (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, and -O(C₁-C₄)alkynyl are optionally substituted with one to six substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine.

[0165] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein A is heterocycloalkyl-C(O)-R^z- or (C₁-C₄)alkyl-N(R^z)₂-; wherein each occurrence of R^z is independently H or (C₁-C₆)alkyl.

[0166] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein A is selected from



wherein R^z is independently selected from H, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl and -O(C₁-C₆)alkynyl,

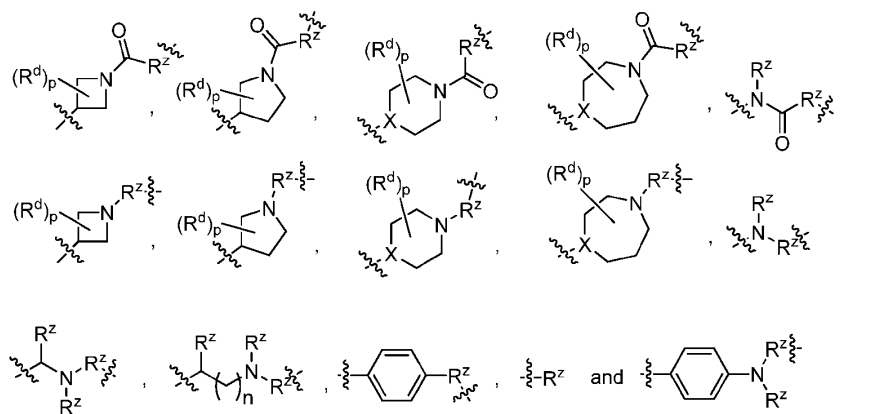
X is CH or N,

R^d is independently selected from H, -OH, oxo, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine,

n is 0, 1, 2, 3 or 4, and

p is 0, 1, 2, 3 or 4.

[0167] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein A is selected from



wherein R^z is independently selected from H, (C₁-C₆)alkyl, (C₁-C₆)alkenyl and (C₁-C₆)alkynyl,

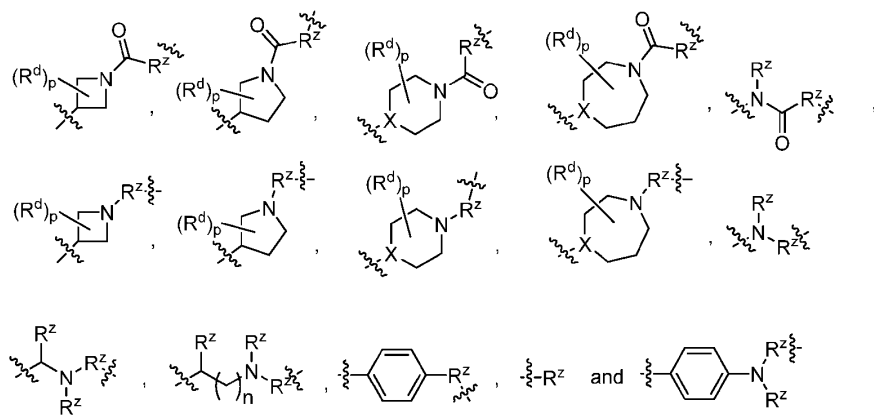
X is CH or N,

R^d is independently selected from -OH, (C₁-C₄)alkyl, -O(C₁-C₄)alkyl, chlorine and fluorine,

n is 0, 1, 2 or 3, and

p is 0, 1 or 2.

[0168] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein A is selected from



wherein R^Z is independently H or (C₁-C₆)alkyl,

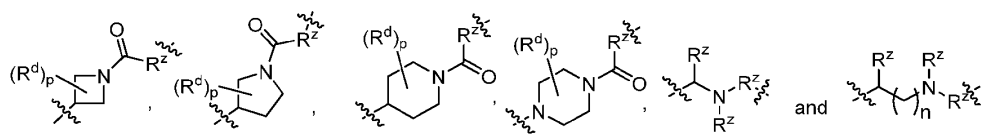
X is CH or N,

R^d is independently selected from -OH, (C₁-C₄)alkyl, -O(C₁-C₄)alkyl, chlorine and fluorine,

n is 0, 1, 2 or 3, and

p is 0, 1 or 2.

[0169] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein A is selected from



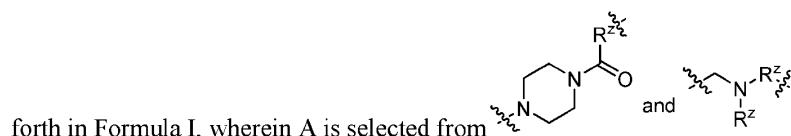
wherein R^Z is independently H or (C₁-C₆)alkyl,

R^d is independently selected from -OH, (C₁-C₄)alkyl, -O(C₁-C₄)alkyl, chlorine and fluorine,

wherein n is 0, 1, 2 or 3, and

wherein p is 0, 1 or 2.

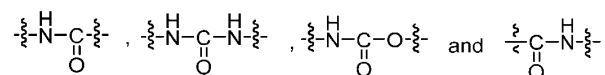
[0170] In some embodiments, a compound of the invention is represented by the structure set



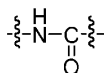
forth in Formula I, wherein A is selected from

wherein R^Z is independently H or (C₁-C₆)alkyl.

[0171] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein B is selected from

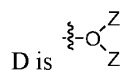


[0172] In some embodiments, a compound of the invention is represented by the structure set



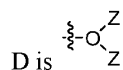
forth in Formula I, wherein B is

[0173] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein D is (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl, or (C₆-C₂₀)alkynyl, wherein said (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl, or (C₆-C₂₀)alkynyl are optionally substituted with one to six substituents independently selected from -OH, -O(CH₃), chlorine and fluorine, or



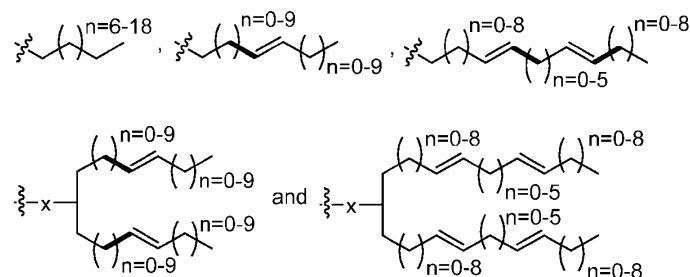
D is $\begin{array}{c} \text{R} \\ | \\ \text{Z} \end{array}$, wherein Z is selected from (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl, wherein said (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl, and (C₆-C₂₀)alkynyl are optionally substituted with one to six substituents independently selected from -OH, -O(CH₃), chlorine and fluorine.

[0174] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein D is (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl, or (C₆-C₂₀)alkynyl, or



D is $\text{--}\overset{\text{O}}{\underset{\text{O}}{\text{C}}}\text{--}$ Z, wherein Z is selected from (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl.

[0175] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein D is selected from



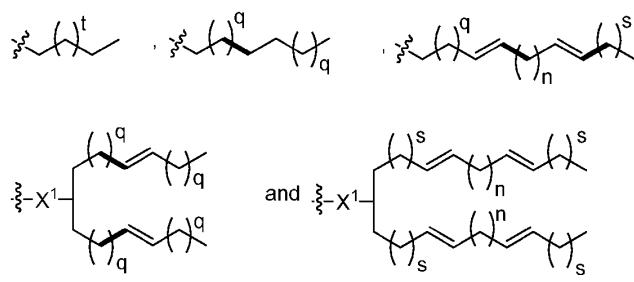
wherein any carbon on the lipid chain is optionally substituted with -OH, -O(CH₃), chlorine or fluorine.

— is cis or trans stereochemistry,

X is $\mathbf{O}$, $\mathbf{C}(\mathbf{R})^2$, or $\mathbf{NR}$, and

R is independently selected from H, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine.

[0176] In some embodiments, a compound of the invention is represented by the structure set forth in Formula I, wherein D is selected from



wherein any carbon on the lipid chain is optionally substituted with -OH, -O(CH₃), chlorine or fluorine,

— is cis or trans stereochemistry,

X¹ is -O-, -C(R)²-, or -NR-, and

each occurrence of R is independently selected from H, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

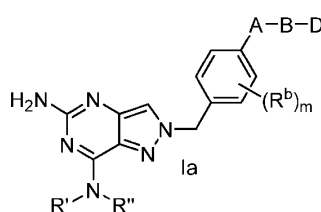
n is 0, 1, 2, 3, 4 or 5;

q is 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9;

s is 0, 1, 2, 3, 4, 5, 6, 7 or 8; and

t is 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18.

[0177] In some embodiments, a compound of the invention is represented by the structure set forth in Formula Ia:



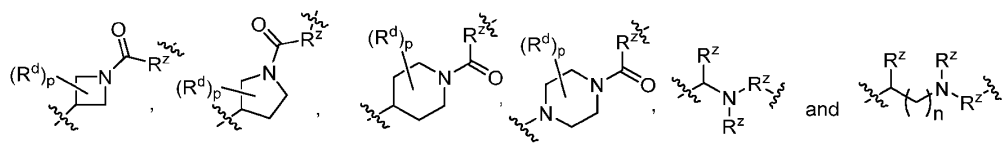
wherein:

R' and R'' are independently selected from H, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, and (C₁-C₆)alkynyl, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl and (C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine, or R' and R'', together with the nitrogen to which they are attached, join together to form a (C₃-

C₆)heterocycloalkyl, wherein said (C₃-C₆)heterocycloalkyl is optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

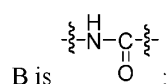
each occurrence of R^b is -O(C₁-C₄)alkyl, wherein said -O(C₁-C₄)alkyl is optionally substituted with one or two substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

A is selected from

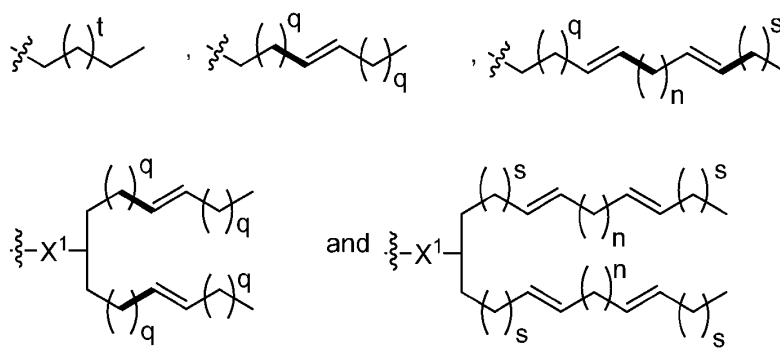


each occurrence of R^z is independently H or (C₁-C₆)alkyl;

each occurrence of R^d is independently selected from -OH, (C₁-C₄)alkyl, -O(C₁-C₄)alkyl, chlorine and fluorine;



D is a lipid chain selected from:



wherein any carbon on the lipid chain is optionally substituted with -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine,

wherein is cis or trans stereochemistry,

X¹ is -O-, -C(R)²-, or -NR-, and

each occurrence of R is independently selected from H, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

m is 0, 1 or 2;

n is 0, 1, 2 or 3;

p is 0, 1 or 2;

q is 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9;

s is, 1, 2, 3, 4, 5, 6, 7 or 8; and

t is 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18.

[0178] In some embodiments, a compound of the invention is represented by the structure set forth in Formula Ia, wherein m is 2.

[0179] In some embodiments, a compound of the invention is represented by the structure set forth in Formula Ia, wherein m is 1.

[0180] In some embodiments, a compound of the invention is represented by the structure set forth in Formula Ia, wherein m is 0.

[0181] In some embodiments, a compound of the invention is represented by the structure set forth in Formula Ia, wherein R' and R'' are independently selected from H and (C₁-C₆)alkyl, wherein said alkyl is optionally substituted with one to four -OH and/or -O(CH₃).

[0182] In some embodiments, a compound of the invention is represented by the structure set forth in Formula Ia, wherein R' and R'' are independently selected from H and (C₁-C₄)alkyl, wherein said alkyl is optionally substituted with one to four -OH and -O(CH₃).

[0183] In some embodiments, a compound of the invention is represented by the structure set forth in Formula Ia, wherein R' is H and R'' is pentyl.

[0184] In some embodiments, a compound of the invention is represented by the structure set forth in Formula Ia, wherein R' is H and R'' is butyl.

[0185] In some embodiments, a compound of the invention is represented by the structure set forth in Formula Ia, wherein R' is H and R'' is propyl.

[0186] In some embodiments, a compound of the invention is represented by the structure set forth in Formula Ia, wherein R' is H and R'' is ethyl.

[0187] In some embodiments, a compound of the invention is represented by the structure set forth in Formula Ia, wherein R' is H and R'' is methyl.

[0188] In some embodiments, a compound of the invention is represented by the structure set forth in Formula Ia, wherein R' is H and R'' is H.

[0189] In some embodiments, a compound of the invention is represented by the structure set forth in Formula Ia, wherein R^{a''} is H or (C₁-C₄)alkyl, wherein said alkyl is optionally substituted with one to two -OH and/or -O(CH₃).

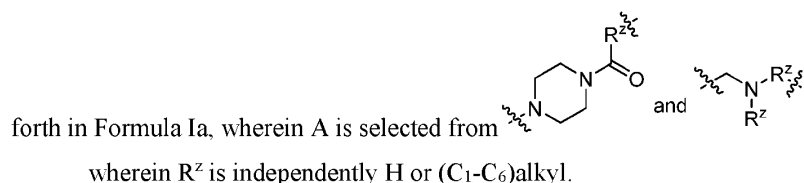
[0190] In some embodiments, a compound of the invention is represented by the structure set forth in Formula Ia, wherein R^{a''} is H or (C₁-C₄)alkyl.

[0191] In some embodiments, a compound of the invention is represented by the structure set forth in Formula Ia, wherein $R^{a''}$ is H.

[0192] In some embodiments, a compound of the invention is represented by the structure set forth in Formula Ia, wherein R^b is $-O(C_1-C_4)alkyl$, wherein said $-O(C_1-C_4)alkyl$ is optionally substituted with one to two $-OH$ and/or $-O(CH_3)$.

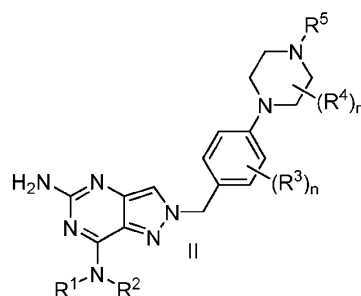
[0193] In some embodiments, a compound of the invention is represented by the structure set forth in Formula Ia, wherein R^b is $-O(CH_3)$.

[0194] In some embodiments, a compound of the invention is represented by the structure set



wherein R^z is independently H or $(C_1-C_6)alkyl$.

[0195] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II:



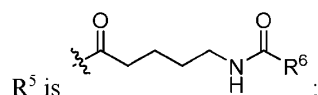
wherein:

R^1 is $(C_1-C_6)alkyl$, wherein said $(C_1-C_6)alkyl$ is optionally substituted with one to four substituents selected from $-OH$ and $-O(CH_3)$;

R^2 is H, methyl or $-O(CH_3)$;

each occurrence of R^3 is independently H, $(C_1-C_4)alkyl$, $(C_1-C_4)alkenyl$, $(C_1-C_4)alkynyl$ or $-O(C_1-C_4)alkyl$ wherein said $(C_1-C_4)alkyl$, $(C_1-C_4)alkenyl$, $(C_1-C_4)alkynyl$ or $-O(C_1-C_4)alkyl$ are optionally substituted with one or two substituents selected from $-OH$ and $-O(CH_3)$;

each occurrence of R^4 is independently H, $(C_1-C_4)alkyl$, $(C_1-C_4)alkenyl$, $(C_1-C_4)alkynyl$ or $-O(C_1-C_4)alkyl$ wherein said $(C_1-C_4)alkyl$, $(C_1-C_4)alkenyl$, $(C_1-C_4)alkynyl$ or $-O(C_1-C_4)alkyl$ are optionally substituted with one or two substituents selected from $-OH$ and $-O(CH_3)$;



R^6 is selected from (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl, wherein said (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl, and (C₆-C₂₀)alkynyl are optionally substituted with one to six substituents independently selected from -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine; and

each occurrence of n is 4.

[0196] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein n is independently 0, 1, 2 or 3.

[0197] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein n is independently 0, 1 or 2.

[0198] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein n is independently 0 or 1.

[0199] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein n is absent.

[0200] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^1 is ethyl, butyl (branched or a straight chain) or pentyl (branched or a straight chain), wherein said ethyl, butyl or pentyl is optionally substituted with one to four substituents independently selected from -OH, -O(CH₃), chlorine and fluorine.

[0201] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^1 is butyl (branched or a straight chain), wherein said butyl is optionally substituted with one to four substituents independently selected from -OH, -O(CH₃), chlorine and fluorine.

[0202] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^1 is ethyl, butyl (branched or a straight chain) or pentyl (branched or a straight chain), wherein said ethyl, butyl or pentyl is optionally substituted with one to two substituents independently selected from -OH and -O(CH₃).

[0203] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^1 is butyl (branched or a straight chain), wherein said butyl is optionally substituted with one to two substituents independently selected from -OH and -O(CH₃).

[0204] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^2 is H or methyl.

[0205] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^2 is H.

[0206] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^3 is independently (C₁-C₄)alkyl, (C₁-C₄)alkenyl or -O(C₁-C₄)alkyl, wherein said (C₁-C₄)alkyl, (C₁-C₄)alkenyl or -O(C₁-C₄)alkyl, is optionally substituted with one to four substituents independently selected from -OH or -O(CH₃).

[0207] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^3 is independently (C₁-C₄)alkyl or -O(C₁-C₄)alkyl, wherein said (C₁-C₄)alkyl or -O(C₁-C₄)alkyl is optionally substituted with one to four substituents independently selected from -OH or -O(CH₃).

[0208] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^3 is independently (C₁-C₄)alkyl or -O(C₁-C₄)alkyl, wherein said (C₁-C₄)alkyl or -O(C₁-C₄)alkyl is optionally substituted with one to two substituents independently selected from -OH or -O(CH₃).

[0209] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^3 is independently methyl or -O(CH₃).

[0210] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^3 is independently -O(CH₃).

[0211] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^4 is independently (C₁-C₄)alkyl or -O(C₁-C₄)alkyl, wherein said (C₁-C₄)alkyl or -O(C₁-C₄)alkyl are optionally substituted with one to four substituents independently selected from -OH, -O(CH₃), chlorine and fluorine.

[0212] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^4 is independently (C₁-C₄)alkyl or -O(C₁-C₄)alkyl.

[0213] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^6 is selected from (C₆-C₂₀)alkyl and (C₆-C₂₀)alkenyl wherein said (C₆-C₂₀)alkyl and (C₆-C₂₀)alkenyl are optionally substituted with one to six -OH and -O(CH₃).

[0214] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^6 is selected from (C₈-C₂₀)alkyl and (C₈-C₂₀)alkenyl wherein said (C₈-C₂₀)alkyl and (C₈-C₂₀)alkenyl are optionally substituted with one to six -OH and -O(CH₃).

[0215] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^6 is selected from (C₁₀-C₂₀)alkyl and (C₁₀-C₂₀)alkenyl wherein said (C₁₀-C₂₀)alkyl and (C₁₀-C₂₀)alkenyl are optionally substituted with one to six -OH and -O(CH₃).

[0216] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^6 is selected from (C₁₂-C₂₀)alkyl and (C₁₂-C₂₀)alkenyl wherein said (C₁₂-C₂₀)alkyl and (C₁₂-C₂₀)alkenyl are optionally substituted with one to six -OH and -O(CH₃).

[0217] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^6 is selected from (C₁₄-C₂₀)alkyl and (C₁₄-C₂₀)alkenyl wherein said (C₁₄-C₂₀)alkyl and (C₁₄-C₂₀)alkenyl are optionally substituted with one to six -OH and -O(CH₃).

[0218] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^6 is selected from (C₁₆-C₂₀)alkyl and (C₁₆-C₂₀)alkenyl wherein said (C₁₆-C₂₀)alkyl and (C₁₆-C₂₀)alkenyl are optionally substituted with one to six -OH and -O(CH₃).

[0219] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^6 is selected from (C₆-C₂₀)alkyl and (C₆-C₂₀)alkenyl.

[0220] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^6 is selected from (C₈-C₂₀)alkyl and (C₈-C₂₀)alkenyl.

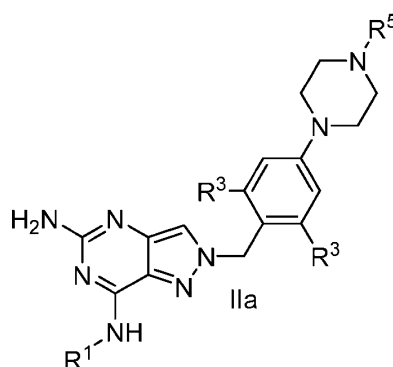
[0221] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^6 is selected from (C₁₀-C₂₀)alkyl and (C₁₀-C₂₀)alkenyl.

[0222] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^6 is selected from (C₁₂-C₂₀)alkyl and (C₁₂-C₂₀)alkenyl.

[0223] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^6 is selected from (C₁₄-C₂₀)alkyl and (C₁₄-C₂₀)alkenyl.

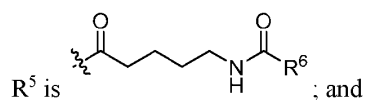
[0224] In some embodiments, a compound of the invention is represented by the structure set forth in Formula II, wherein R^6 is selected from (C₁₆-C₂₀)alkyl and (C₁₆-C₂₀)alkenyl.

[0225] The invention further provides a compound having the structure set forth in Formula IIa:



wherein:

R^1 is butyl, wherein said butyl is optionally substituted with one or two -OH;
each occurrence of R^3 is independently H or -O(CH₃); and



R^6 is selected from (C₁₀-C₂₀)alkyl, (C₁₀-C₂₀)alkenyl and (C₁₀-C₂₀)alkynyl.

[0226] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIa, wherein R^6 is selected from (C₁₀-C₂₀)alkyl and (C₁₀-C₂₀)alkenyl wherein said (C₁₀-C₂₀)alkyl and (C₁₀-C₂₀)alkenyl are optionally substituted with one or two -OH and -O(CH₃).

[0227] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIa, wherein R^6 is selected from (C₁₂-C₂₀)alkyl and (C₁₂-C₂₀)alkenyl wherein said (C₁₂-C₂₀)alkyl and (C₁₂-C₂₀)alkenyl are optionally substituted with one or two -OH and -O(CH₃).

[0228] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIa, wherein R^6 is selected from (C₁₄-C₂₀)alkyl and (C₁₄-C₂₀)alkenyl wherein said (C₁₄-C₂₀)alkyl and (C₁₄-C₂₀)alkenyl are optionally substituted with one or two -OH and -O(CH₃).

[0229] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIa, wherein R^6 is selected from (C₁₆-C₂₀)alkyl and (C₁₆-C₂₀)alkenyl wherein said (C₁₆-C₂₀)alkyl and (C₁₆-C₂₀)alkenyl are optionally substituted with one or two -OH and -O(CH₃).

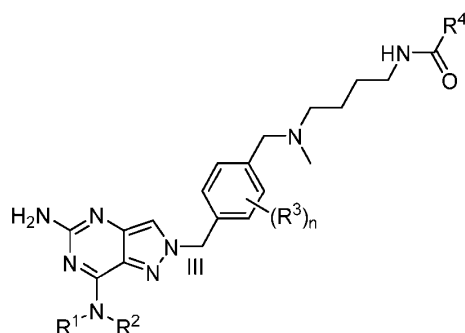
[0230] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIa, wherein R^6 is selected from (C₁₀-C₂₀)alkyl and (C₁₀-C₂₀)alkenyl.

[0231] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIa, wherein R^6 is selected from (C₁₂-C₂₀)alkyl and (C₁₂-C₂₀)alkenyl.

[0232] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIa, wherein R^6 is selected from (C₁₄-C₂₀)alkyl and (C₁₄-C₂₀)alkenyl.

[0233] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIa, wherein R^6 is selected from (C₁₆-C₂₀)alkyl and (C₁₆-C₂₀)alkenyl.

[0234] The invention provides a compound having the structure set forth in Formula III:



wherein:

R¹ is (C₁-C₆)alkyl, wherein said (C₁-C₆)alkyl is optionally substituted with one to four substituents selected from -OH and -O(CH₃);

R² is H, methyl or -O(CH₃);

each occurrence of R³ is independently H, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl or -O(C₁-C₄)alkyl wherein said (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl or -O(C₁-C₄)alkyl are optionally substituted with one or two substituents selected from -OH and -O(CH₃);

R⁴ is selected from (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl, wherein said (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl are optionally substituted with one to six substituents selected from -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine; and

n is 4.

[0235] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein n is 0, 1, 2 or 3.

[0236] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein n is 0, 1 or 2.

[0237] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein n is 0 or 1.

[0238] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein n is absent.

[0239] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein R¹ is ethyl, butyl (branched or a straight chain) or pentyl (branched or a straight chain), wherein said ethyl, butyl or pentyl is optionally substituted with one to four substituents independently selected from -OH, -O(CH₃), chlorine and fluorine.

[0240] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein R¹ is butyl (branched or a straight chain), wherein said butyl is optionally substituted with one to four substituents independently selected from -OH, -O(CH₃), chlorine and fluorine.

[0241] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein R¹ is ethyl, butyl (branched or a straight chain) or pentyl (branched or a straight chain), wherein said ethyl, butyl or pentyl is optionally substituted with one to two substituents independently selected from -OH and -O(CH₃).

[0242] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein R¹ is butyl (branched or a straight chain), wherein said butyl is

optionally substituted with one to two substituents independently selected from -OH and -O(CH₃).

[0243] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein R² is H or methyl.

[0244] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein R² is H.

[0245] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein each occurrence of R³ is independently (C₁-C₄)alkyl, (C₁-C₄)alkenyl or -O(C₁-C₄)alkyl, wherein said (C₁-C₄)alkyl, (C₁-C₄)alkenyl or -O(C₁-C₄)alkyl, is optionally substituted with one to four substituents independently selected from -OH and -O(CH₃).

[0246] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein each occurrence of R³ is independently (C₁-C₄)alkyl or -O(C₁-C₄)alkyl, wherein said (C₁-C₄)alkyl or -O(C₁-C₄)alkyl is optionally substituted with one to four substituents independently selected from -OH and -O(CH₃).

[0247] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein each occurrence of R³ is independently (C₁-C₄)alkyl or -O(C₁-C₄)alkyl, wherein said (C₁-C₄)alkyl or -O(C₁-C₄)alkyl is optionally substituted with one to two substituents independently selected from -OH and -O(CH₃).

[0248] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein each occurrence of R³ is independently methyl or -O(CH₃).

[0249] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein each occurrence of R³ is -O(CH₃).

[0250] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein R⁴ is selected from (C₆-C₂₀)alkyl and (C₆-C₂₀)alkenyl wherein said (C₆-C₂₀)alkyl and (C₆-C₂₀)alkenyl are optionally substituted with one to six substituents independently selected from -OH and -O(CH₃).

[0251] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein R⁴ is selected from (C₈-C₂₀)alkyl and (C₈-C₂₀)alkenyl wherein said (C₈-C₂₀)alkyl and (C₈-C₂₀)alkenyl are optionally substituted with one to six substituents independently selected from -OH and -O(CH₃).

[0252] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein R⁴ is selected from (C₁₀-C₂₀)alkyl and (C₁₀-C₂₀)alkenyl wherein

said (C₁₀-C₂₀)alkyl and (C₁₀-C₂₀)alkenyl are optionally substituted with one to six substituents independently selected from -OH and -O(CH₃).

[0253] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein R⁴ is selected from (C₁₂-C₂₀)alkyl and (C₁₂-C₂₀)alkenyl wherein said (C₁₂-C₂₀)alkyl and (C₁₂-C₂₀)alkenyl are optionally substituted with one to six substituents independently selected from -OH and -O(CH₃).

[0254] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein R⁴ is selected from (C₁₄-C₂₀)alkyl and (C₁₄-C₂₀)alkenyl wherein said (C₁₄-C₂₀)alkyl and (C₁₄-C₂₀)alkenyl are optionally substituted with one to six substituents independently selected from -OH and -O(CH₃).

[0255] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein R⁴ is selected from (C₁₆-C₂₀)alkyl and (C₁₆-C₂₀)alkenyl wherein said (C₁₆-C₂₀)alkyl and (C₁₆-C₂₀)alkenyl are optionally substituted with one to six substituents independently selected from -OH and -O(CH₃).

[0256] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein R⁴ is selected from (C₆-C₂₀)alkyl and (C₆-C₂₀)alkenyl.

[0257] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein R⁴ is selected from (C₈-C₂₀)alkyl and (C₈-C₂₀)alkenyl.

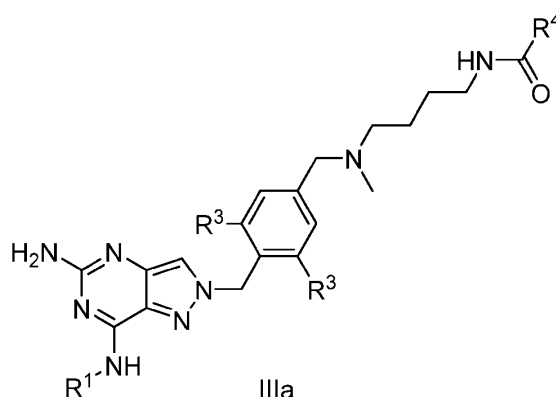
[0258] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein R⁴ is selected from (C₁₀-C₂₀)alkyl and (C₁₀-C₂₀)alkenyl.

[0259] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein R⁴ is selected from (C₁₂-C₂₀)alkyl and (C₁₂-C₂₀)alkenyl.

[0260] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein R⁴ is selected from (C₁₄-C₂₀)alkyl and (C₁₄-C₂₀)alkenyl.

[0261] In some embodiments, a compound of the invention is represented by the structure set forth in Formula III, wherein R⁴ is selected from (C₁₆-C₂₀)alkyl and (C₁₆-C₂₀)alkenyl.

[0262] The invention also provides a compound having the structure set forth in Formula IIIa:



wherein:

R^1 is butyl, wherein said butyl is optionally substituted with one or two -OH;

each occurrence of R^3 is independently H or -O(CH₃); and

R^4 is selected from (C₁₀-C₂₀)alkyl, (C₁₀-C₂₀)alkenyl and (C₁₀-C₂₀)alkynyl.

[0263] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIIa, wherein R^4 is selected from (C₆-C₂₀)alkyl and (C₆-C₂₀)alkenyl wherein said (C₆-C₂₀)alkyl and (C₆-C₂₀)alkenyl are optionally substituted with one to six -OH and -O(CH₃).

[0264] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIIa, wherein R^4 is selected from (C₈-C₂₀)alkyl and (C₈-C₂₀)alkenyl wherein said (C₈-C₂₀)alkyl and (C₈-C₂₀)alkenyl are optionally substituted with one to six substituents independently selected from -OH and -O(CH₃).

[0265] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIIa, wherein R^4 is selected from (C₁₀-C₂₀)alkyl and (C₁₀-C₂₀)alkenyl wherein said (C₁₀-C₂₀)alkyl and (C₁₀-C₂₀)alkenyl are optionally substituted with one to six substituents independently selected from -OH and -O(CH₃).

[0266] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIIa, wherein R^4 is selected from (C₁₂-C₂₀)alkyl and (C₁₂-C₂₀)alkenyl wherein said (C₁₂-C₂₀)alkyl and (C₁₂-C₂₀)alkenyl are optionally substituted with one to six substituents independently selected from -OH and -O(CH₃).

[0267] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIIa, wherein R^4 is selected from (C₁₄-C₂₀)alkyl and (C₁₄-C₂₀)alkenyl wherein said (C₁₄-C₂₀)alkyl and (C₁₄-C₂₀)alkenyl are optionally substituted with one to six substituents independently selected from -OH and -O(CH₃).

[0268] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIIa, wherein R^4 is selected from (C₁₆-C₂₀)alkyl and (C₁₆-C₂₀)alkenyl wherein said (C₁₆-C₂₀)alkyl and (C₁₆-C₂₀)alkenyl are optionally substituted with one to six substituents independently selected from -OH and -O(CH₃).

[0269] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIIa, wherein R^4 is selected from (C₆-C₂₀)alkyl and (C₆-C₂₀)alkenyl.

[0270] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIIa, wherein R^4 is selected from (C₈-C₂₀)alkyl and (C₈-C₂₀)alkenyl.

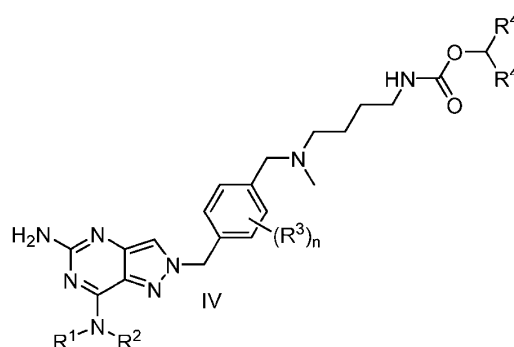
[0271] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIIa, wherein R^4 is selected from (C₁₀-C₂₀)alkyl and (C₁₀-C₂₀)alkenyl.

[0272] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIIa, wherein R^4 is selected from (C₁₂-C₂₀)alkyl and (C₁₂-C₂₀)alkenyl.

[0273] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIIa, wherein R^4 is selected from (C₁₄-C₂₀)alkyl and (C₁₄-C₂₀)alkenyl.

[0274] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IIIa, wherein R^4 is selected from (C₁₆-C₂₀)alkyl and (C₁₆-C₂₀)alkenyl.

[0275] The invention provides a compound having the structure set forth in Formula IV:



wherein:

R^1 is (C₁-C₆)alkyl, wherein said (C₁-C₆)alkyl is optionally substituted with one to four substituents independently selected from -OH and -O(CH₃);

R^2 is H, methyl or -O(CH₃);

each occurrence of R^3 is independently H, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, or -O(C₁-C₄)alkyl; wherein said (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, and -O(C₁-C₄)alkyl are optionally substituted with one or two substituents independently selected from -OH and -O(CH₃);

each occurrence of R^4 is independently selected from (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl, wherein said (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl are optionally substituted with one to six substituents independently selected from -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine or fluorine; and

n is 4.

[0276] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein n is 0, 1, 2 or 3.

[0277] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein n is 0, 1 or 2.

[0278] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein n is 0 or 1.

[0279] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein n is absent.

[0280] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein R^1 is ethyl, butyl (branched or a straight chain) or pentyl (branched or a straight chain), wherein said ethyl, butyl or pentyl is optionally substituted with one to four substituents independently selected from -OH, -O(CH₃), chlorine and fluorine.

[0281] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein R^1 is butyl (branched or a straight chain), wherein said butyl is optionally substituted with one to four substituents independently selected from -OH, -O(CH₃), chlorine and fluorine.

[0282] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein R^1 is ethyl, butyl (branched or a straight chain) or pentyl (branched or a straight chain), wherein said ethyl, butyl or pentyl is optionally substituted with one to two substituents independently selected from -OH and -O(CH₃).

[0283] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein R^1 is butyl (branched or a straight chain), wherein said butyl is optionally substituted with one to two substituents independently selected from -OH and -O(CH₃).

[0284] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein R^2 is H or methyl.

[0285] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein R^2 is H.

[0286] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein each occurrence of R^3 is independently (C₁-C₄)alkyl, (C₁-C₄)alkenyl or -O(C₁-C₄)alkyl, wherein said (C₁-C₄)alkyl, (C₁-C₄)alkenyl or -O(C₁-C₄)alkyl, is optionally substituted with one to four substituents independently selected from -OH or -O(CH₃).

[0287] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein each occurrence of R^3 is independently (C₁-C₄)alkyl or -O(C₁-C₄)alkyl, wherein said (C₁-C₄)alkyl or -O(C₁-C₄)alkyl is optionally substituted with one to four substituents independently selected from -OH or -O(CH₃).

[0288] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein each occurrence of R^3 is independently (C₁-C₄)alkyl or -O(C₁-C₄)alkyl, wherein said (C₁-C₄)alkyl or -O(C₁-C₄)alkyl is optionally substituted with one to two substituents independently selected from -OH or -O(CH₃).

[0289] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein each occurrence of R^3 is independently methyl or -O(CH₃).

[0290] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein each occurrence of R^3 is independently -O(CH₃).

[0291] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein each occurrence of R^4 is independently selected from (C₆-C₂₀)alkyl and (C₆-C₂₀)alkenyl wherein said (C₆-C₂₀)alkyl and (C₆-C₂₀)alkenyl are optionally substituted with one to six -OH and -O(CH₃).

[0292] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein each occurrence of R^4 is independently selected from (C₈-C₂₀)alkyl and (C₈-C₂₀)alkenyl wherein said (C₈-C₂₀)alkyl and (C₈-C₂₀)alkenyl are optionally substituted with one to six -OH and -O(CH₃).

[0293] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein each occurrence of R^4 is independently selected from (C₁₀-C₂₀)alkyl and (C₁₀-C₂₀)alkenyl wherein said (C₁₀-C₂₀)alkyl and (C₁₀-C₂₀)alkenyl are optionally substituted with one to six -OH and -O(CH₃).

[0294] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein each occurrence of R^4 is independently selected from (C₁₂-C₂₀)alkyl and (C₁₂-C₂₀)alkenyl wherein said (C₁₂-C₂₀)alkyl and (C₁₂-C₂₀)alkenyl are optionally substituted with one to six -OH and -O(CH₃).

[0295] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein each occurrence of R^4 is independently selected from (C₁₄-C₂₀)alkyl

and (C₁₄-C₂₀)alkenyl wherein said (C₁₄-C₂₀)alkyl and (C₁₄-C₂₀)alkenyl are optionally substituted with one to six -OH and -O(CH₃).

[0296] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein each occurrence of R⁴ is independently selected from (C₁₆-C₂₀)alkyl and (C₁₆-C₂₀)alkenyl wherein said (C₁₆-C₂₀)alkyl and (C₁₆-C₂₀)alkenyl are optionally substituted with one to six -OH and -O(CH₃).

[0297] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein each occurrence of R⁴ is independently selected from (C₆-C₂₀)alkyl and (C₆-C₂₀)alkenyl.

[0298] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein each occurrence of R⁴ is independently selected from (C₈-C₂₀)alkyl and (C₈-C₂₀)alkenyl.

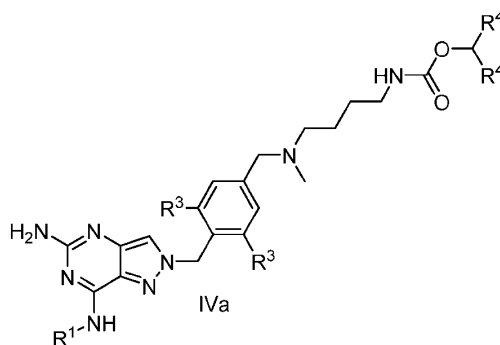
[0299] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein each occurrence of R⁴ is independently selected from (C₁₀-C₂₀)alkyl and (C₁₀-C₂₀)alkenyl.

[0300] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein each occurrence of R⁴ is independently selected from (C₁₂-C₂₀)alkyl and (C₁₂-C₂₀)alkenyl.

[0301] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein each occurrence of R⁴ is independently selected from (C₁₄-C₂₀)alkyl and (C₁₄-C₂₀)alkenyl.

[0302] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IV, wherein each occurrence of R⁴ is independently selected from (C₁₆-C₂₀)alkyl and (C₁₆-C₂₀)alkenyl.

[0303] The invention provides a compound having the structure set forth in Formula IVa:



wherein:

R¹ is butyl, wherein said butyl is optionally substituted with one or two -OH;
each occurrence of R³ is independently H or -O(CH₃); and
each occurrence of R⁴ is independently selected from (C₁₀-C₂₀)alkyl, (C₁₀-C₂₀)alkenyl and (C₁₀-C₂₀)alkynyl.

[0304] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IVa, wherein R⁴ is independently selected from (C₆-C₂₀)alkyl and (C₆-C₂₀)alkenyl wherein said (C₆-C₂₀)alkyl and (C₆-C₂₀)alkenyl are optionally substituted with one to six substituents independently selected from -OH and -O(CH₃).

[0305] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IVa, wherein R⁴ is independently selected from (C₈-C₂₀)alkyl and (C₈-C₂₀)alkenyl wherein said (C₈-C₂₀)alkyl and (C₈-C₂₀)alkenyl are optionally substituted with one to six substituents independently selected from -OH and -O(CH₃).

[0306] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IVa, wherein R⁴ is independently selected from (C₁₀-C₂₀)alkyl and (C₁₀-C₂₀)alkenyl wherein said (C₁₀-C₂₀)alkyl and (C₁₀-C₂₀)alkenyl are optionally substituted with one to six substituents independently selected from -OH and -O(CH₃).

[0307] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IVa, wherein R⁴ is independently selected from (C₁₂-C₂₀)alkyl and (C₁₂-C₂₀)alkenyl wherein said (C₁₂-C₂₀)alkyl and (C₁₂-C₂₀)alkenyl are optionally substituted with one to six substituents independently selected from -OH and -O(CH₃).

[0308] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IVa, wherein R⁴ is independently selected from (C₁₄-C₂₀)alkyl and (C₁₄-C₂₀)alkenyl wherein said (C₁₄-C₂₀)alkyl and (C₁₄-C₂₀)alkenyl are optionally substituted with one to six substituents independently selected from -OH and -O(CH₃).

[0309] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IVa, wherein R⁴ is independently selected from (C₁₆-C₂₀)alkyl and (C₁₆-C₂₀)alkenyl wherein said (C₁₆-C₂₀)alkyl and (C₁₆-C₂₀)alkenyl are optionally substituted with one to six substituents independently selected from -OH and -O(CH₃).

[0310] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IVa, wherein each occurrence of R⁴ is independently selected from (C₆-C₂₀)alkyl and (C₆-C₂₀)alkenyl.

[0311] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IVa, wherein each occurrence of R⁴ R⁴ is independently selected from (C₈-C₂₀)alkyl and (C₈-C₂₀)alkenyl.

[0312] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IVa, wherein each occurrence of R⁴ R⁴ is independently selected from (C₁₀-C₂₀)alkyl and (C₁₀-C₂₀)alkenyl.

[0313] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IVa, wherein each occurrence of R⁴ R⁴ is independently selected from (C₁₂-C₂₀)alkyl and (C₁₂-C₂₀)alkenyl.

[0314] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IVa, wherein each occurrence of R⁴ R⁴ is independently selected from (C₁₄-C₂₀)alkyl and (C₁₄-C₂₀)alkenyl.

[0315] In some embodiments, a compound of the invention is represented by the structure set forth in Formula IVa, wherein each occurrence of R⁴ R⁴ is independently selected from (C₁₆-C₂₀)alkyl and (C₁₆-C₂₀)alkenyl.

[0316] In some embodiments, the invention provides a compound selected from:

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide;

S-(*N*-(5-(4-(4-((5-amino-7-((1-hydroxypentan-2-yl)amino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide;

S-(1-(4-(4-((5-amino-7-((1-hydroxypentan-2-yl)amino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)octadecan-1-one;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)tetradecanamide;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)oleamide;

(9*Z*,12*Z*)-*N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)octadeca-9,12-dienamide;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-1,4-diazepan-1-yl)-5-oxopentyl)stearamide;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperidin-1-yl)-5-oxopentyl)stearamide;

N-(5-(3-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)azetidin-1-yl)-5-oxopentyl)stearamide;

1-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-*N*-(3-stearamidopropyl)piperidine-4-carboxamide;

(1*s*,3*s*)-3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-*N*-octadecylcyclobutane-1-carboxamide;

(1*s*,3*s*)-3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-*N*-hexadecylcyclobutane-1-carboxamide;

N-(3-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-3-oxopropyl)stearamide;

N-(7-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-7-oxoheptyl)stearamide;

N-(3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)cyclobutyl)stearamide;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-4,4-dimethyl-5-oxopentyl)stearamide;

N-(6-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-methyl-6-oxohexan-2-yl)stearamide;

1-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-(octadecyloxy)pentan-1-one;

1-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-(octadecylamino)pentan-1-one;

N-(5-(4-(4-((5-amino-7-(butylamino)-3-methyl-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide;

(9*Z*,12*Z*)-*N*-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)octadeca-9,12-dienamide;

N-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)tetradecanamide;

N-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)oleamide;

N-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)stearamide;

N-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)-4-oxobutyl)stearamide;

(6*Z*,9*Z*,28*Z*,31*Z*)-heptatriaconta-6,9,28,31-tetraen-19-yl (4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)carbamate;

4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-*N*-(3-stearamidopropyl)piperazine-1-carboxamide;
3-stearamidopropyl 4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazine-1-carboxylate;
N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-6,6,6-trifluorohexyl)stearamide;
N-(4-((4-(7-(butylamino)-5-hydroxy-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)stearamide; and
(6*Z*,9*Z*,28*Z*,31*Z*)-heptatriaconta-6,9,28,31-tetraen-19-yl (5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)carbamate;
or a pharmaceutically acceptable salt thereof.

[0317] In some embodiments, a compound of the invention is *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, or a pharmaceutically acceptable salt thereof.

[0318] In some embodiments, a compound of the invention is *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, or a pharmaceutically acceptable salt thereof.

[0319] In some embodiments, the compound of the invention is (*S*)-*N*-(5-(4-(4-((5-amino-7-(1-hydroxypentan-2-yl)amino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, or a pharmaceutically acceptable salt thereof.

[0320] In some embodiments, a compound of the invention is (*S*)-1-(4-(4-((5-amino-7-(1-hydroxypentan-2-yl)amino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)octadecan-1-one, or a pharmaceutically acceptable salt thereof.

[0321] In some embodiments, a compound of the invention is *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, or a pharmaceutically acceptable salt thereof.

[0322] In some embodiments, a compound of the invention is *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)tetradecanamide, or a pharmaceutically acceptable salt thereof.

[0323] In some embodiments, a compound of the invention is *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)oleamide, or a pharmaceutically acceptable salt thereof.

[0324] In some embodiments, a compound of the invention is (9*Z*,12*Z*)-*N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)octadeca-9,12-dienamide, or a pharmaceutically acceptable salt thereof.

[0325] In some embodiments, a compound of the invention is *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-1,4-diazepan-1-yl)-5-oxopentyl)stearamide, or a pharmaceutically acceptable salt thereof.

[0326] In some embodiments, a compound of the invention is *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperidin-1-yl)-5-oxopentyl)stearamide, or a pharmaceutically acceptable salt thereof.

[0327] In some embodiments, a compound of the invention is *N*-(5-(3-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)azetidin-1-yl)-5-oxopentyl)stearamide, or a pharmaceutically acceptable salt thereof.

[0328] In some embodiments, a compound of the invention is 1-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-*N*-(3-stearamidopropyl)piperidine-4-carboxamide, or a pharmaceutically acceptable salt thereof.

[0329] In some embodiments, a compound of the invention is (1*s*,3*s*)-3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-*N*-octadecylcyclobutane-1-carboxamide, or a pharmaceutically acceptable salt thereof.

[0330] In some embodiments, a compound of the invention is (1*s*,3*s*)-3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-*N*-hexadecylcyclobutane-1-carboxamide, or a pharmaceutically acceptable salt thereof.

[0331] In some embodiments, a compound of the invention is *N*-(3-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-3-oxopropyl)stearamide, or a pharmaceutically acceptable salt thereof.

[0332] In some embodiments, a compound of the invention is *N*-(7-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-7-oxoheptyl)stearamide, or a pharmaceutically acceptable salt thereof.

[0333] In some embodiments, a compound of the invention is *N*-(3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)cyclobutyl)stearamide, or a pharmaceutically acceptable salt thereof.

[0334] In some embodiments, a compound of the invention is *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-4,4-dimethyl-5-oxopentyl)stearamide, or a pharmaceutically acceptable salt thereof.

[0335] In some embodiments, a compound of the invention is *N*-(6-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-methyl-6-oxohexan-2-yl)stearamide, or a pharmaceutically acceptable salt thereof.

[0336] In some embodiments, a compound of the invention is 1-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-(octadecyloxy)pentan-1-one, or a pharmaceutically acceptable salt thereof.

[0337] In some embodiments, a compound of the invention is 1-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-(octadecylamino)pentan-1-one, or a pharmaceutically acceptable salt thereof.

[0338] In some embodiments, a compound of the invention is *N*-(5-(4-(4-((5-amino-7-(butylamino)-3-methyl-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, or a pharmaceutically acceptable salt thereof.

[0339] In some embodiments, a compound of the invention is (9*Z*,12*Z*)-*N*-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)octadeca-9,12-dienamide, or a pharmaceutically acceptable salt thereof.

[0340] In some embodiments, a compound of the invention is *N*-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)tetradecanamide, or a pharmaceutically acceptable salt thereof.

[0341] In some embodiments, a compound of the invention is *N*-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)oleamide, or a pharmaceutically acceptable salt thereof.

[0342] In some embodiments, a compound of the invention is *N*-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)stearamide, or a pharmaceutically acceptable salt thereof.

[0343] In some embodiments, a compound of the invention is *N*-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)-4-oxobutyl)stearamide, or a pharmaceutically acceptable salt thereof.

[0344] In some embodiments, a compound of the invention is (6*Z*,9*Z*,28*Z*,31*Z*)-heptatriaconta-6,9,28,31-tetraen-19-yl (4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)carbamate, or a pharmaceutically acceptable salt thereof.

[0345] In some embodiments, a compound of the invention is 4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-*N*-(3-stearamidopropyl)piperazine-1-carboxamide, or a pharmaceutically acceptable salt thereof.

[0346] In some embodiments, a compound of the invention is 3-stearamidopropyl 4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazine-1-carboxylate, or a pharmaceutically acceptable salt thereof.

[0347] In some embodiments, a compound of the invention is *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-6,6,6-trifluorohexyl)stearamide, or a pharmaceutically acceptable salt thereof.

[0348] In some embodiments, a compound of the invention is *N*-(4-((4-((7-(butylamino)-5-hydroxy-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)stearamide, or a pharmaceutically acceptable salt thereof.

[0349] In some embodiments, a compound of the invention is (6*Z*,9*Z*,28*Z*,31*Z*)-heptatriacont-6,9,28,31-tetraen-19-yl (5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)carbamate, or a pharmaceutically acceptable salt thereof.

Adjuvants

[0350] Adjuvant-based approaches are being investigated to enhance vaccine immunogenicity and help address residual unmet medical need, particularly for populations with increased susceptibility to infectious disease (i.e., immunocompromised, elderly, or pediatric populations).

[0351] For example, while invasive pneumococcal disease (IPD) incidence has dramatically declined in both children and adults following the introduction of pneumococcal conjugate vaccines, residual disease caused by persistent vaccine-type serotypes remains. Further, serotype-specific immune responses vary and the introduction of expanded valency PCVs has resulted in an overall decrease in serotype-specific immune responses. Adjuvant-based strategies to enhance immunogenicity of PCVs may increase vaccine efficacy for challenging serotypes, provide more durable immune responses, or allow for reduction in dosing schedule.

[0352] Preclinical data indicates that a toll-like receptor 7/8 (TLR7/8) agonist can increase the immunogenicity of PCVs. [Dowling, D.J. et al.] have reported both accelerated and enhanced serotype specific antibody responses, including both binding antibody titers and functional opsonophagocytic killing, in neonatal and infant rhesus monkeys immunized with PCV13 plus the TLR7/8 agonist 3M-052 compared to PCV13 alone (PCV13 contains alum adjuvant). The addition of the TLR7/8 agonist to PCV13 also induced Th1-polarized CRM197-specific CD4 T cells and early life *S. pneumonia* antigen-specific B cells in infant rhesus monkeys and enhanced

type II IFN and Th1 polarizing cytokine production after in vitro stimulation of human neonatal cord blood (Dowling, D.J. et al., JCI Insight 2017; 2(2) e91020).

Adjuvant Formulations

[0353] The disclosure provides an adjuvant formulation that comprises 1) one or more compounds of the invention or pharmaceutically acceptable salt(s) thereof; 2) one or more sorbitan-based surfactants; and 3) one or more terpenes. The adjuvant formulations of the invention are also referred to as stable nanoemulsions or SNEs.

[0354] The disclosure provides an adjuvant formulation that comprises 1) one or more compounds of the invention or pharmaceutically acceptable salt(s) thereof; 2) sorbitan trioleate (SPAN-85); 3) polysorbate-20 (PS-20) or polysorbate-80 (PS-80), and 4) squalene.

[0355] The disclosure provides an adjuvant formulation that comprises 1) one or more compounds of the invention or pharmaceutically acceptable salt(s) thereof; 2) SPAN-85; 3) PS-20, and 4) squalene.

[0356] The disclosure provides an adjuvant formulation that comprises 1) a compound of the invention or a pharmaceutically acceptable salt thereof; 2) SPAN-85; 3) PS-20 or PS-80, and 4) squalene.

[0357] The disclosure provides an adjuvant formulation that comprises 1) a compound of the invention or a pharmaceutically acceptable salt thereof; 2) SPAN-85; 3) PS-20, and 4) squalene.

General Methods of Making SNE Formulations

[0358] Generally, SNEs may be formed, for example, by initially combining and mixing components together. Once mixed and blended, an aqueous buffer is added and mixed with initial compound components to form a blended emulsion mixture. In some embodiments, the blended emulsion components are first subjected to coarse homogenization followed by fine homogenization. Then, the resulting formulation is subjected to a final filtration step and stored at 4°C. The solution may include one or more compounds; one or more sorbitan based surfactants (e.g., PS-20; PS-80; SPAN-85); and one or more terpenes (e.g., squalene) at specific molar ratios.

[0359] Alternatively, a process of preparing an SNE of the invention consists of 4 primary steps: 1) solution preparation of a component mixture including functional and non-functional ingredients and an aqueous buffer; 2) SNE formation by means of split stream mixing; 3) ultra-filtration; and 4) filtration.

[0360] Generally, components are dissolved in ethanol before being sterile filtered to form a mixture. Several aqueous buffers are also prepared. The mixture and buffer streams are then combined using a T-tube or Y mixer and then, immediately after exit, are diluted and mixed with

an aqueous buffer to form an SNE intermediate. An SNE intermediate is then subjected to ultra-filtration or dialysis to both concentrate the material as well as exchange the material against a suitable buffer to remove residual ethanol. After the diafiltration, there is a final concentration step performed in order to achieve a final target concentration. SNE bulk is then filtered with a sterilizing filter.

SNE Formulations

[0361] In some embodiments, an SNE formulation is provided comprising: 1) about 10-14 mol% of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) about 1-4 mol% of SPAN-85; 3) about 1-4 mol% of PS-20 or PS-80; and 4) about 50-80 mol% of squalene.

[0362] In some embodiments, an SNE formulation is provided comprising: 1) about 30-65 mol% of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) about 5-30 mol% of SPAN-85; 3) about 0.5-4 mol% of PS-20 or PS-80; and 4) about 10-40 mol% of squalene.

[0363] In some embodiments, an SNE formulation is provided comprising: 1) about 55-65 mol% of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) about 5-15 mol% SPAN-85; 3) about 1-2.5 mol% of PS-20 or PS-80; and 4) about 25-35 mol% of squalene.

[0364] In some embodiments, an SNE formulation is provided comprising: 1) about 13-45 mol% of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) about 2-4 mol% SPAN-85; 3) about 1.5-3 mol% of PS-20 or PS-80; and 4) about 50-82 mol% of squalene.

[0365] In some embodiments, an SNE formulation is provided comprising: 1) about 13-14 mol% of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) about 1-2 mol% SPAN-85; 3) about 1-2 mol% PS-20 or PS-80; and 4) about 79-81 mol% of squalene.

[0366] In some embodiments, an SNE formulation is provided comprising: about 1-60 mol% of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) about 1-4 mol% SPAN-85; 3) about 1-4 mol% of PS-20 or PS-80; and 4) about 32-97 mol% of squalene.

[0367] In some embodiments, an SNE formulation is provided comprising: 1) about 0-45 mol% of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) about 1-10 mol% of a non-ionic surfactant; and 3) about 50-85 mol%

of squalene. In one aspect of this embodiment, the non-ionic surfactant comprises a mixture of SPAN-85 and PS-20 or PS-80.

[0368] In some embodiments, an SNE formulation is provided comprising: 1) about 10-14 mol% of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) about 1-4 mol% of SPAN-85; 3) about 1-4 mol% of PS-20; and 4) about 50-80 mol% of squalene.

[0369] In some embodiments, an SNE formulation is provided comprising: 1) about 30-65 mol% of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) about 5-30 mol% of SPAN-85; 3) about 0.5-4 mol% of PS-20; and 4) about 10-40 mol% of squalene.

[0370] In some embodiments, an SNE formulation is provided comprising: 1) about 55-65 mol% of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) about 5-15 mol% SPAN-85; 3) about 1-2.5 mol% of PS-20; and 4) about 25-35 mol% of squalene.

[0371] In some embodiments, an SNE formulation is provided comprising: 1) about 13-45 mol% of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) about 2-4 mol% SPAN-85; 3) about 1.5-3 mol% of PS-20; and 4) about 50-82 mol% of squalene.

[0372] In some embodiments, an SNE formulation is provided comprising: 1) about 13-14 mol% of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) about 1-2 mol% SPAN-85; 3) about 1-2 mol% PS-20; and 4) about 79-81 mol% of squalene.

[0373] In some embodiments, an SNE formulation is provided comprising: about 1-60 mol% of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) about 1-4 mol% SPAN-85; 3) about 1-4 mol% of PS-20; and 4) about 32-97 mol% of squalene.

[0374] In some embodiments, an SNE formulation is provided comprising: 1) about 0-45 mol% of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) about 1-10 mol% of a non-ionic surfactant; and 3) about 50-85 mol% of squalene. In one aspect of this embodiment, the non-ionic surfactant comprises a mixture of SPAN-85 and PS-20.

[0375] In some embodiments, an SNE formulation is provided comprising: 0.01-0.1 mol% of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt

thereof; 2) 1.5-15.0 mol% SPAN-85; 3) 1-10.0 mol% of PS-20; and 4) 80.0-98.0 mol% of squalene.

[0376] In some embodiments, an SNE formulation is provided comprising: 0.1-1.0 mol% of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) 8.0-11.9 mol% SPAN-85; 3) 6-10 mol% of PS-20; and 4) 78-85 mol% of squalene.

[0377] In some embodiments, an SNE formulation is provided comprising: 0.01-0.1 mol% of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) 8.0-11.9 mol% SPAN-85; 3) 6-10 mol% of PS-20; and 4) 78-85 mol% of squalene.

[0378] In some embodiments, an SNE formulation is provided comprising: 200-400 µg of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) 8-16 mg of SPAN-85; 3) 8-16 mg of PS-20; and 4) 40-80 mg of squalene.

[0379] In some embodiments, an SNE formulation is provided comprising: 10-20 µg of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) 8-16 mg of SPAN-85; 3) 8-16 mg of PS-20; and 4) 40-80 mg of squalene.

[0380] In some embodiments, an SNE formulation is provided comprising: 0.5-3.0 µg of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) 0.6-3.6 mg of SPAN-85; 3) 0.6-3.6 mg of PS-20; and 4) 2.0-12.0 mg of squalene.

[0381] In some embodiments, an SNE formulation is provided comprising: 0.5-3.0 µg of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) 0.15-0.18 mg of SPAN-85; 3) 0.15-0.18 mg of PS-20; and 4) 0.5-0.6 mg of squalene.

[0382] In some embodiments, an SNE formulation is provided comprising: 0.5-3.0 µg of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) 0.15-0.95 mg of SPAN-85; 3) 0.15-0.95 mg of PS-20; and 4) 0.5-3.0 mg of squalene.

[0383] In some embodiments, an SNE formulation is provided comprising: 4.0-20 µg of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) 0.24-1.3 mg of SPAN-85; 3) 0.24-1.3 mg of PS-20; and 4) 0.8-4.0 mg of squalene.

[0384] In some embodiments, an SNE formulation is provided comprising: 4.0-20 µg of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) 1.2-6.5 mg of SPAN-85; 3) 1.2-6.5 mg of PS-20; and 4) 4.0-20.0 mg of squalene.

[0385] In some embodiments, an SNE formulation is provided comprising: 20-100 µg of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) 1.2-6.5 mg of SPAN-85; 3) 1.2-6.5 mg of PS-20; and 4) 4.0-20.0 mg of squalene.

[0386] In some embodiments, an SNE formulation is provided comprising: 20-40 µg of a compound of Formula I, Ia, II, IIa, III, IIIa, IV and IVa or a pharmaceutically acceptable salt thereof; 2) 5-8.0 mg of SPAN-85; 3) 5-8.0 mg of PS-20; and 4) 20.0-40.0 mg of squalene.

[0387] In some embodiments, any one of the formulations provided above comprise a particular compound selected from:

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, also referred to as Compound A-1;

(S)-*N*-(5-(4-(4-((5-amino-7-((1-hydroxypentan-2-yl)amino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, also referred to as Compound A-2;

(S)-1-(4-(4-((5-amino-7-((1-hydroxypentan-2-yl)amino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)octadecan-1-one, also referred to as Compound A-3;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, also referred to as Compound B-1;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)tetradecanamide, also referred to as Compound B-2;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)oleamide, also referred to as Compound B-3;

(9*Z*,12*Z*)-*N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)octadeca-9,12-dienamide, also referred to as Compound B-4;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-1,4-diazepan-1-yl)-5-oxopentyl)stearamide, also referred to as Compound B-5;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperidin-1-yl)-5-oxopentyl)stearamide, also referred to as Compound B-6;

N-(5-(3-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)azetidin-1-yl)-5-oxopentyl)stearamide, also referred to as Compound B-7;

1-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-*N*-(3-stearamidopropyl)piperidine-4-carboxamide, also referred to as Compound B-8;

(1*s*,3*s*)-3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-*N*-octadecylcyclobutane-1-carboxamide, also referred to as Compound B-9;

(1*s*,3*s*)-3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-*N*-hexadecylcyclobutane-1-carboxamide, also referred to as Compound B-10;

N-(3-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-3-oxopropyl)stearamide, also referred to as Compound B-11;

N-(7-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-7-oxoheptyl)stearamide, also referred to as Compound B-12;

N-(3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)cyclobutyl)stearamide, also referred to as Compound B-13;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-4,4-dimethyl-5-oxopentyl)stearamide, also referred to as Compound B-14;

N-(6-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-methyl-6-oxohexan-2-yl)stearamide, also referred to as Compound B-15;

1-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-(octadecyloxy)pentan-1-one, also referred to as Compound B-16;

1-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-(octadecylamino)pentan-1-one, also referred to as Compound B-17;

N-(5-(4-(4-((5-amino-7-(butylamino)-3-methyl-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, also referred to as Compound B-18;

(9*Z*,12*Z*)-*N*-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)octadeca-9,12-dienamide, also referred to as Compound C-1;

N-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)tetradecanamide, also referred to as Compound C-2;

N-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)oleamide, also referred to as Compound C-3;

N-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)stearamide, also referred to as Compound C-4;

N-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)-4-oxobutyl)stearamide, also referred to as Compound C-5; (6*Z*,9*Z*,28*Z*,31*Z*)-heptatriaconta-6,9,28,31-tetraen-19-yl (4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)carbamate, also referred to as Compound D-1; 4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-*N*-(3-stearamidopropyl)piperazine-1-carboxamide, also referred to as Compound D-2; 3-stearamidopropyl 4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazine-1-carboxylate, also referred to as Compound D-3; *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-6,6,6-trifluorohexyl)stearamide, also referred to as Compound D-4; *N*-(4-((4-((7-(butylamino)-5-hydroxy-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)stearamide, also referred to as Compound D-5; and (6*Z*,9*Z*,28*Z*,31*Z*)-heptatriaconta-6,9,28,31-tetraen-19-yl (5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)carbamate, also referred to as Compound D-6; or a pharmaceutically acceptable salt thereof.

[0388] In some embodiments of the invention, a formulation is provided wherein the SNE further comprises one or more additional components which are selected from a surfactant, a mixture of surfactants, a phospholipid, a terpene, a terpenoid, a triterpene or a combination thereof.

[0389] In some embodiments of the invention, the surfactant includes, but is not limited to: the polyoxyethylene sorbitan esters surfactants (commonly referred to as the Tweens), especially PS-20 and PS-80; copolymers of ethylene oxide (EO), propylene oxide (PO), and/or butylene oxide (BO), sold under the DOWFAX™ tradename, such as linear EO/PO block copolymers; octoxynols, which can vary in the number of repeating ethoxy (oxy-1,2-ethanediyl) groups, with octoxynol-9 (Triton X-100, or t-octylphenoxypolyethoxyethanol) being of particular interest; (octylphenoxy)polyethoxyethanol (IGEPAL CA-630/NP-40); nonylphenol ethoxylates, such as the Tergitol™ NP series; polyoxyethylene fatty ethers derived from lauryl, cetyl, stearyl and oleyl alcohols (known as Brij surfactants), such as triethyleneglycol monolauryl ether (Brij 30); and sorbitan esters (commonly known as the SPANs), such as sorbitan trioleate (Span-85, Tween-85 or [2-[(2*R*,3*S*,4*R*)-4-hydroxy-3-[(*Z*)-octadec-9-enoyl]oxyoxolan-2-yl]-2-[(*Z*)-octadec-9-enoyl]oxyethyl] (Z)-octadec-9-enoate) and sorbitan monolaurate.

[0390] In some embodiments of the invention, mixtures of surfactants are used, e.g., PS-20/Span 85 mixtures. A combination of a polyoxyethylene sorbitan ester such as polyoxyethylene sorbitan monooleate (PS-80) and an octoxynol such as t-octylphenoxy polyethoxyethanol (Triton X-100) are also suitable. Another useful combination comprises laureth 9 plus a polyoxyethylene sorbitan ester and/or an octoxynol.

[0391] In some embodiments of the invention, the amounts of surfactants or emulsifiers are: polyoxyethylene sorbitan esters (such as PS-20) 0.01 to 10 mole %, in particular about 1 to 4 mol%; octyl- or nonylphenoxy polyoxyethanols (such as Triton X-100, or other detergents in the Triton series) 0.001 to 10 mol%, in particular about 1 to 4 mol%; w/v, in particular 0.01 to 0.1% w/v; polyoxyethylene ethers (such as laureth 9) 0.1 to 20 mol%, preferably 0.5 to 10 mol% and in particular 1 to 4 mol% or about 10 % by mass.

[0392] In some embodiments of the invention, the phospholipid is selected from, a natural phospholipid including phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylglycerol (PG), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidic acid (phosphatidate) (PA), dipalmitoylphosphatidylcholine, monoacyl-phosphatidylcholine (lyso PC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), N-Acyl-PE, a phosphoinositide, and a phosphosphingolipid. Phospholipid derivatives include phosphatidic acid (DMPA, DPPA, DSPA), phosphatidylcholine (DDPC, DLPC, DMPC, DPPC, DSPC, DOPC, POPC, DEPC), phosphatidylglycerol (DMPG, DPPG, DSPG, POPG), phosphatidylethanolamine (DMPE, DPPE, DSPE, DOPE), phosphatidylserine (DOPS). Fatty acids include C14:0, palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), and arachidonic acid (C20:4), C20:0, C22:0 and lethicin. In certain embodiments of the invention, the phospholipid is phosphatidylserine, 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dipalmitoleoyl-sn-glycero-3-phosphocholine, 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), dilauroylphosphatidylcholine (DLPC), 1,2-dieicosenoyl-sn-glycero-3-phosphocholine, or 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC).

[0393] In some embodiments of the invention, the terpene is selected from a monoterpene, such as geraniol, terpineol, limonene, myrcene, linalool or pinene. In some embodiments, the formulation comprises a sesquiterpene consisting of humulene, a farnesene, or farnesol; a diterpene such as cafestol, kahweol, cembrene or taxadiene; A triterpene such as squalene or squalante; a tetraterpene such as acyclic lycopene, monocyclic gamma-carotene, or bicyclic alpha- and beta-carotenes; a polyterpene or a norisoprenoids,

Vaccine Compositions

[0394] Vaccines (vaccine or immunogenic compositions) are well known in the art and include adenovirus vaccines, anthrax vaccines, cholera vaccines, dengue vaccines, diphtheria vaccines, hepatitis vaccines, *Haemophilus influenzae* type b (Hib) vaccines, human papillomavirus (HPV) vaccines, seasonal influenza (Flu) vaccines, Japanese encephalitis vaccines, measles vaccines, meningococcal vaccines, mumps vaccines, pertussis vaccines, pneumococcal vaccines, polio vaccines, rabies vaccines, rotavirus vaccines, rubella vaccines, shingles vaccines, smallpox vaccines, tetanus vaccines, tuberculosis vaccines, typhoid fever vaccines, varicella vaccines and yellow fever vaccines.

[0395] Pneumococcal vaccines or compositions are well known (for example PNEUMOVAX®, Merck & Co., Inc., Rahway, NJ, USA). Pneumococcal conjugate vaccines or compositions have been previously disclosed. See WO2011/100151, WO2019/139692 and WO2020/131763.

[0396] Bacterial capsular polysaccharides, particularly those that have been used as antigens, are suitable for use in the invention and can readily be identified by methods for identifying immunogenic and/or antigenic polysaccharides. Example bacterial capsular polysaccharides from *S. pneumoniae* are serotypes: 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19A, 19F, 20 (20A and 20B), 22F, 23A, 23B, 23F, 24F, 33F, 35B, 35F, or 38, among others.

Administration/Dosage

[0397] The compositions and formulations of the invention can be used to protect or treat a patient susceptible to infection by means of administering the composition, formulation or vaccine via a systemic or mucosal route to the patient. In one embodiment, the invention provides a method of inducing an immune response to an antigen, comprising administering to a patient an immunologically effective amount of an immunogenic composition of the invention. In another embodiment, the invention provides a method of vaccinating a against an infection, comprising the step of administering to the patient an immunologically effective amount of an immunogenic composition, formulation or vaccine of the invention.

[0398] In some embodiments of the methods above, the patient is a human.

[0399] In other embodiments of the methods above, the patient is a non-human animal.

[0400] Optimal amounts of components for a particular composition, formulation or vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. For example, in one embodiment, the dosage for human vaccination is determined by extrapolation from animal studies to human data. In another embodiment, the dosage is determined empirically.

[0401] The methods of the invention can be used for the prevention and/or reduction of primary clinical syndromes caused by infective agents in a subject; i.e., a human or non-human animal.

[0402] Administration of the compositions, formulations or vaccines of the invention can include one or more of: injection via the intramuscular, intraperitoneal, intradermal or subcutaneous routes; or via mucosal administration to the oral/alimentary, respiratory or genitourinary tracts. In one embodiment, intranasal administration is used for the treatment of disease.

[0403] According to any of the methods of the invention and in some embodiments, the subject is human. In certain embodiments, the human patient is an infant (less than 1 year of age), toddler (approximately 12 to 24 months), or young child (approximately 2 to 5 years). In other embodiments, the human patient is an elderly patient (> 65 years). The compositions of the invention are also suitable for use with older children, adolescents and adults (e.g., aged 18 to 45 years or 18 to 65 years).

[0404] In one embodiment of the methods of the invention, a composition, formulation or vaccine of the invention is administered to the subject as a single inoculation. In another embodiment, the composition, formulation or vaccine is administered twice, three times or four times or more, adequately spaced apart. For example, the composition, formulation or vaccine may be administered at 1-, 2-, 3-, 4-, 5-, or 6-month intervals or any combination thereof.

Formulations

[0405] In the embodiments of the formulations below, a composition refers to a pharmaceutical composition and/or an immunogenic composition and/or a single dose vaccine composition. In some embodiments, a composition is provided that includes one or more compounds as described herein and one or more antigens.

[0406] In some embodiments, a composition is provided that includes about 1 µg to about 200 mg of a compound of the invention, or a pharmaceutically acceptable salt thereof, and at least one antigen, wherein the antigen is present in a concentration of about 0.01 µg to about 100 µg per 0.5 mL of the vaccine formulation.

[0407] In some embodiments, a composition is provided that includes about 0.02 µg to about 40 mg of a compound of the invention, or a pharmaceutically acceptable salt thereof, and at least one antigen, wherein the antigen is present in a concentration of about 0.002 µg to about 20 µg per 0.1 mL of the vaccine formulation.

[0408] In some embodiments, a composition is provided that includes about 50 µg to about 2.1 mg of a compound of the invention, or a pharmaceutically acceptable salt thereof, and at least

one antigen, wherein the antigen is present in a concentration of about 0.002 µg to about 20 µg per 0.5 mL of the vaccine formulation.

[0409] In some embodiments, a composition is provided that includes about 50 µg to about 10 mg of a compound of the invention, or a pharmaceutically acceptable salt thereof, and at least one antigen, wherein the antigen is present in a concentration of about 0.002 µg to about 20 µg per 0.5 mL of the vaccine formulation.

[0410] In some embodiments, a composition is provided that includes about 50 µg to about 10 mg of a compound of the invention, or a pharmaceutically acceptable salt thereof, and at least one antigen, wherein the antigen is present in a concentration of about 0.002 µg to about 20 µg per 0.5 mL of the vaccine formulation.

[0411] In some embodiments, a composition is provided that includes about 1 µg to about 200 mg of a compound of the invention, or a pharmaceutically acceptable salt thereof, and at least one antigen, wherein the antigen is present in a concentration of about 0.01 µg to about 100 µg per 0.5 mL of the vaccine formulation prepared as a lyophilized formulation.

[0412] In some embodiments, a composition is provided that includes about 1 µg to about 200 mg of a compound of the invention, or a pharmaceutically acceptable salt thereof, 1 µg to about 1 mg of Aluminum in the form of APA and at least one antigen, wherein the antigen is present in a concentration of about 0.01 µg to about 100 µg per 0.5 mL of the vaccine formulation prepared as a lyophilized formulation.

[0413] In some embodiments, a composition, as highlighted in the various embodiments above, is provided that includes about 0.05 µg to about 200 mg of a compound of the invention, or a pharmaceutically acceptable salt thereof, and further includes SPAN-85, PS-20 or PS-80 and squalene. In some embodiments, a composition, as highlighted in the various embodiments above, is provided that includes about 0.05 µg to about 200 mg of a compound of the invention, or a pharmaceutically acceptable salt thereof, and further includes SPAN-85, PS-20 and squalene. In another embodiment, the compound is Compound A-1, or A-2, or A-3. In another embodiment, the compound is Compound B-1, or B-2, or B-3, or B-4, or B-5, or B-6, or B-7, or B-8, or B-9, or B-10, or B-11, or B-12, or B-13, or B-14, or B-15, or B-16, or B-17, or B-18. In another embodiment, the compound is Compound C-1, or C-2, or C-3, or C-4, or C-5. In another embodiment, the compound is Compound D-1, or D-2, or D-3, or D-4, or D-5, or D-6.

[0414] In some embodiments, a composition of the invention is administered subcutaneously, topically, orally, on the mucosa, intravenously, or intramuscularly. The compositions are administered in an amount sufficient to elicit a protective response. Compositions can also be administered by various other routes, for example, orally, parenterally, subcutaneously, on the

mucosa, or intramuscularly. The dose administered may vary depending on the general condition, sex, weight and age of the patient, and the route of administration.

[0415] Compositions of the invention, as highlighted in the various embodiments above, may be referred to as immunogenic compositions.

[0416] Compositions of the invention, as highlighted in the various embodiments above may be referred to as vaccines or vaccine compositions.

[0417] In each of the embodiments described above the composition further comprises one or more antigens.

[0418] The invention provides methods of treating or preventing diseases by administration of the compositions described above.

[0419] The invention provides the use of the compositions described above for treating or preventing diseases.

[0420] All publications mentioned herein are incorporated by-reference for the purpose of describing and disclosing methodologies and materials that might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0421] Having described certain embodiments of the invention with reference to the accompanying drawings, it is to be understood that the invention is not limited to those precise embodiments, and that various changes and modifications may be used by one skilled in the art without departing from the scope or spirit of the invention as defined in the appended claims.

[0422] The following examples illustrate, but do not limit the invention.

EXAMPLE 1

General Methods For Making the Compounds of Formula I, Ia, II, IIa, III, IIIa, IV and IVa

[0423] The compounds may be prepared from known or readily prepared starting materials, following methods known to one skilled in the art of organic synthesis. Representative methods useful for making the compounds are set forth in the Examples below. Alternative synthetic pathways and analogous structures will be apparent to those skilled in the art of organic synthesis.

[0424] One skilled in the art of organic synthesis will recognize that the synthesis of multicyclic and/or heterocyclic cores contained in compounds of the invention may require protection of certain functional groups (*i.e.*, derivatization for the purpose of chemical compatibility with a particular reaction condition). Suitable protecting groups for the various functional groups of these compounds and methods for their installation and removal are well

known in the art of organic chemistry. A summary of many of these methods can be found in Greene et al., *Protective Groups in Organic Synthesis*, Wiley-Interscience, New York, (1999).

[0425] One skilled in the art of organic synthesis will also recognize that one route for the synthesis of the multicyclic heterocycle cores of the compounds of the invention may be more desirable depending on the choice of appendage substituents.

[0426] Additionally, one skilled in the art will recognize that in some cases, the order of reactions may differ from that presented herein to avoid functional group incompatibilities and thus adjust the synthetic route accordingly.

[0427] The preparation of multicyclic intermediates useful for making the multicyclic and/or heterocyclic cores of the compounds of the invention have been described in the literature and in compendia such as "Comprehensive Heterocyclic Chemistry" editions I, II and III, published by Elsevier and edited by A.R. Katritzky & R. JK Taylor. Manipulation of the required substitution patterns have also been described in the available chemical literature as summarized in compendia such as "Comprehensive Organic Chemistry" published by Elsevier and edited by DH R. Barton and W. D. Ollis; "Comprehensive Organic Functional Group Transformations" edited by edited by A.R. Katritzky & R. JK Taylor and "Comprehensive Organic Transformation" published by Wiley-CVH and edited by R. C. Larock.

[0428] The starting materials used and the intermediates prepared using the methods set forth in the Examples below may be isolated and purified if desired using conventional techniques, including but not limited to filtration, distillation, crystallization, chromatography and alike. Such materials can be characterized using conventional means, including physical constants and spectral data.

[0429] One skilled in the art will be aware of standard formulation techniques as set forth in the open literature as well as in textbooks such as Zheng, "Formulation and Analytical Development for Low-Dose Oral Drug Products," Wiley, 2009, ISBN.

Preparation of Compounds and Intermediates

[0430] The invention is illustrated by the following Examples. For all of the Examples, standard work-up and purification methods known to those skilled in the art can be utilized. Unless otherwise indicated, all temperatures are expressed in °C (degrees Celsius). All reactions are conducted at room temperature unless otherwise noted. Synthetic methodologies illustrated herein are intended to exemplify the applicable chemistry through the use of specific examples and are not indicative of the scope of the disclosure.

General Methods

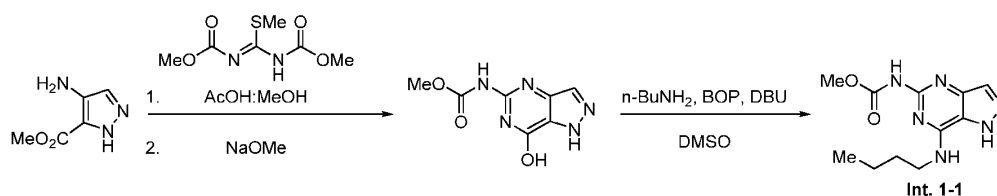
[0431] Solvents, reagents, and intermediates that are commercially available were used as received. Intermediates that are not commercially available were prepared in the manner as described below. ¹H NMR spectra are reported as ppm downfield from Me₄Si with number of protons, multiplicities, and coupling constants in Hertz indicated parenthetically. Where LC/MS data are presented, the observed parent ion is given. Flash column chromatography was performed using pre-packed normal phase silica or bulk silica.

EXAMPLE 2

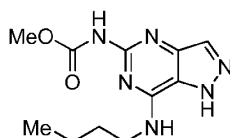
Methods For Making the Intermediates of Compounds of the Invention

Intermediate 1-1

Preparation of Compound Int. 1-1



methyl (7-(butylamino)-1H-pyrazolo[4,3-d]pyrimidin-5-yl)carbamate



Step 1: methyl (7-hydroxy-1H-pyrazolo[4,3-d]pyrimidin-5-yl)carbamate

[0432] To a stirred mixture of methyl 4-amino-1H-pyrazole-5-carboxylate (2 g, 14.17 mmol) and 1,3-bis(methoxycarbonyl)-2-methyl-2-thiopseudothiuron (2.92 g, 14.17 mmol) in MeOH (20 mL) was added AcOH (4.87 mL, 85 mmol) at ambient temperature. The resulting mixture was stirred for 16 h then NaOMe (25.5 g, 142 mmol) in MeOH was added dropwise. After the addition was complete, the reaction mixture was acidified to pH 1-2 with AcOH. The resulting solid was collected by filtration then washed with CH₃CN (200 mL) then dried *in vacuo* to give the title compound. MS *m/z* (M+H)⁺: calculated 210.1, observed 210.1. ¹H-NMR (400 MHz, DMSO-d₆) δ 7.83 (s, 1H), 3.67 (s, 3H).

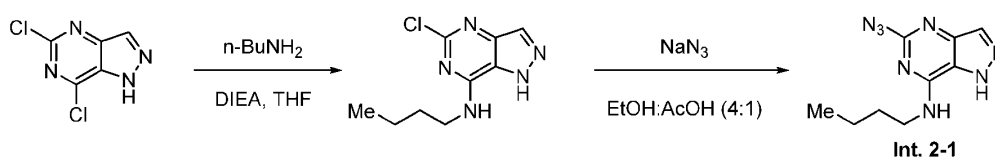
Step 2: methyl (7-(butylamino)-1H-pyrazolo[4,3-d]pyrimidin-5-yl)carbamate

[0433] To a stirred mixture of methyl (7-hydroxy-1H-pyrazolo[4,3-d]pyrimidin-5-yl)carbamate (5 g, 23.90 mmol), butan-1-amine (4.73 mL, 47.8 mmol) and DBU (3.92 mL, 26.3 mmol) in DMSO (100 mL) was added BOP (12.69 g, 28.7 mmol) at ambient temperature. The resulting

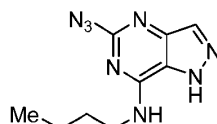
mixture was warmed to 60 °C for 3 h. The resulting mixture was cooled to ambient temperature, then filtered, then purified directly by reverse-phase chromatography (0-50% CH₃CN/water with 0.1% formic acid modifier) to give the title compound. MS m/z (M+H)⁺: calculated 265.1, observed 265.1. ¹H-NMR (400 MHz, DMSO-d₆) δ 12.94 (br., 1H), 9.89 (s, 1H), 8.14 – 7.99 (m, 2H), 3.67 (s, 3H), 3.56 – 3.51 (m, 2H), 1.66 – 1.58 (m, 2H), 1.42 – 1.33 (m, 2H), 0.92 (t, J = 7.2 Hz, 3H).

Intermediate 2-1

Preparation of Compound Int. 2-1



5-azido-N-butyl-1H-pyrazolo[4,3-d]pyrimidin-7-amine



Step 1: N-butyl-5-chloro-1H-pyrazolo[4,3-d]pyrimidin-7-amine

[0434] To a mixture of 5,7-dichloro-1H-pyrazolo[4,3-d]pyrimidine (3 g, 15.87 mmol) in THF (30 mL) at 0 °C was added DIEA (3.08 g, 23.81 mmol) and butan-1-amine (1.741 g, 23.81 mmol). After the addition was complete, the mixture was warmed to ambient temperature and stirred for 2 h. The resulting mixture was diluted with water (200 mL), then extracted with EtOAc (3 x 300 mL). The combined organic extracts were washed with brine (2 x 300 mL), then dried (Na₂SO₄), then filtered. The filtrate was concentrated to give the title compound, which was used directly in the next step without purification. MS m/z (M+H)⁺: calculated 226.1, observed 226.2.

Step 2: 5-azido-N-butyl-1H-pyrazolo[4,3-d]pyrimidin-7-amine

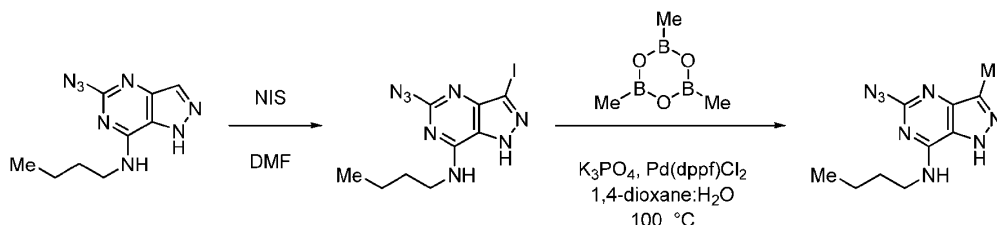
[0435] To a mixture of N-butyl-5-chloro-1H-pyrazolo[4,3-d]pyrimidin-7-amine (3.5 g, 15.51 mmol) in AcOH (6 mL) and EtOH (24 mL) at ambient temperature was added sodium azide (1.512 g, 23.26 mmol), then the mixture was heated to 100 °C for 3 h. The resulting mixture was cooled to ambient temperature, then diluted with water (200 mL), then extracted with EtOAc (3 x 300 mL). The combined organic extracts were washed with brine (2 x 300 mL), then dried (Na₂SO₄), then filtered, then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-20% EtOAc/petroleum ether) to give the title compound. MS m/z (M+H)⁺: calculated 233.1, observed 233.1.

[0436] Utilizing the procedures described for **Intermediate 2-1**, the following compounds were prepared substituting the appropriate reagents for butan-1-amine.

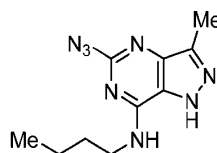
Cmpd/ Int. #	Structure	Name	Calc'd [M+H] ⁺	Observed [M+H] ⁺	Comments
Int. 2-2		(S)-2-((5-azido-1H-pyrazolo[4,3-d]pyrimidin-7-yl)amino)pentan-1-ol	263.3	263.3	

Intermediate 2-3

Preparation of Compound Int. 2-3



5-azido-N-butyl-3-methyl-1H-pyrazolo[4,3-d]pyrimidin-7-amine



Step 1: 5-azido-N-butyl-3-iodo-1H-pyrazolo[4,3-d]pyrimidin-7-amine

[0437] To a solution of 5-azido-N-butyl-2H-pyrazolo[4,3-d]pyrimidin-7-amine (1g, 4.31 mmol) in DMF (30 mL) was added *N*-iodosuccinimide (1.937 g, 8.61 mmol) then the mixture was heated to 40 °C. After heating overnight, the mixture was cooled to rt then diluted 10% Na₂SO₃ (40 mL). The resulting solid was collected by filtration then washed with water (10 mL x 3) then dried under vacuum to give the title compound. MS *m/z* (M+H)⁺: calculated 359.0, observed 359.0.

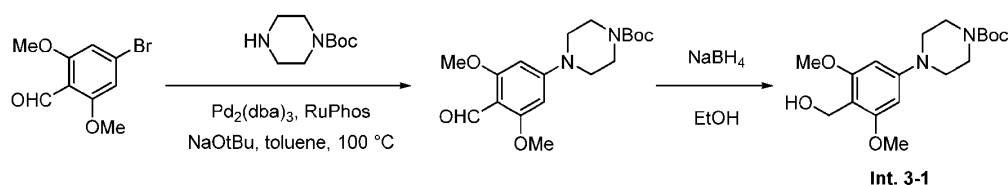
Step 2: 5-azido-N-butyl-3-methyl-1H-pyrazolo[4,3-d]pyrimidin-7-amine

[0438] To a solution of 5-azido-N-butyl-3-iodo-2H-pyrazolo[4,3-d]pyrimidin-7-amine (1 g, 2.79 mmol) in 1,4-dioxane (10 mL) and H₂O (1 mL) was added Pd(dppf)Cl₂ (0.204 g, 0.279 mmol), K₃PO₄ (1.185 g, 5.58 mmol) and 2,4,6-trimethyl-1,3,5,2,4,6-trioxatriborinane (3.51 g,

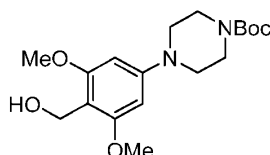
13.96 mmol) under N₂ then the mixture was heated to 100 °C overnight. The resulting mixture was cooled to rt then diluted with EtOAc (30 mL) then dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (30-70% EtOAc/pet. ether) to give the title compound. MS *m/z* (M+H)⁺: calculated 247.1, observed 247.4.

Intermediate 3-1

Preparation of Compound Int. 3-1



tert-butyl 4-(4-(hydroxymethyl)-3,5-dimethoxyphenyl)piperazine-1-carboxylate

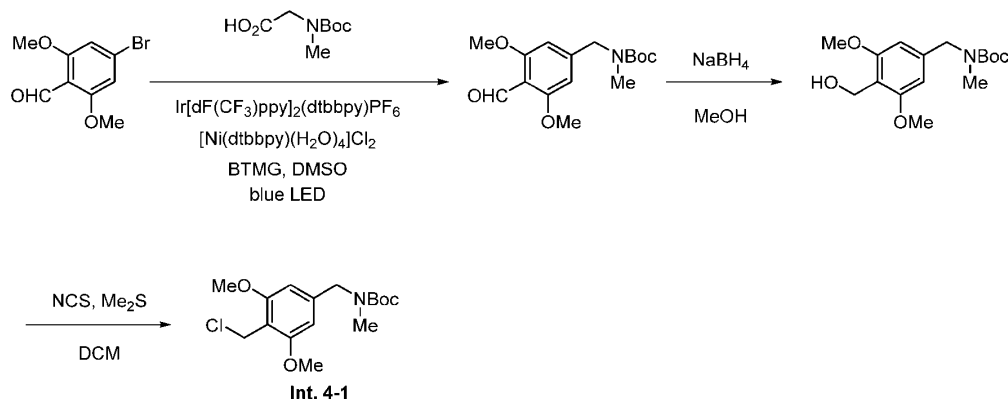
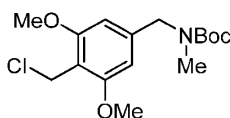


Step 1: tert-butyl 4-(4-formyl-3,5-dimethoxyphenyl)piperazine-1-carboxylate

[0439] To a stirred mixture of 4-bromo-2,6-dimethoxybenzaldehyde (5g, 20.40 mmol) in toluene (20 mL) was added *tert*-butyl piperazine-1-carboxylate (5.70 g, 30.6 mmol), RuPhos (1.904 g, 4.08 mmol), Pd₂(dba)₃ (1.868 g, 2.040 mmol), and NaOtBu (5.88 g, 61.2 mmol) under an argon atmosphere, then the mixture was heated to 100 °C. After 6 h the mixture was cooled to ambient temperature, then diluted with water (50 mL), then extracted with EtOAc (3 x 80 mL). The combined organic extracts were washed with brine (3 x 50 mL), then dried (Na₂SO₄), then filtered, then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-100% EtOAc/DCM) to give the title compound. MS *m/z* (M+H)⁺: calculated 351.2, observed 351.3.

Step 2: tert-butyl 4-(4-(hydroxymethyl)-3,5-dimethoxyphenyl)piperazine-1-carboxylate

[0440] To a stirred mixture of *tert*-butyl 4-(4-formyl-3,5-dimethoxyphenyl)piperazine-1-carboxylate (3 g, 8.56 mmol) in EtOH (10 mL) was added NaBH₄ (0.389 g, 10.27 mmol) at ambient temperature. After 15 minutes the mixture was diluted with water (15 mL) then extracted with Et₂O (3 x 50 mL). The combined organic extracts were washed with brine (3 x 50 mL), then dried (Na₂SO₄), then filtered. The filtrate was then concentrated give the title compound which was used directly in the next step without purification. MS *m/z* (M+H)⁺: calculated 353.2, observed 353.3.

Intermediate 4-1**Preparation of Compound Int. 4-1*****tert*-butyl (4-(chloromethyl)-3,5-dimethoxybenzyl)(methyl)carbamate**

Step 1: tert-butyl (4-(formyl)-3,5-dimethoxybenzyl)(methyl)carbamate

[0441] 4-bromo-2,6-dimethoxybenzaldehyde (6 g, 24.48 mmol), *N*-(*tert*-butoxycarbonyl)-*N*-methylglycine (9.26 g, 49.0 mmol), $\text{Ir}[\text{dF}(\text{CF}_3)\text{ppy}]_2(\text{dtbbpy})\text{PF}_6$ (0.275 g, 0.245 mmol), and $[\text{Ni}(\text{dtbbpy})(\text{H}_2\text{O})_4]\text{Cl}_2$ (0.575 g, 1.224 mmol) were combined in DMSO (120 mL). To this was added BTMG (10 mL, 49.0 mmol). The mixture was sonicated until all solids dissolved. The resulting mixture was divided evenly into 4 screw cap vials with stir bars. N_2 was bubbled through each mixture for 5 minutes. The vials were capped then the mixtures were irradiated in a PennOC Photoreactor[®] (wavelength: 420 nm; LED intensity: 100%; fan speed: 5000 rpm; stir: 1200 rpm) overnight. The separated mixtures were combined then diluted with H_2O then extracted with EtOAc (3 x). The combined organic extracts were washed with H_2O and brine then dried (MgSO_4) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-50% 3:1 EtOAc:EtOH/heptane) to give the title compound. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 10.47 (s, 1H), 6.44 (bs, 2H), 4.41 (s, 2H), 3.88 (s, 6H), 2.87 (bs, 3H), 1.49 (bs, 9H).

Step 2: tert-butyl (4-(hydroxymethyl)-3,5-dimethoxybenzyl)(methyl)carbamate

[0442] A solution of *tert*-butyl (4-(formyl)-3,5-dimethoxybenzyl)(methyl)carbamate (6.98 g, 22.56 mmol) in MeOH (100 mL) was cooled to 0°C . To this was added NaBH_4 (1.03 g, 27.2 mmol) in portions. After 1 h gas evolution had ceased. The cooling bath was removed and the

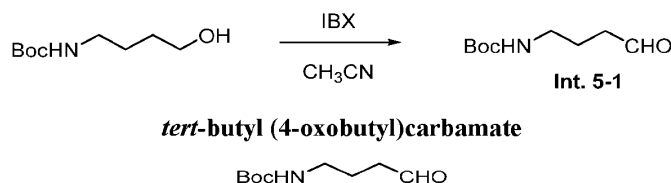
mixture warmed to ambient temperature. After 90 minutes the mixture was concentrated. The residue was taken up in DCM, filtered through a pad of Celite® washing with DCM, then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-15% 3:1 EtOAc:EtOH/heptane) to give the title compound. ¹H-NMR (400 MHz, CDCl₃) δ 6.45 (bs, 2H), 4.76 (d, J = 6.6 Hz, 2H), 4.39 (s, 2H), 3.83 (s, 6H), 2.86 (bs, 3H), 1.49 (s, 9H).

Step 3: tert-butyl (4-(chloromethyl)-3,5-dimethoxybenzyl)(methyl)carbamate

[0443] A solution of *N*-chlorosuccinimide (0.472 g, 3.53 mmol) in DCM (1 mL) was cooled to 0°C. To this was added dimethylsulfide (0.239 g, 3.85 mmol) slowly resulting in a precipitate. The reaction mixture was cooled to -20 °C then a solution of *tert*-butyl (4-(hydroxymethyl)-3,5-dimethoxybenzyl)(methyl)carbamate (1 g, 3.21 mmol) in DCM was added dropwise. The mixture was stirred for 2 h allowing the temperature to reach 0 °C during which time all the solid precipitate had dissolved giving a clear solution. The solution was poured over cold brine then extracted with Et₂O (2x). The combined organic extracts were washed with cold brine then dried (Na₂SO₄) then filtered then the filtrate was concentrated give the title compound which was used directly in the next step without purification. MS *m/z* (M+H)⁺: calculated 330.1, observed 329.2.

Intermediate 5-1

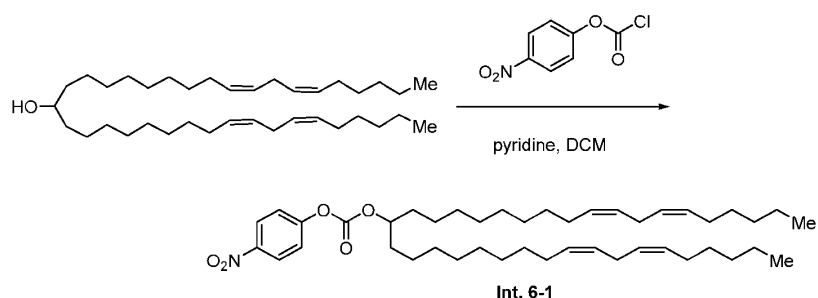
Preparation of Compound **Int. 5-1**



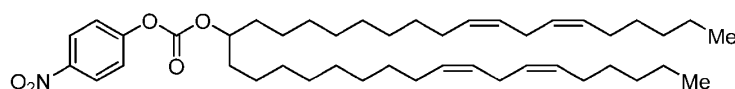
[0444] To a mixture of *tert*-butyl (4-hydroxybutyl)carbamate (1 g, 5.28 mmol) in CH₃CN (30 mL) at ambient temperature was added IBX (1.231 g, 6.34 mmol) then the mixture was heated to 80°C. After 1 h the mixture was cooled to ambient temperature then diluted with water (100 mL) then extracted with EtOAc (3 x 200 mL). The combined organic extracts were washed with brine (2 x 200 mL) then dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-70% EtOAc/petroleum ether) to give the title compound. ¹H-NMR (400 MHz, CDCl₃): δ 3.76 – 3.73 (m, 2H), 2.53 – 2.49 (t, 2H), 2.03 – 1.97 (m, 2H), 1.53 – 1.45 (s, 9H).

Intermediate 6-1

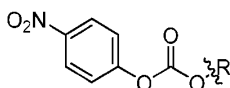
Preparation of Compound **Int. 6-1**



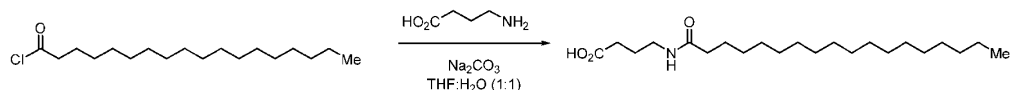
(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl (4-nitrophenyl) carbonate

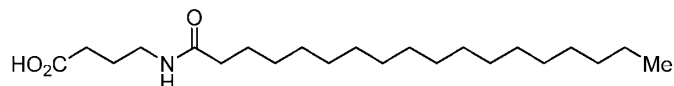


[0445] A mixture of (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-ol (300 mg, 0.567 mmol) and hypochlorous 4-nitrobenzoic anhydride (229 mg, 1.134 mmol) in DCM (4.5 mL) and pyridine (1.5 mL) was stirred at ambient temperature for 5 h. The resulting mixture was concentrated then purified by prep-TLC (petroleum ether:DCM = 1:1) to give the title compound. Utilizing the procedures described for **Intermediate 6-1**, the following compounds were prepared substituting the appropriate reagents for (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-ol.



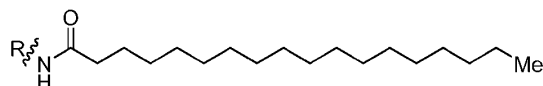
Cmpd/ Int. #	R	Name	Calc'd [M+H] ⁺	Observed [M+H] ⁺	Comments
Int. 6-2		4-nitrophenyl (3-stearamidopropyl) carbonate	507.3	507.4	from Int. 8-2 ; substitute DIEA for pyridine as base

Intermediate 7-1Preparation of Compound **Int. 7-1**

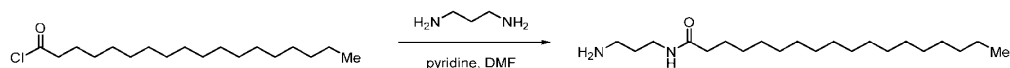
4-Stearamidobutanoic acid

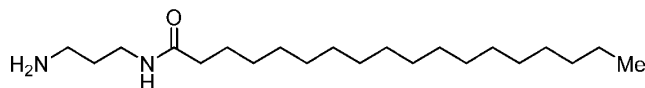
[0446] A mixture of 4-aminobutanoic acid (1 g, 9.70 mmol) and Na₂CO₃ (1.542 g, 14.55 mmol) in THF (30 mL) and water (30 mL) was cooled to 0 °C. To this was added stearoyl chloride (2.94 g, 9.70 mmol). After the addition was complete the mixture was stirred for 16 h at 25 °C. The resulting mixture was quenched with saturated KHSO₄ then extracted with CHCl₃ (500 mL). The combined organic layers were washed with brine (3 x 100 mL) then dried (Na₂SO₄) then filtered then the filtrate was concentrated to give the title compound. MS *m/z* (M-H)⁻: calculated 368.6, observed 368.3.

[0447] Utilizing the procedures described for **Intermediate 7-1**, the following compounds were prepared substituting the appropriate reagents for 4-aminobutanoic acid.



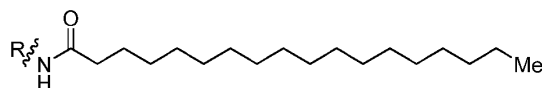
Cmpd/ Int. #	R	Name	Calc'd [M-H] ⁻	Observed [M-H] ⁻	Comments
Int. 7-2	HO ₂ C-CH ₂ -CH ₂ -	3-stearamidopropanoic acid	354.4	354.3	
Int. 7-3	HO ₂ C-CH ₂ -CH ₂ -CH ₂ -CH ₂ -CH ₂ -CH ₂ -	7-stearamidoheptanoic acid	410.7	410.4	methyl 7-aminoheptanoate hydrochloride was used as the amine; ester hydrolysis was achieved with NaOH in THF:H ₂ O (5:1)
Int. 7-4	HO ₂ C-CH ₂ -CH ₂ -CH ₂ -CH ₂ -	5-stearamidopentanoic acid	382.6	382.3	
Int. 7-5	HO ₂ C-CH ₂ -cyclobutyl-	2-(3-stearamidocyclobutyl) acetic acid	396.6	396.6	MS [M+H] ⁺

Intermediate 8-1Preparation of Compound **Int. 8-1**

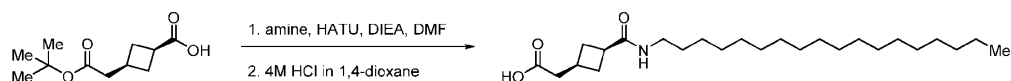
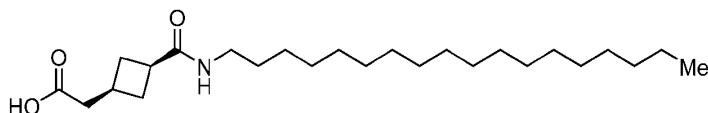
***N*-(3-aminopropyl)stearamide**

[0448] A mixture of propane-1,3-diamine (1 g, 13.49 mmol) and pyridine (1.091 mL, 13.49 mmol) in DMF (15 mL) was cooled to 0 °C. To this was added stearoyl chloride (4.09 g, 13.49 mmol). After the addition was complete the mixture was stirred for 16 h at 25 °C. The resulting mixture was diluted with ice water resulting in a solid. The solid was collected by filtration then washed with water (3 x 100 mL) then dried to give the title compound. MS m/z (M+H)⁺: calculated 341.6, observed 341.3.

[0449] Utilizing the procedures described in **Intermediate 8-1** the following compounds were prepared substituting the appropriate reagents for propane-1,3-diamine.



Cmpd/ Int. #	R	Name	Calc'd [M+H] ⁺	Observed [M+H] ⁺	Comments
Int. 8-2		<i>N</i> -(3-hydroxypropyl)stearamide	342.3	342.3	
Int. 8-3		<i>N</i> -(5-hydroxy-4,4-dimethylpentyl)stearamide	398.4	398.7	

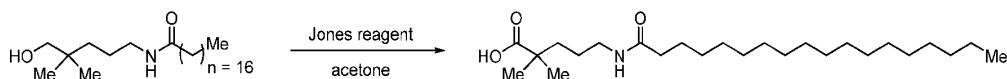
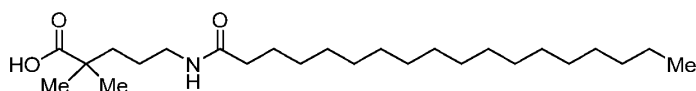
Intermediate 9-1**Preparation of Compound Int. 9-1****2-((1s,3s)-3-(octadecylcarbamoyl)cyclobutyl)acetic acid**

Step 1: tert-butyl 2-((1s,3s)-3-(octadecylcarbamoyl)cyclobutyl)acetate

[0450] To a stirred mixture of (1s,3s)-3-(2-(*tert*-butoxy)-2-oxoethyl)cyclobutane-1-carboxylic acid (0.5 g, 2.334 mmol), HATU (1.78 g, 4.67 mmol), and DIEA (1.22 mL, 7.00 mmol) in DMF (10 mL) was added a solution of octadecan-1-amine (0.818 g, 3.03 mmol) in DMF (10 mL) at 25 °C. After 12 hr the mixture was diluted with water (10 mL) then extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (3 x 10 mL) then dried (Na₂SO₄) then filtered then the filtrate was concentrated to give the title compound as an 80:20 mixture with *tert*-butyl 2-((1s,3s)-3-(hexadecylcarbamoyl)cyclobutyl)acetate. MS *m/z* (M+H)⁺: calculated 466.4, observed 466.2.

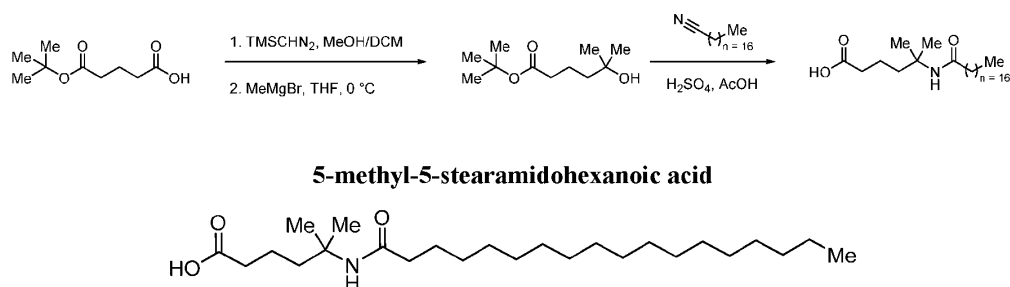
Step 2: 2-((1s,3s)-3-(octadecylcarbamoyl)cyclobutyl)acetic acid

[0451] To a stirred mixture of *tert*-butyl 2-(3-(octadecylcarbamoyl)cyclobutyl)acetate (0.932 g, 2 mmol) in 1,4-dioxane (16 mL) was added 4M HCl (4 mL) in 1,4-dioxane at 25 °C. After 6 hr the mixture was diluted with EtOAc then washed with brine (3 x 30 mL). The organic layer was dried (Na₂SO₄) then filtered then the filtrate was concentrated to give the title compound as an 80:20 mixture with 2-((1s,3s)-3-(hexadecylcarbamoyl)cyclobutyl)acetic acid. MS *m/z* (M+H)⁺: calculated 410.4, observed 410.4.

Intermediate 10-1Preparation of Compound **Int. 10-1****2,2-dimethyl-5-stearamidopentanoic acid**

[0452] To a mixture of *N*-(5-hydroxy-4,4-dimethylpentyl)stearamide (**Int. 8-3**, 500 mg, 1.257 mmol) in acetone (10 mL) was added chromium trioxide solution in sulfuric acid (0.691 mL, 1.383 mmol) at 0 °C. After 6 hr the mixture was diluted with water. The resulting solid was collected by filtration then washed with water then air dried. MS *m/z* (M+H)⁺: calculated 412.4, observed 412.1.

Intermediate 11-1Preparation of Compound **Int. 11-1**



Step 1: tert-butyl methyl glutarate

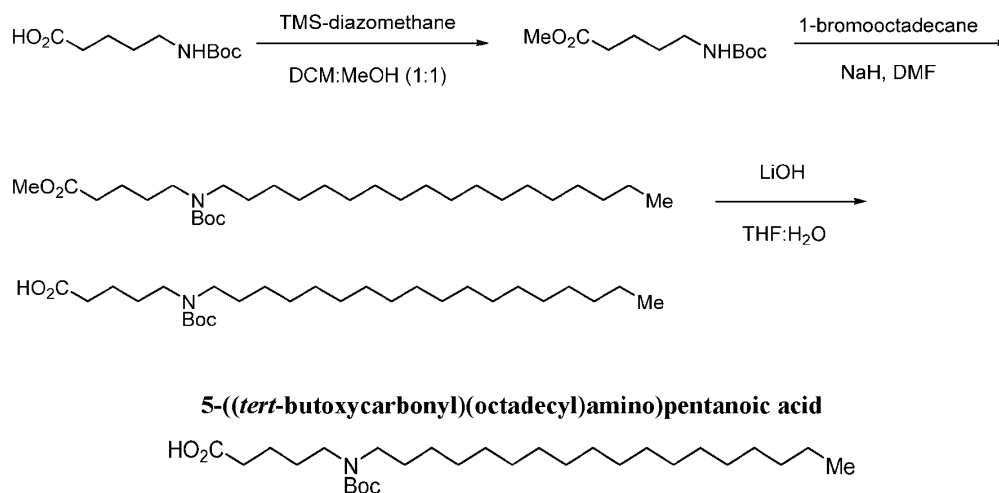
[0453] A solution of 5-(*tert*-butoxy)-5-oxopentanoic acid (5 g, 26.6 mmol) in DCM (40 mL) and MeOH (40 mL) was cooled to 0 °C. To this was added (diazomethyl)trimethylsilane (39.8 mL, 80 mmol, 2M in hexane). After the addition was complete the mixture was warmed to rt. After 3 hr the mixture was concentrated. The crude product was subjected to silica gel chromatography (0-5% EtOAc/pet. ether) to give the title compound. ¹H NMR (400 MHz, CDCl₃) δ 3.68 (s, 3H), 2.37 (t, *J* = 7.2 Hz, 2H), 2.28 (t, *J* = 7.6 Hz, 2H), 1.91 (quin, *J* = 7.2 Hz, 2H), 1.44 (s, 9H).

Step 2: tert-butyl 5-hydroxy-5-methylhexanoate

[0454] To a solution of *tert*-butyl methyl glutarate (1 g, 4.94 mmol) in THF (16 mL) was added MeMgBr (4.12 mL, 12.36 mmol, 3M in THF) at 0 °C. After 2 hr the mixture was quenched with saturated NH₄Cl (25 mL) then warmed to rt then extracted with EtOAc (10 mL x 3). The combined organic layers were washed with brine (30 mL) then dried (Na₂SO₄) then filtered then the filtrate concentrated. The crude product was subjected to silica gel chromatography (0-20% EtOAc/pet. ether) to give the title compound. ¹H NMR (400 MHz, CDCl₃) δ 2.25 (t, *J* = 7.2 Hz, 2H), 1.69-1.64 (m, 2H), 1.50-1.48 (m, 2H), 1.45 (s, 9H), 1.23 (s, 6H).

Step 3: 5-methyl-5-stearamido-hexanoic acid

[0455] To a solution of *tert*-butyl 5-hydroxy-5-methylhexanoate (300 mg, 1.483 mmol) in AcOH (2 mL) was added stearonitrile (787 mg, 2.97 mmol) at rt then the mixture was cooled to 0 °C. H₂SO₄ (0.5 mL) was added. After the addition was complete the cooling bath was removed and the mixture warmed to rt. After stirring overnight the mixture was quenched with saturated NaHCO₃ to pH = 5. The resulting mixture was extracted with EtOAc (2 mL x 3). The combined organic layers were dried (Na₂SO₄) then filtered then the filtrate was concentrated. The residue was taken up in MeCN (5 mL). The resulting precipitated solid was collected then recrystallized from EtOAc to give the title compound. MS *m/z* (M+H)⁺: calculated 412.7, observed 412.3.

Intermediate 12-1**Preparation of Compound Int. 12-1**

Step 1: methyl 5-((tert-butoxycarbonyl)amino)pentanoate

[0456] To a solution of 5-((*tert*-butoxycarbonyl)amino)pentanoic acid (2 g, 9.21 mmol) in MeOH (15 mL) and DCM (15 mL) was added 2M (trimethylsilyl)diazomethane (23.01 mL, 46.0 mmol) in hexane at rt. After 1 hr the mixture was concentrated to give the title compound which was used without purification. ¹H NMR (400 MHz, CDCl₃) δ 4.56 (s, 1H), 3.68 (s, 3H), 3.13 (q, J = 6.4 Hz, 2H), 2.34 (t, J = 7.2 Hz, 2H), 1.70 - 1.62 (m, 2H), 1.49 - 1.56 (m, 2H), 1.45 (s, 9H).

Step 2: methyl 5-((tert-butoxycarbonyl)(octadecyl)amino)pentanoate

[0457] To a solution of methyl 5-((*tert*-butoxycarbonyl)amino)pentanoate (1 g, 4.32 mmol) in DMF (20 mL) was added NaH (0.259 g, 6.49 mmol, 60% in mineral oil) at 0 °C. After the addition was complete the mixture was warmed to rt. After 30 minutes 1-bromooctadecane (4.32 g, 12.97 mmol) was added then the mixture was heated to 50 °C. After 2 hr the mixture was cooled to rt then diluted with water (100 mL) then extracted with EtOAc (3x). The combined organic layers were washed with brine (50 mL) then dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-20% EtOAc/pet. ether) to give the title compound. ¹H NMR (400 MHz, CD₃OD) δ 3.67 - 3.64 (m, 3H), 3.23-3.15 (m, 4H), 2.39-2.33 (m, 2H), 1.57 (d, J = 4.4 Hz, 6H), 1.46 (s, 9H), 1.29 (s, 30H), 0.92 - 0.88 (m, 3H).

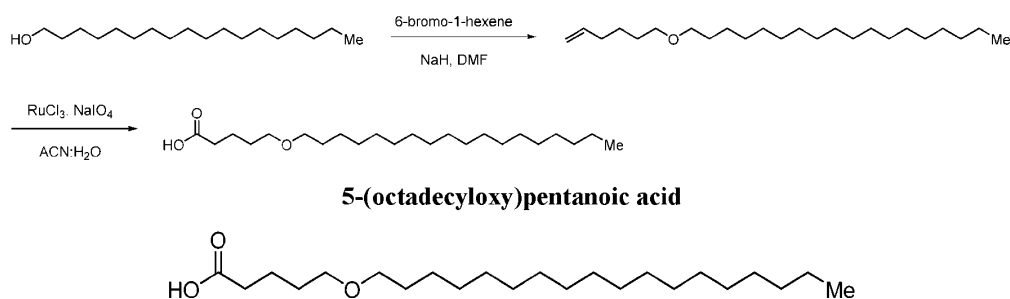
Step 3: 5-((tert-butoxycarbonyl)(octadecyl)amino)pentanoic acid

[0458] To a stirred solution of methyl 5-((*tert*-butoxycarbonyl)(octadecyl)amino)pentanoate (240 mg, 0.496 mmol) in THF (6 mL) and H₂O (2 mL) was added LiOH monohydrate (62.5 mg,

1.488 mmol) at rt then the mixture was warmed to 40 °C. After stirring overnight the mixture was cooled to rt then the pH was adjusted to 7 with 1N HCl. The resulting mixture was extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with brine (3 x 5 mL) then dried (Na₂SO₄) then filtered then the filtrate was concentrated to give the title compound which was used in the next step without purification. ¹H NMR (400 MHz, CD₃OD) δ 3.23 - 3.14 (m, 4H), 2.35 - 2.28 (m, 2H), 1.65 - 1.52 (m, 6H), 1.46 (s, 9H), 1.29 (s, 30H), 0.92-0.88 ppm (m, 3H).

Intermediate 13-1

Preparation of Compound Int. 13-1



Step 1: 1-(hex-5-en-1-yloxy)octadecane

[0459] To a solution of octadecan-1-ol (5 g, 18.48 mmol) in DMF (100 mL) was added NaH (3.70 g, 92 mmol) (60% in mineral oil) in portions at rt. After stirring for 30 minutes 6-bromo-1-hexene (24.70 mL, 185 mmol) was added then the mixture was heated to 80 °C. After heating overnight the mixture was cooled to rt then diluted with water (200 mL) then extracted with EtOAc (100 mL x 3). The combined organic layers were dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-100% EtOAc/pet. ether) to give the title compound. ¹H NMR (400 MHz, CDCl₃) δ 5.82 (ddt, J = 17.2, 10.4, 6.8, 6.8 Hz, 1H), 5.01 (dq, J = 17.6, 1.2 Hz, 1H), 4.95 (d, J = 10.0 Hz, 1H), 3.45 - 3.36 (m, 4H), 2.08 (d, J = 7.2 Hz, 2H), 1.65 - 1.55 (m, 5H), 1.49 - 1.44 (m, 2H), 1.39 - 1.17 (m, 29H), 0.85 - 0.92 (m, 3H).

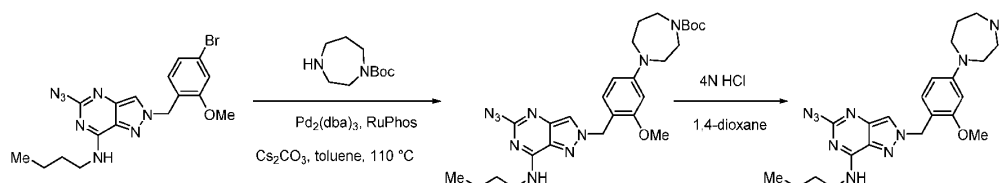
Step 2: 5-(octadecyloxy)pentanoic acid

[0460] To a solution of 1-(hex-5-en-1-yloxy) octadecane (1 g, 2.84 mmol) in MeCN (10 mL) was added a solution of RuCl₃ (0.059 g, 0.284 mmol) and NaIO₄ (2.426 g, 11.34 mmol) in H₂O (10 mL) at rt. After 2 hr the mixture was diluted with Na₂SO₃ (25 mL) then extracted with EtOAc (25 mL x 3). The combined organic layers were washed with 1N HCl (20 mL), saturated NaHCO₃ (20 mL), and brine (20 mL) then dried (Na₂SO₄) then filtered then the filtrate was

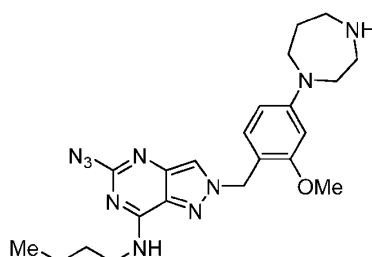
concentrated to give the title compound which was used in the next step without purification. ^1H NMR (400 MHz, CDCl_3) δ ppm 3.39 - 3.44 (m, 4 H), 2.40 (t, $J=7.27$ Hz, 2 H), 1.70 - 1.75 (m, 2 H), 1.65 (br d, $J=7.51$ Hz, 2 H), 1.55 - 1.58 (m, 2 H), 1.26 (s, 30 H), 0.87 - 0.89 (m, 3 H).

Intermediate 14-1

Preparation of Compound Int. 14-1



2-(4-(1,4-diazepan-1-yl)-2-methoxybenzyl)-5-azido-N-butyl-2H-pyrazolo[4,3-d]pyrimidin-7-amine



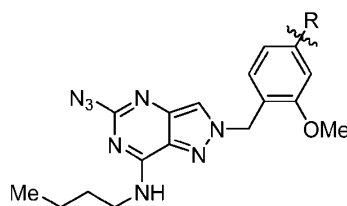
Step 1: Tert-butyl 1-(4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperidine-4-carboxylate

[0461] To a mixture of 5-azido-2-(4-bromo-2-methoxybenzyl)-N-butyl-2H-pyrazolo[4,3-d]pyrimidin-7-amine (from Compound B-1 step 1, 500 mg, 1.159 mmol) and *tert*-butyl 1,4-diazepane-1-carboxylate (302 mg, 1.507 mmol) in toluene (10 mL) was added RuPhos (216 mg, 0.464 mmol), $\text{Pd}_2(\text{dba})_3$ (212 mg, 0.232 mmol) and Cs_2CO_3 (1133 mg, 3.48 mmol) under N_2 then the mixture was heated to 110 °C. After heating overnight the mixture was cooled to rt then diluted with water (100 mL) then the resulting mixture was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (3 x 300 mL) then dried (Na_2SO_4) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-52% 3:1 EtOAc/pet. ether) to give the title compound. MS m/z ($\text{M}+\text{H}^+$): calculated 551.7, observed 551.1.

Step 2: 2-(4-(1,4-diazepan-1-yl)-2-methoxybenzyl)-5-azido-N-butyl-2H-pyrazolo[4,3-d]pyrimidin-7-amine

[0462] A solution of *tert*-butyl 4-(4-((5-azido-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-1,4-diazepane-1-carboxylate (350 mg, 0.636 mmol) in DCE (2.00 mL) was cooled to 0 °C. To this was added 4M HCl in 1,4-dioxane (2 mL) at 0 °C. After 1 hr the mixture was warmed to rt then concentrated to give the title compound as the HCl salt which was used without purification. MS *m/z* (M+H)⁺: calculated 451.3, observed 451.3.

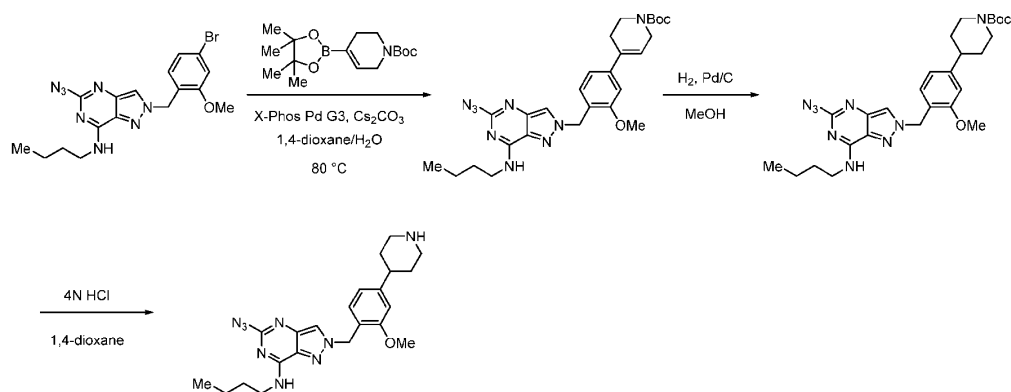
[0463] Utilizing the procedures described for **Intermediate 14-1**, the following compounds were prepared substituting the appropriate reagents for *tert*-butyl 1,4-diazepane-1-carboxylate.



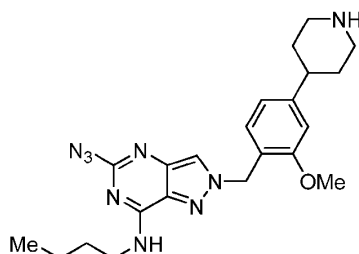
Cmpd/ Int. #	R	Name	Calc'd [M+H] ⁺	Observed [M+H] ⁺	Comments
Int. 14-2		1-(4-((5-azido-7-(butylamino)-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperidine-4-carboxylic acid	480.2	480.2	

Intermediate 15-1

Preparation of Compound Int. 15-1



5-azido-*N*-butyl-2-(2-methoxy-4-(piperidin-4-yl)benzyl)-2*H*-pyrazolo[4,3-*d*]pyrimidin-7-amine



*Step 1: Tert-butyl 4-(4-((5-azido-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-3,6-dihydropyridine-1(2*H*)-carboxylate*

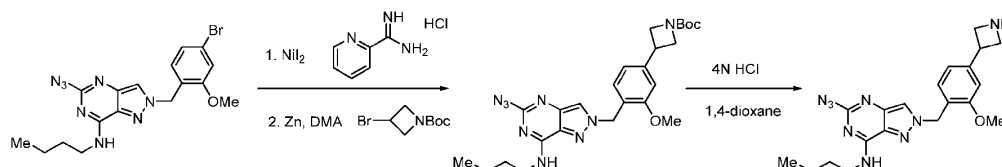
[0464] To a mixture of 5-azido-2-(4-bromo-2-methoxybenzyl)-*N*-butyl-2*H*-pyrazolo[4,3-*d*]pyrimidin-7-amine (**from Compound B-1 step 1**, 400 mg, 0.927 mmol) and *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (430 mg, 1.391 mmol) in 1,4-dioxane (4 mL)/H₂O (0.4 mL) was added Cs₂CO₃ (604 mg, 1.855 mmol) and X-Phos Pd G3 (39.3 mg, 0.046 mmol) under N₂ then the mixture was heated to 80 °C. After 2 hr the mixture was cooled to rt then diluted with EtOAc (200 mL). The resulting mixture was washed with brine (3 x 100 mL) then dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-50% EtOAc/pet. ether) to give the title compound. MS *m/z* (M+H)⁺: calculated 534.3, observed 534.4.

*Step 2: tert-butyl 4-(4-((5-azido-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperidine-1-carboxylate*

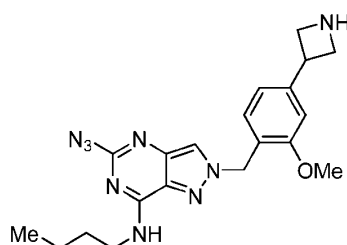
[0465] To a solution of *tert*-butyl 4-(4-((5-azido-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-3,6-dihydropyridine-1(2*H*)-carboxylate (290 mg, 0.543 mmol) in MeOH (10 mL) was added Pd/C (150 mg, 1.410 mmol) under N₂. The resulting mixture was placed under and atmosphere of H₂ (3 x vacuum/H₂) and stirred at rt. After 16 hr the mixture was degassed (3 x vacuum/N₂) then filtered washing with MeOH then the filtrate was concentrated to give the title compound. MS *m/z* (M+H)⁺: calculated 536.3, observed 536.3.

*Step 3: 5-azido-*N*-butyl-2-(2-methoxy-4-(piperidin-4-yl)benzyl)-2*H*-pyrazolo[4,3-*d*]pyrimidin-7-amine*

[0466] To a solution of *tert*-butyl 4-(4-((5-azido-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperidine-1-carboxylate (250 mg, 0.467 mmol) in DCM (2 mL) was added 4M HCl in 1,4-dioxane (2 mL) at rt. After 1 hr the mixture was concentrated to give the title compound as the HCl salt which was used without purification. MS *m/z* (M+H)⁺: calculated 436.3, observed 436.6.

Intermediate 16-1**Preparation of Compound Int. 16-1**

2-(4-(azetidin-3-yl)-2-methoxybenzyl)-5-azido-N-butyl-2H-pyrazolo[4,3-d]pyrimidin-7-amine

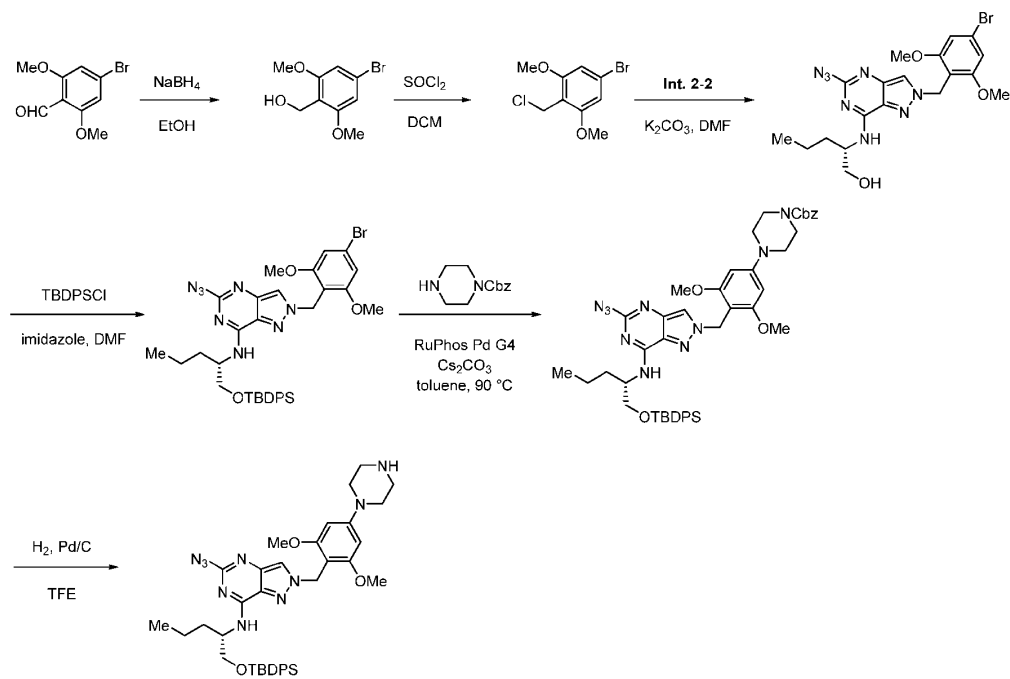


Step 1: Tert-butyl 3-(4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)azetidine-1-carboxylate

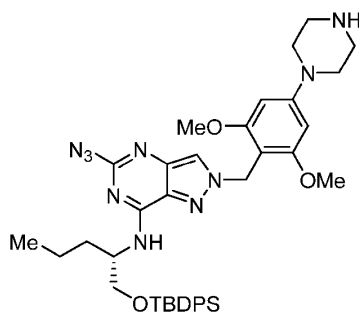
[0467] To a solution of NiI_2 (36.2 mg, 0.116 mmol) in DMA (1 mL) was added picolinimidamide hydrochloride (36.5 mg, 0.232 mmol) then the mixture was heated to 50 °C. After 30 minutes zinc powder (152 mg, 2.319 mmol) was added then a solution of 5-azido-2-(4-bromo-2-methoxybenzyl)-N-butyl-2H-pyrazolo[4,3-d]pyrimidin-7-amine (**from Compound B-1 step 1**, 500 mg, 1.159 mmol) and *tert*-butyl 3-bromoazetidine-1-carboxylate (411 mg, 1.739 mmol) in DMA (10 mL). After stirring overnight at rt the mixture was purified directly by reverse-phase chromatography (CH_3CN /water with 0.1% TFA modifier) to give the title compound. MS m/z ($\text{M}+\text{H}^+$): calculated 508.3, observed 508.4.

Step 2: 2-(4-(azetidin-3-yl)-2-methoxybenzyl)-5-azido-N-butyl-2H-pyrazolo[4,3-d]pyrimidin-7-amine

[0468] To a solution of *tert*-butyl 3-(4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)azetidine-1-carboxylate (60 mg, 0.118 mmol) in DCM (0.5 mL) was added 4M HCl in 1,4-dioxane (0.5 mL) at rt. After 1 hr the mixture was concentrated to give the title compound as the HCl salt which was used without purification. MS m/z ($\text{M}+\text{H}^+$): calculated 408.2, observed 408.3.

Intermediate 17-1**Preparation of Compound Int. 17-1**

(S)-5-azido-N-(1-((*tert*-butyldiphenylsilyl)oxy)pentan-2-yl)-2-(2,6-dimethoxy-4-(piperazin-1-yl)benzyl)-2H-pyrazolo[4,3-*d*]pyrimidin-7-amine



Step 1: (4-bromo-2,6-dimethoxyphenyl)methanol

[0469] To a solution of 4-bromo-2,6-dimethoxybenzaldehyde (5 g, 20.40 mmol) in EtOH (60 mL) was added NaBH_4 (0.926 g, 24.48 mmol) at rt. After 1 hr the mixture was diluted with water (50 mL) then extracted with EtOAc (30 mL x 2). The combined organic layers were washed with brine (50 mL) then dried (Na_2SO_4) then filtered then the filtrate was concentrated. The crude

product was subjected to silica gel chromatography (15-50% EtOAc/pet. ether) to give the title compound. ¹H NMR (400 MHz, CDCl₃) δ 6.72 (s, 2H), 4.72 (d, J = 6.8 Hz, 2H), 3.84 (s, 6H), 2.32 (t, J = 6.8 Hz, 1H).

Step 2: 5-bromo-2-(chloromethyl)-1,3-dimethoxybenzene

[0470] To a solution of (4-bromo-2,6-dimethoxyphenyl)methanol (1 g, 4.05 mmol) in DCM (10 mL) was added SOCl₂ (0.591 mL, 8.09 mmol) at 0 °C. After the addition was complete the mixture was warmed to rt and stirred overnight. The resulting mixture was concentrated to give the title compound which was used in the next step without purification. ¹H NMR (400 MHz, CDCl₃) δ 6.72 (s, 2H), 4.70 (s, 2H), 3.87 (s, 6H).

Step 3: (S)-2-((5-azido-2-(4-bromo-2,6-dimethoxybenzyl)-2H-pyrazolo[4,3-d]pyrimidin-7-yl)amino)pentan-1-ol

[0471] To a solution of (S)-2-((5-azido-2H-pyrazolo[4,3-d]pyrimidin-7-yl)amino)pentan-1-ol (**Int. 2-2**, 800 mg, 3.05 mmol) in DMF (15 mL) was added 5-bromo-2-(chloromethyl)-1,3-dimethoxybenzene (800 mg, 3.01 mmol) and K₂CO₃ (1.25 g, 9.04 mmol) at rt. After stirring overnight the mixture was diluted with water (10 mL). The resulting solid was collected by filtration then dried under vacuum to give the title compound. MS *m/z* (M+H)⁺: calculated 491.1, observed 491.2. ¹H NMR (400 MHz, CD₃OD) δ 8.53 (s, 1H), 6.93 (s, 2H), 5.64 (d, J = 1.6 Hz, 2H), 4.50-4.63 (m, 1H), 3.88 (s, 6H), 3.68-3.76 (m, 2H), 1.61-1.86 (m, 2H), 1.38-1.54 (m, 2H), 0.97 (t, J = 7.2 Hz, 3H).

Step 4: (S)-5-azido-2-(4-bromo-2,6-dimethoxybenzyl)-N-(1-((tert-butyl)diphenylsilyl)oxy)pentan-2-yl)-2H-pyrazolo[4,3-d]pyrimidin-7-amine

[0472] To a solution of (S)-2-((5-azido-2-(4-bromo-2,6-dimethoxybenzyl)-2H-pyrazolo[4,3-d]pyrimidin-7-yl)amino)pentan-1-ol (900 mg, 1.832 mmol) in DMF (15 mL) was added imidazole (374 mg, 5.50 mmol) and TBDPSCl (0.565 mL, 2.198 mmol) at rt. After stirring overnight the mixture was diluted with water (15 mL) then extracted with EtOAc (15 mL x 2). The combined organic layers were washed with brine (20 mL) then dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-10% MeOH/DCM) to give the title compound. MS *m/z* (M+H)⁺: calculated 731.2, observed 731.2.

Step 5: benzyl (S)-4-(4-((5-azido-7-((1-((tert-butyl)diphenylsilyl)oxy)pentan-2-yl)amino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazine-1-carboxylate

[0473] To a solution of (S)-5-azido-2-(4-bromo-2,6-dimethoxybenzyl)-N-(1-((tert-butyl)diphenylsilyl)oxy)pentan-2-yl)-2H-pyrazolo[4,3-d]pyrimidin-7-amine (500 mg, 0.685 mmol) in toluene (10 mL) was added benzyl piperazine-1-carboxylate (226 mg, 1.028 mmol),

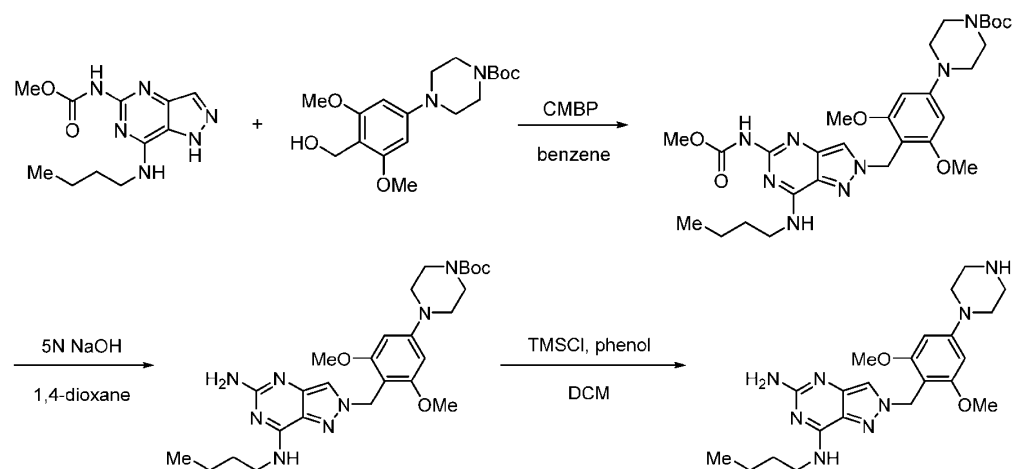
RuPhos Pd G4 (58.3 mg, 0.069 mmol), and Cs_2CO_3 (670 mg, 2.055 mmol). The mixture was purged with N_2 then heated to 90 °C. After heating overnight the mixture was cooled to rt then diluted with water (10 mL) then extracted with DCM (15 mL x 2). The combined organic layers were dried (Na_2SO_4) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-5% MeOH/DCM) to give the title compound. MS m/z ($\text{M}+\text{H}^+$): calculated 869.4, observed 869.4.

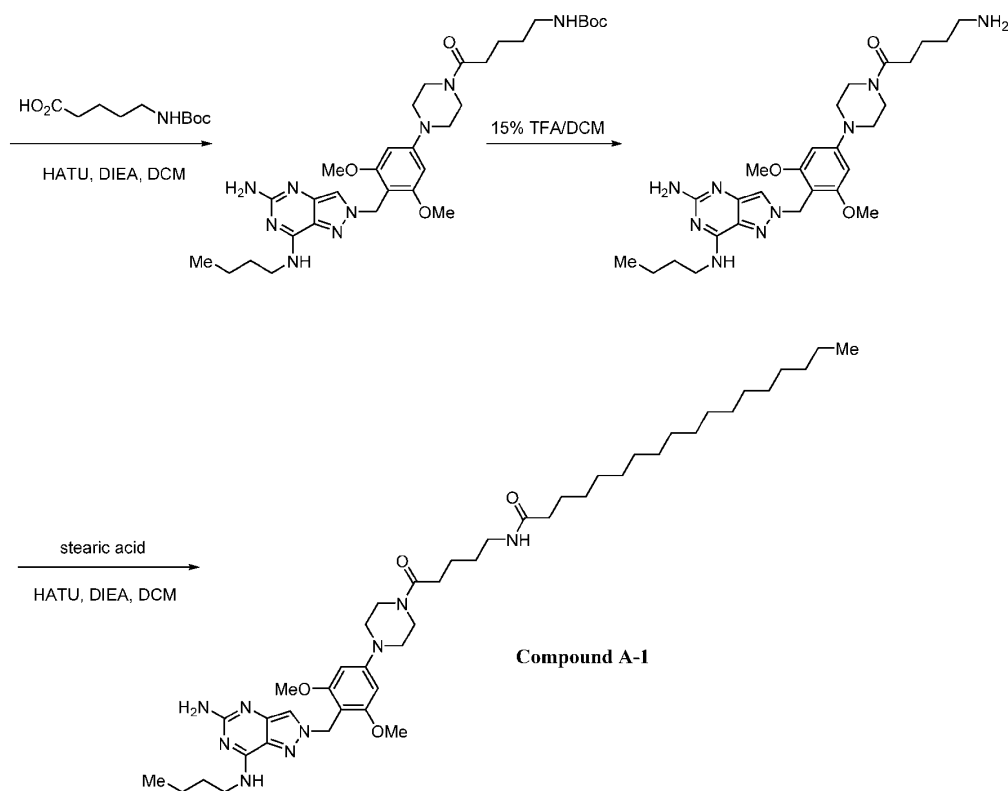
Step 6: (S)-5-azido-N-(1-((tert-butyldiphenylsilyl)oxy)pentan-2-yl)-2-(2,6-dimethoxy-4-(piperazin-1-yl)benzyl)-2H-pyrazolo[4,3-d]pyrimidin-7-amine

[0474] To a solution of benzyl (S)-4-(4-((5-amino-7-((1-((tert-butyldiphenylsilyl)oxy)pentan-2-yl)amino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazine-1-carboxylate (370 mg, 0.439 mmol) in TFE (8 mL) was added Pd/C (47 mg). The mixture was degassed (3 x vacuum/ N_2) then placed under an atmosphere of H_2 (balloon). After 2 hr the mixture was filtered then the filtrate was concentrated to give the title compound which was used in the next step without purification. MS m/z ($\text{M}+\text{H}^+$): calculated 735.4, observed 735.4.

EXAMPLE 3

Preparation of Compound A-1





***N*-(5-(4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide (Compound A-1)**

Step 1: tert-butyl 4-(4-((7-(butylamino)-5-((methoxycarbonyl)amino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazine-1-carboxylate

[0475] To a stirred mixture of *tert*-butyl 4-(4-(hydroxymethyl)-3,5-dimethoxyphenyl)piperazine-1-carboxylate (**Int. 3-1**, 13.87 g, 39.4 mmol) in benzene (80 mL) was added methyl (7-(butylamino)-1H-pyrazolo[4,3-d]pyrimidin-5-yl)carbamate (**Int. 1-1**, 8 g, 30.3 mmol) and cyanomethylenetriethylphosphorane (10.96 g, 45.4 mmol) under an argon atmosphere, then the mixture was heated to 80 °C. After 3 h, the mixture was cooled to ambient temperature, then diluted with water (10 mL), then extracted with EtOAc (3 x 20 mL). The combined organic extracts were washed with brine (3 x 10 mL) then dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-100% EtOAc/DCM) to give the title compound. MS *m/z* (M+H)⁺: calculated 599.3, observed 599.3.

Step 2: tert-butyl 4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazine-1-carboxylate

[0476] To a stirred mixture of *tert*-butyl 4-(4-((7-(butylamino)-5-((methoxycarbonyl)amino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazine-1-carboxylate (2.6 g, 4.34 mmol) in 1,4-dioxane (5 mL) was added NaOH (10M, 20 mL, 200 mmol) then the mixture was heated to 60°C. After 12 h the mixture was cooled to ambient temperature then diluted with water (20 mL) then extracted with EtOAc (3 x 80 mL). The combined organic extracts were washed with brine (50 mL) then dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-10% MeOH/DCM) to give the title compound. MS *m/z* (M+H)⁺: calculated 541.3, observed 541.4.

Step 3: N⁷-butyl-2-(2,6-dimethoxy-4-(piperazin-1-yl)benzyl)-2H-pyrazolo[4,3-d]pyrimidine-5,7-diamine

[0477] To a stirred mixture of *tert*-butyl 4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazine-1-carboxylate (1.8 g, 3.33 mmol) in DCM (25 mL) was added a solution of phenol (9.40 g, 100 mmol) in DCM (5 mL) at ambient temperature. The mixture was cooled to 5°C then a solution of chlorotrimethylsilane (0.24 mL, 1.878 mmol) in DCM (3 mL) was added dropwise. After the addition was complete the mixture was warmed to ambient temperature. After 25 minutes, the mixture was poured into ice-cold 2 N NaOH (30 mL), then extracted with ethyl EtOAc (5 x 150 mL). The combined organic extracts were dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to reverse-phase chromatography (0-60% MeCN/water with 0.5% NH₄OH modifier) to give the title compound. MS *m/z* (M+H)⁺: calculated 441.3, observed 441.2.

Step 4: tert-butyl (5-(4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)carbamate

[0478] To a stirred mixture of 5-((*tert*-butoxycarbonyl)amino)pentanoic acid (225 mg, 1.035 mmol) in DMF (2 mL) was added a solution of HATU (738 mg, 1.941 mmol) in DMF at ambient temperature. After 30 minutes *N*⁷-butyl-2-(2,6-dimethoxy-4-(piperazin-1-yl)benzyl)-2H-pyrazolo[4,3-d]pyrimidine-5,7-diamine (570 mg, 1.294 mmol) and DIEA (0.452 mL, 2.59 mmol) were added. After 5 h the mixture was cooled to ambient temperature then diluted with water (30 mL) then extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with brine (3 x 25 mL) then dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (1-10% MeOH/DCM) to give the title compound. MS *m/z* (M+H)⁺: calculated 640.4, observed 640.5.

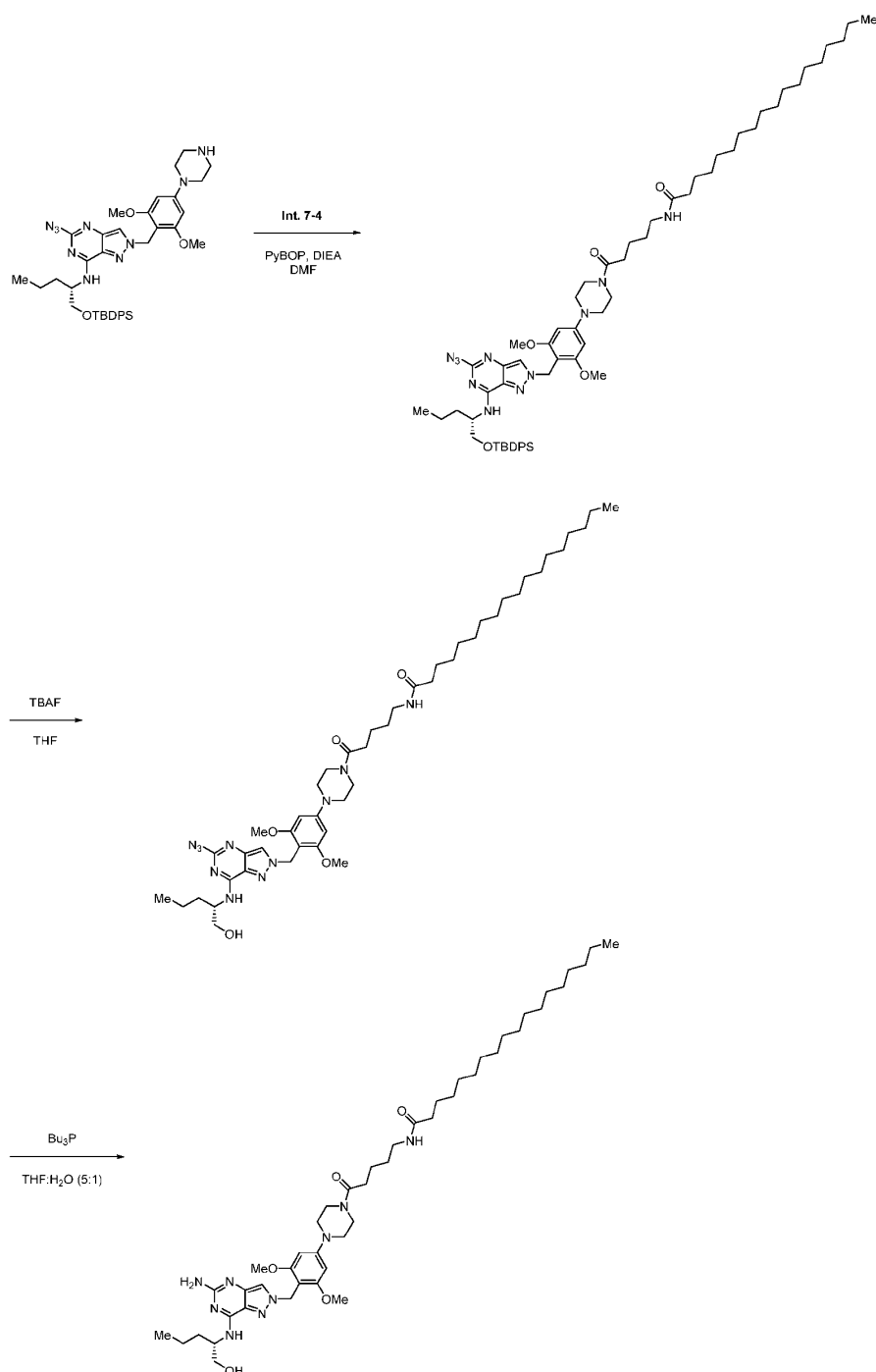
Step 5: 5-amino-1-(4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)pentan-1-one

[0479] A solution of *tert*-butyl (5-(4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)carbamate (550mg, 0.860 mmol) in 10% TFA/DCM (2 mL) was stirred at ambient temperature. After 30 minutes the mixture was diluted with toluene (30 mL) then concentrated. The crude product was subjected to reverse-phase chromatography (0-100% MeCN/water with 1% NH₄OH modifier) to give the title compound. MS *m/z* (M+H)⁺: calculated 540.3, observed 540.5.

Step 6: N-(5-(4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide

[0480] To a stirred mixture of stearic acid (274 mg, 0.964 mmol) in DMF (7 mL) was added a solution of HATU (423 mg, 1.112 mmol) in DMF at ambient temperature. After 30 minutes 5-amino-1-(4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)pentan-1-one (400 mg, 0.741 mmol) and DIEA (0.259 mL, 1.482 mmol) were added. After 2 h the mixture was cooled to ambient temperature then diluted with water (20 mL) then extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with brine (3 x 20 mL) then dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (1-10% MeOH/DCM) to give the title compound. **Compound A-1:** MS *m/z* (M+H)⁺: calculated 806.6, observed 806.6. ¹H-NMR (400 MHz, CDCl₃) δ 12.41 (s, 1H), 7.41 (s, 1H), 6.71 – 6.68 (m, 1H), 6.11 (s, 2H), 5.78 – 5.76 (m, 1H), 5.38 (s, 2H), 3.85 – 3.82 (m, 9H), 3.65 – 3.60 (m, 5H), 3.28 – 3.25 (m, 7H), 2.41 (t, *J* = 7.2 Hz, 2H), 2.16 (t, *J* = 7.2 Hz, 2H), 1.70 – 1.56 (m, 18H), 1.51 – 1.49 (m, 4H), 1.25 (s, 9H), 1.00 – 0.90 (m, 4H), 0.89 – 0.80 (m, 6H).

Preparation of Compound A-2



(S)-N-(5-(4-(4-((5-amino-7-((1-hydroxypentan-2-yl)amino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide (Compound A-2)

Step 1: (S)-N-(5-(4-(4-((5-azido-7-((1-((tert-butyl)diphenylsilyl)oxy)pentan-2-yl)amino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide

[0481] To a solution of 5-stearamidopentanoic acid (**Int. 7-4**, 129 mg, 0.337 mmol) in DMF (10 mL) was added DIEA (109 mg, 0.842 mmol) and PyBOP (175 mg, 0.337 mmol) at rt. After 10 minutes (S)-5-azido-N-(1-((tert-butyl)diphenylsilyl)oxy)pentan-2-yl)-2-(2,6-dimethoxy-4-(piperazin-1-yl)benzyl)-2H-pyrazolo[4,3-d]pyrimidin-7-amine (**Int. 17-1**, 300 mg, 0.281 mmol) was added at rt. After 30 minutes the mixture was diluted with water (10 mL) then extracted with EtOAc (15 mL x 2). The combined organic layers were dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was purified by prep-TLC (DCM/MeOH=10:1) to give the title compound and (S)-1-(4-(4-((5-azido-7-((1-((tert-butyl)diphenylsilyl)oxy)pentan-2-yl)amino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)octadecan-1-one (**Int. 18-1**). **Title Compound**: MS *m/z* (M+H)⁺: calculated 1100.7, observed 1100.7. **Int. 18-1**: MS *m/z* (M+H)⁺: calculated 1001.6, observed 1001.7.

Step 2: (S)-N-(5-(4-(4-((5-azido-7-((1-hydroxypentan-2-yl)amino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide

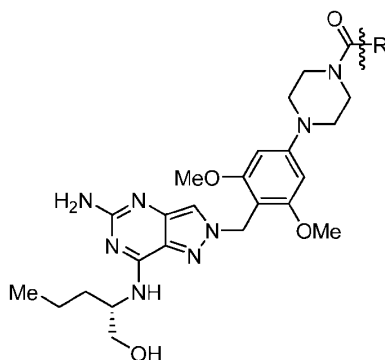
[0482] To a solution of (S)-N-(5-(4-(4-((5-azido-7-((1-((tert-butyl)diphenylsilyl)oxy)pentan-2-yl)amino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide (150 mg, 0.075 mmol) in THF (2 mL) was added TBAF (0.112 mL, 0.112 mmol, 1M in THF) at rt. After 1 hr the mixture was diluted with water (10 mL) then extracted with EtOAc (15 mL x 2). The combined organic layers were dried (Na₂SO₄) then filtered then the filtrate was concentrated to give the title compound which was used in the next step without purification. MS *m/z* (M+H)⁺: calculated 862.6, observed 862.5.

Step 3: (S)-N-(5-(4-(4-((5-amino-7-((1-hydroxypentan-2-yl)amino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide

[0483] To a solution of (S)-N-(5-(4-(4-((5-azido-7-((1-hydroxypentan-2-yl)amino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide (55 mg, 0.064 mmol) in THF (1.5 mL) and H₂O (0.3 mL) was added BusP (0.079 mL, 0.319 mmol) then the mixture was heated to 50 °C. After 1 hr the mixture was cooled to rt then concentrated. The crude product was purified by reverse-phase chromatography (CH₃CN/water with 0.2% FA modifier) to give the title compound. **Compound A-2**: MS *m/z* (M+H)⁺: calculated 836.6, observed 836.5. ¹H NMR (400 MHz, CD₃OD) δ 7.57 (s, 1H), 6.27 (s, 2H), 5.42 (s, 2H), 4.49 - 4.40 (m, 1H), 3.84 (s, 6H), 3.72 (dd, *J* = 5.2, 10.0 Hz, 4H), 3.68 - 3.61 (m, 2H), 3.27 - 3.18 (m, 4H), 2.48 (t, *J* = 7.2 Hz, 2H), 2.17 (t, *J* = 7.2 Hz, 2H), 1.73 - 1.53 (m,

10H), 1.50 - 1.36 (m, 4H), 1.30 - 1.24 (m, 27H), 0.97 (t, $J = 7.2$ Hz, 4H), 0.89 (t, $J = 6.8$ Hz, 3H).

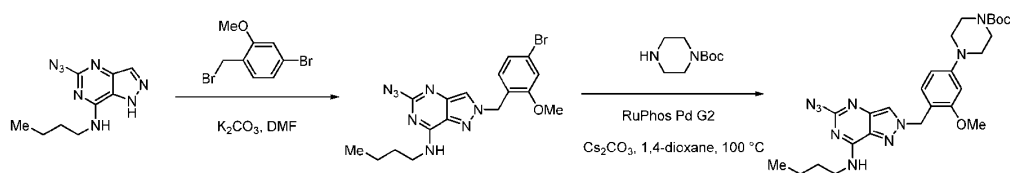
[0484] Utilizing the procedures described for **Compound A-2**, the following compounds were prepared substituting the appropriate reagents for 5-stearamidopentanoic acid in step 1.

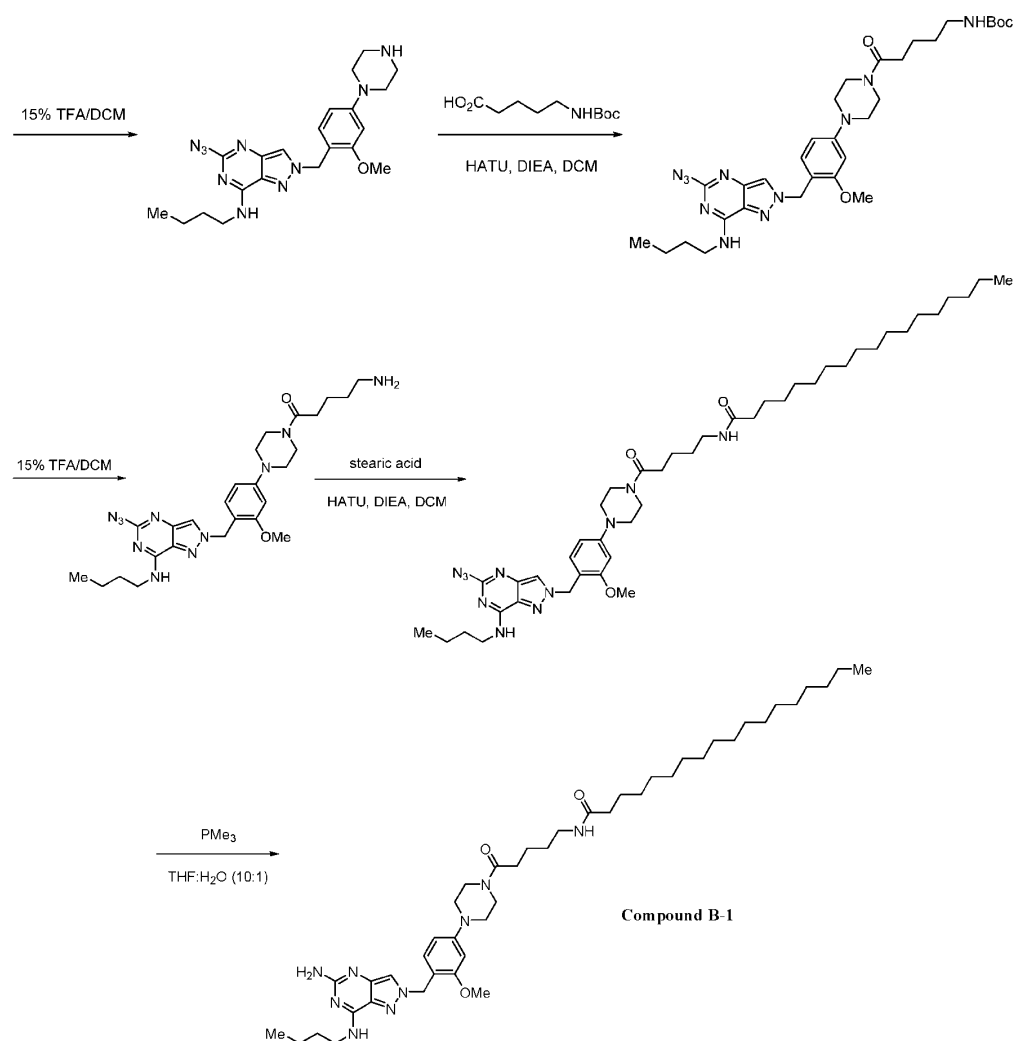


Cmpd/ Ex. #	R	Name	Calc'd [M+H] ⁺	Observed [M+H] ⁺
A-3		(S)-1-(4-(4-((5-amino-7-((1-hydroxypent-2-yl)amino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)octadecan-1-one	737.5	737.5

EXAMPLE 4

Preparation of Compound B-1





***N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide (Compound B-1)**

*Step 1: 5-Azido-2-(4-bromo-2-methoxybenzyl)-N-butyl-2H-pyrazolo[4,3-*d*]pyrimidin-7-amine [0485]* 5-Azido-*N*-butyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-7-amine (**Int. 2-1**, 938 mg, 4.04 mmol), 4-bromo-1-(bromomethyl)-2-methoxybenzene (1.36 g, 4.86 mmol), and K₂CO₃ (1.2 g, 8.68 mmol) were combined in DMF (20 mL) at ambient temperature. After stirring overnight the mixture was diluted with H₂O then extracted with EtOAc (3x). The combined organic extracts were washed with brine then dried (Na₂SO₄) then filtered then the filtrate was concentrated. The

crude product was subjected to silica gel chromatography (0-50% 3:1 EtOAc:EtOH/heptane) to give the title compound. MS m/z (M+H)⁺: calculated 431.3, observed 431.0.

Step 2: tert-butyl 4-(4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazine-1-carboxylate

[0486] 5-Azido-2-(4-bromo-2-methoxybenzyl)-*N*-butyl-2H-pyrazolo[4,3-*d*]pyrimidin-7-amine (1.38 g, 3.20 mmol), *tert*-butyl piperazine-1-carboxylate (0.775 g, 4.16 mmol), and Cs₂CO₃ (3.13 g, 9.60 mmol) were combined in 1,4-dioxane (20 mL). The mixture was degassed (3 x pump/N₂). RuPhos Pd G2 (0.25 g, 0.322 mmol) was added then the mixture was heated to 100 °C. After stirring overnight at 100 °C, the mixture was cooled to ambient temperature then diluted with EtOAc then filtered through a pad of Celite®, washing with EtOAc, then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-50% 3:1 EtOAc:EtOH/heptane) to give the title compound. MS m/z (M+H)⁺: calculated 537.3, observed 537.1.

Step 3: 5-azido-N-butyl-2-(2-methoxy-4-(piperazin-1-yl)benzyl)-2H-pyrazolo[4,3-d]pyrimidin-7-amine

[0487] To a solution of *tert*-butyl 4-(4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazine-1-carboxylate (799 mg, 1.489 mmol) in DCM (8 mL) was added TFA (1.5 mL, 19.47 mmol) at ambient temperature. After 2 h the mixture was concentrated. The residue was taken up in 1:1 DCM:heptane then concentrated (2 x) then dried under vacuum to give the TFA salt of the title compound which was used directly in the next step. MS m/z (M+H)⁺: calculated 437.2, observed 437.1.

Step 4: tert-butyl (5-(4-(4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)carbamate

[0488] 5-((*tert*-butoxycarbonyl)amino)pentanoic acid (421 mg, 1.936 mmol) and HATU (736 mg, 1.936 mmol) were combined in DCM (5 mL) then DIEA (1.300 mL, 7.45 mmol) was added at ambient temperature. After 1 h a solution of crude 5-azido-*N*-butyl-2-(2-methoxy-4-(piperazin-1-yl)benzyl)-2H-pyrazolo[4,3-*d*]pyrimidin-7-amine (TFA salt, 650 mg, 1.489 mmol) in DCM (10 mL) was added. After stirring overnight at ambient temperature the mixture was concentrated. The crude product was subjected to silica gel chromatography (0-100% 9:1 DCM:MeOH/DCM) to give impure product which was further purified by silica gel chromatography (0-100% 3:1 EtOAc:EtOH/heptane) to give the title compound. MS m/z (M+H)⁺: calculated 636.4, observed 636.2.

Step 5: 5-amino-1-(4-(4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)pentan-1-one

[0489] *Tert*-butyl (5-(4-(4-((5-azido-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)carbamate (848 mg, 1.334 mmol) was taken up in DCM (10 mL) then TFA (1.1 mL, 14.28 mmol) as added at ambient temperature. After 90 minutes the mixture was concentrated give the TFA salt of the title compound which was used directly in the next step. MS *m/z* (M+H)⁺: calculated 536.3, observed 536.3.

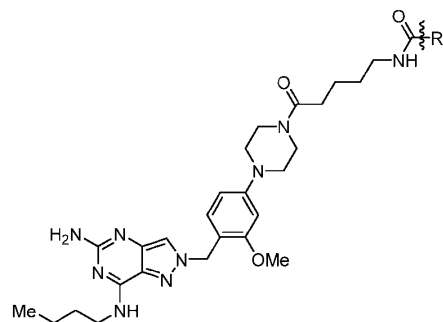
Step 6: N-(5-(4-(4-((5-azido-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide

[0490] Stearic acid (493 mg, 1.734 mmol) and HATU (659 mg, 1.734 mmol) were combined in DCM (5 mL) then DIEA (1.2 mL, 6.87 mmol) was added at ambient temperature. After 30 minutes the mixture was transferred to a flask containing crude 5-amino-1-(4-(4-((5-azido-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)pentan-1-one (TFA salt, 715 mg, 1.334 mmol) and DCM (10 mL). After stirring overnight at ambient temperature the mixture was concentrated. The crude product was subjected to silica gel chromatography (0-100% 9:1 DCM:MeOH/DCM) to give impure product which was further purified by silica gel chromatography (0-100% 3:1 EtOAc:EtOH/heptane) to give the title compound. MS *m/z* (M+H)⁺: calculated 802.6, observed 802.9.

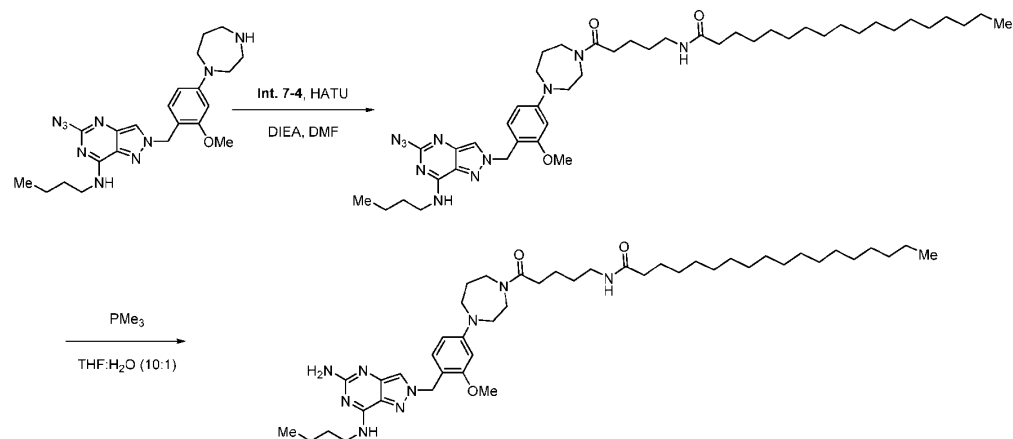
Step 7: N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide

[0491] *N*-(5-(4-(4-((5-azido-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide (641 mg, 0.799 mmol) was taken up in THF (8 mL):H₂O (0.800 mL) resulting in a thick suspension. The mixture was heated to 50 °C and as the mixture was heating the suspension became a solution. To this was added 1M trimethylphosphine (2.4 mL, 2.400 mmol) in THF and heating continued at 50 °C. After 3 h the mixture was cooled to ambient temperature then quenched with MeOH then concentrated. The crude product was subjected to silica gel chromatography (0-15% MeOH:DCM) to give the title compound. **Compound B-1**: MS *m/z* (M+H)⁺: calculated 776.6, observed 776.5. ¹H-NMR (400 MHz, DMSO-*d*₆) δ 11.5 (br., 1H), 8.11 (s, 1H), 7.72 – 7.65 (m, 2H), 7.06 – 6.80 (m, 1H), 6.60 (s, 1H), 6.49 – 6.47 (m, 1H), 6.32 (s, 1H), 5.32 (s, 2H), 3.81 (s, 3H), 3.57 – 3.42 (m, 6H), 3.32 – 3.02 (m, 4H), 2.36 – 2.32 (m, 2H), 2.07 – 1.99 (m, 4H), 1.47 – 1.34 (m, 8H), 1.29 – 1.18 (m, 30H), 0.91 – 0.83 (m, 6H).

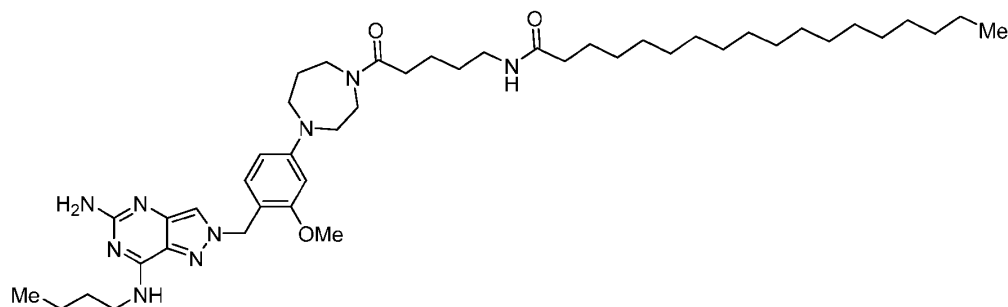
[0492] Utilizing the procedures described for **Compound B-1**, the following compounds were prepared substituting the appropriate reagents for stearic acid.



Cmpd.	R	Name	Calc'd [M+H] ⁺	Observed [M+H] ⁺
B-2		<i>N</i> -(5-(4-(4-((5-amino-7-(butylamino)-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)tetradecanamide	720.5	720.5
B-3		<i>N</i> -(5-(4-(4-((5-amino-7-(butylamino)-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)oleamide	774.6	774.6
B-4		(9 <i>Z</i> ,12 <i>Z</i>)- <i>N</i> -(5-(4-(4-((5-amino-7-(butylamino)-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)octadeca-9,12-dienamide	772.6	772.5

Preparation of Compound B-5

***N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-1,4-diazepan-1-yl)-5-oxopentyl)stearamide**



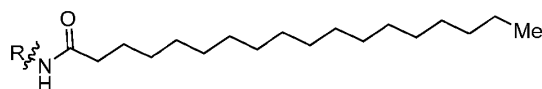
Step 1: N-(5-(4-(4-((5-azido-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-1,4-diazepan-1-yl)-5-oxopentyl)stearamide

[0493] To a mixture of 2-(4-(1,4-diazepan-1-yl)-2-methoxybenzyl)-5-azido-*N*-butyl-2*H*-pyrazolo[4,3-*d*]pyrimidin-7-amine hydrochloride (**Int. 14-1**, 300 mg, 0.666 mmol) and 5-stearamidopentanoic acid (**Int. 7-4**, 383 mg, 0.999 mmol) in DMF (5 mL) was added HATU (506 mg, 1.332 mmol) and DIEA (0.349 mL, 1.998 mmol) at rt. After 2 hr the mixture was diluted with water (100 mL) then extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (3 x 100 mL) then dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-6% MeOH/DCM) to give the title compound. MS *m/z* (M+H)⁺: calculated 816.6, observed 816.7.

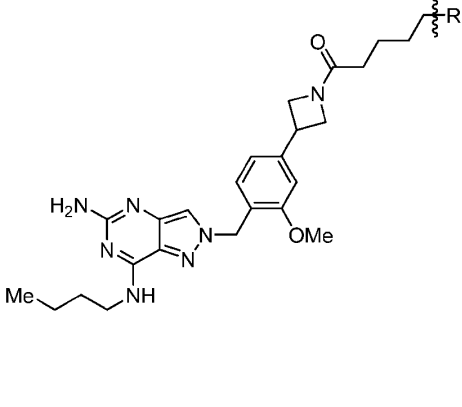
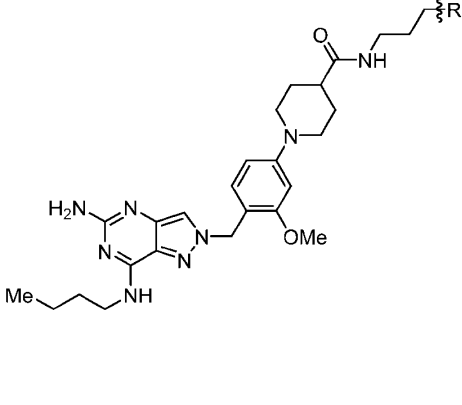
Step 2: *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-1,4-diazepan-1-yl)-5-oxopentyl)stearamide

[0494] To a solution of *N*-(5-(4-(4-((5-azido-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-1,4-diazepan-1-yl)-5-oxopentyl)stearamide (290 mg, 0.355 mmol) in THF (3 mL):H₂O (0.60 mL) was added PMe₃ (1.066 mL, 1.066 mmol) then the mixture was heated to 50 °C. After heating overnight the mixture was cooled to rt then quenched with MeOH (5 mL) then the resulting mixture was concentrated. The crude product was subjected to silica gel chromatography (0-6% MeOH/DCM) to give the title compound. **Compound B-5**: MS *m/z* (M+H)⁺: calculated 790.6, observed 790.6. ¹H-NMR (400 MHz, DMSO-*d*₆) δ 8.16 (s, 1H), 7.72 (d, *J* = 7.2 Hz, 1H), 7.60 – 7.56 (m, 1H), 6.93 (d, *J* = 8.0 Hz, 1H), 6.31 – 6.27 (m, 2H), 6.19 (s, 2H), 5.26 (s, 2H), 3.79 (s, 3H), 3.66 – 3.39 (m, 8H), 3.29 – 3.27 (m, 2H), 2.99 – 2.95 (m, 2H), 2.26 (t, *J* = 7.2 Hz, 1H), 2.16 (t, *J* = 7.2 Hz, 1H), 2.03 – 1.98 (m, 2H), 1.87 – 1.76 (m, 2H), 1.59 – 1.55 (m, 2H), 1.47 – 1.22 (m, 36H), 0.90 (t, *J* = 7.2 Hz, 3H), 0.85 (t, *J* = 7.2 Hz, 3H).

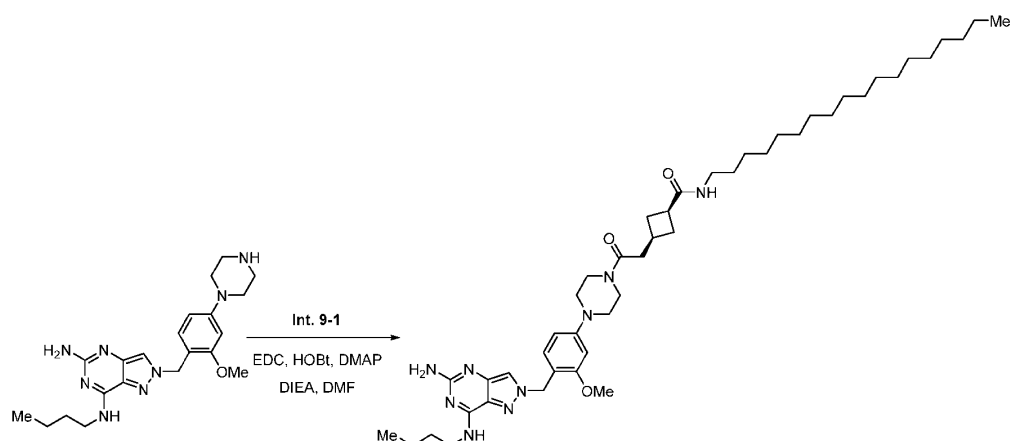
[0495] Utilizing the procedures described for **Compound B-5**, the following compounds were prepared substituting the appropriate reagents for 2-(4-(1,4-diazepan-1-yl)-2-methoxybenzyl)-5-azido-*N*-butyl-2*H*-pyrazolo[4,3-*d*]pyrimidin-7-amine hydrochloride and 5-stearamidopentanoic acid in step 1.



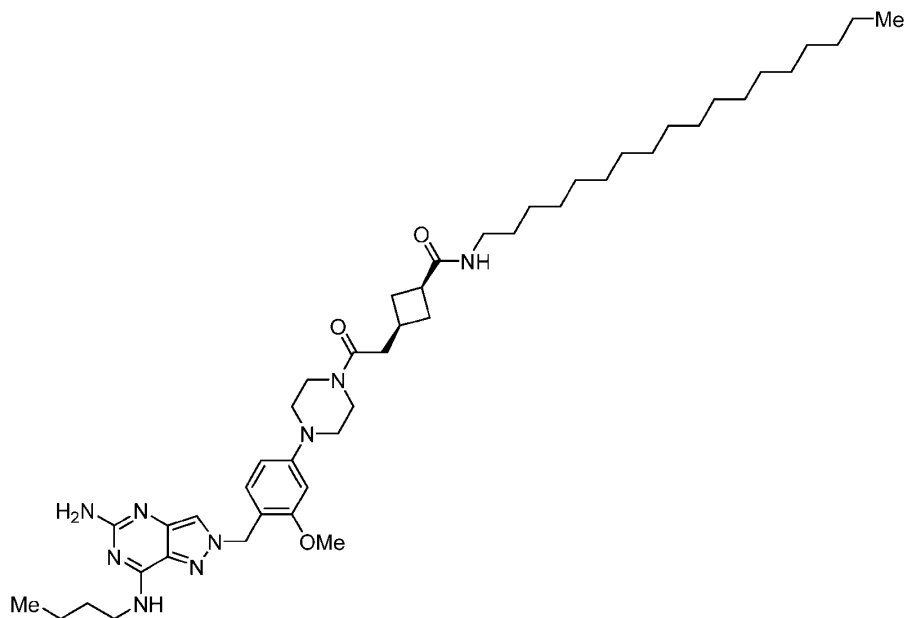
Cmpd/ Ex. #	R	Name	Calc'd [M+H] ⁺	Observed [M+H] ⁺
B-6		<i>N</i> -(5-(4-(4-((5-amino-7-(butylamino)-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperidin-1-yl)-5-oxopentyl)stearamide	775.6	775.6

B-7		N-(5-(3-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)azetidin-1-yl)-5-oxopentyl)stearamide	747.6	747.6
B-8		1-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-N-(3-stearamidopropyl)piperidine-4-carboxamide	776.6	776.6

Preparation of Compound B-9 and B-10

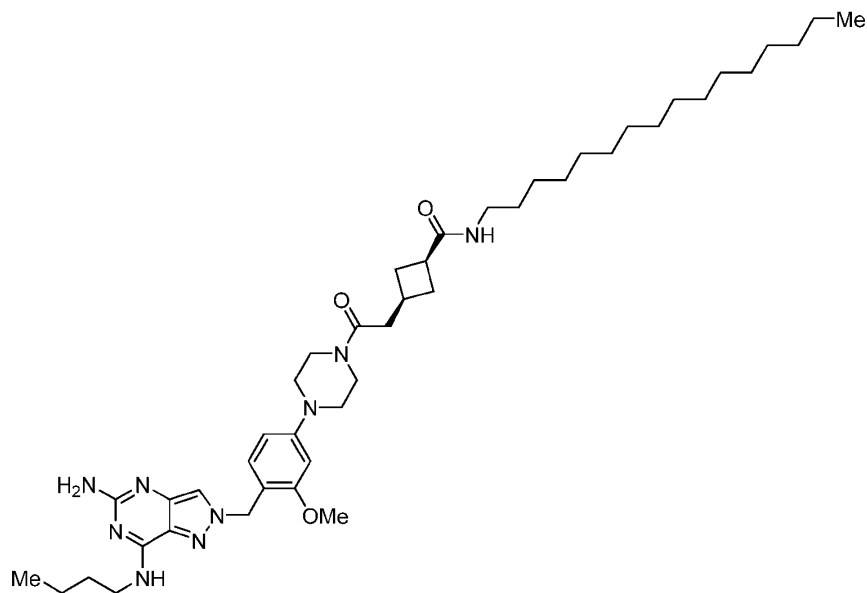


(1*s*,3*s*)-3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-*N*-octadecylcyclobutane-1-carboxamide (B-9)



and

(1*s*,3*s*)-3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-*N*-hexadecylcyclobutane-1-carboxamide (B-10)



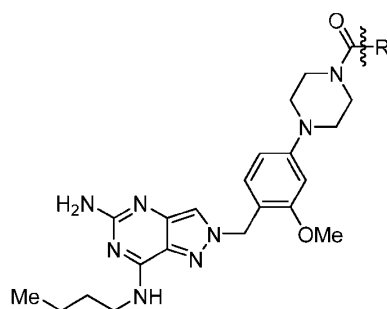
Step 1: (1s,3s)-3-(2-(4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-N-octadecylcyclobutane-1-carboxamide and (1s,3s)-3-(2-(4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-N-hexadecylcyclobutane-1-carboxamide

[0496] To a stirred mixture of 2-(3-(octadecylcarbamoyl)cyclobutyl)acetic acid (**Int. 9-1 as an 80:20 mixture with 2-((1s,3s)-3-(hexadecylcarbamoyl)cyclobutyl)acetic acid**, 0.3 g, 0.732 mmol), DMAP (8.95 mg, 0.073 mmol), DIEA (0.192 mL, 1.098 mmol), EDC (0.211 g, 1.098 mmol) and HOBt (0.148 g, 1.098 mmol) in DMF (10 mL) was added a solution of *N*⁷-butyl-2-(2-methoxy-4-(piperazin-1-yl)benzyl)-2H-pyrazolo[4,3-*d*]pyrimidine-5,7-diamine (0.391 g, 0.952 mmol) in DMF (10 mL) at 25 °C. After 6 hr the mixture was diluted with H₂O then extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (3 × 10 mL) then dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-20% MeOH/DCM) to give a purified mixture of title compounds. The mixture was further purified by chiral chromatography (CHIRALPAK OD, 30% EtOH in hexanes containing 0.1% TFA modifier) to give P1 and P2.

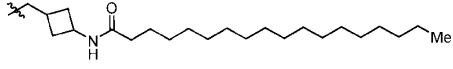
P1: (1s,3s)-3-(2-(4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-N-hexadecylcyclobutane-1-carboxamide **Compound B-10**: MS *m/z* (M+H)⁺: calculated 774.6, observed 774.6. ¹H-NMR (400 MHz, DMSO-*d*₆) δ 8.37 (br., 1H), 7.67 (s, 1H), 7.58 (t, *J* = 5.6 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 6.60 (d, *J* = 2.4 Hz, 1H), 6.50 – 6.43 (m, 1H), 5.32 (s, 2H), 3.80 (s, 3H), 3.55 – 3.43 (m, 5H), 3.16 – 3.11 (m, 2H), 2.99 – 2.95 (m, 2H), 2.85 – 2.73 (m, 1H), 2.46 – 2.43 (m, 4H), 2.14 – 2.13 (m, 2H), 1.76 – 1.75 (m, 2H), 1.58 – 1.55 (m, 2H), 1.23 (s, 32H), 0.95 – 0.85 (m, 6H).

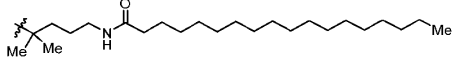
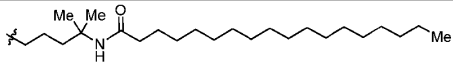
P2: (1s,3s)-3-(2-(4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-N-octadecylcyclobutane-1-carboxamide **Compound B-9**: MS *m/z* (M+H)⁺: calculated 802.6, observed 802.6. ¹H-NMR (400 MHz, DMSO-*d*₆) δ 8.11 (br., 1H), 7.67 (s, 1H), 7.58 (t, *J* = 5.6 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 6.60 (d, *J* = 2.4 Hz, 1H), 6.50 – 6.43 (m, 1H), 6.18 (br., 1H), 5.32 (s, 2H), 3.80 (s, 3H), 3.55 (t, *J* = 5.2 Hz, 4H), 3.43 (d, *J* = 6.8 Hz, 1H), 3.16 – 3.11 (m, 4H), 2.99 – 2.95 (m, 2H), 2.85 – 2.73 (m, 1H), 2.46 – 2.43 (m, 4H), 2.14 – 2.13 (m, 2H), 1.76 – 1.75 (m, 2H), 1.58 – 1.55 (m, 2H), 1.23 (s, 34H), 0.93 – 0.80 (m, 6H).

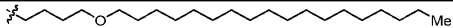
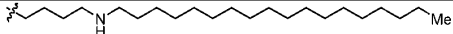
[0497] Utilizing the procedures described for **Compounds B-9 and B-10**, the following compounds were prepared substituting the appropriate reagents for 2-(3-(octadecylcarbamoyl)cyclobutyl)acetic acid.



Cmpd /Ex. #	R	Name	Calc'd [M+H] ⁺	Observed [M+H] ⁺	Comments
B-11		<i>N</i> -(3-(4-(4-((5-amino-7-(butylamino)-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimidin-2-yl)methyl)-3-methoxyphenyl)perazin-1-yl)-3-oxopropyl)stearamide	748.6	748.6	
B-12		<i>N</i> -(7-(4-(4-((5-amino-7-(butylamino)-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimi	804.6	804.7	

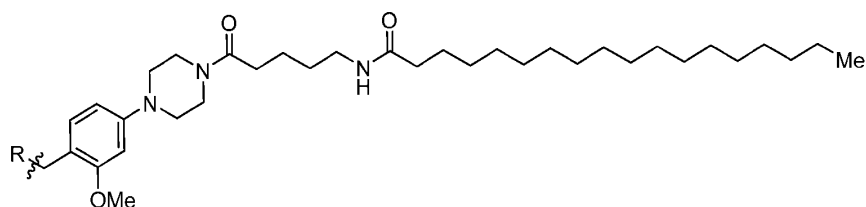
		din-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-7-oxoheptyl)stearamide			
B-13		<i>N</i> -(3-(2-(4-(4-((5-amino-7-(butylamino)-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)cyclobutyl)stearamide	788.6	788.8	

B-14		<i>N</i> -(5-(4-(4-((5-amino-7-(butylamino)-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimidin-2-yl)methyl)-3-methoxyphenyl)perazin-1-yl)-4,4-dimethyl-5-oxopentyl)stearamide	804.6	804.7	
B-15		<i>N</i> -(6-(4-(4-((5-amino-7-(butylamino)-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimidin-2-yl)methyl)-3-methoxyphenyl)perazin-1-yl)-2-methyl-	804.6	804.8	

		6-oxohexan-2-yl)stearamide			
B-16		1-(4-(4-((5-amino-7-(butylamino)-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimidin-2-yl)methyl)-3-methoxyphenyl)perazin-1-yl)-5-(octadecyloxy)pentan-1-one	763.6	763.6	
B-17		1-(4-(4-((5-amino-7-(butylamino)-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimidin-2-yl)methyl)-3-methoxyphenyl)perazin-1-yl)-5-(octadecyloxy)pentan-1-one	762.6	762.6	from Int. 12-1 then deprotected using HCl in 1,4-dioxane

		perazin-1-yl)-5-(octadecylamino)pentan-1-one			
--	--	--	--	--	--

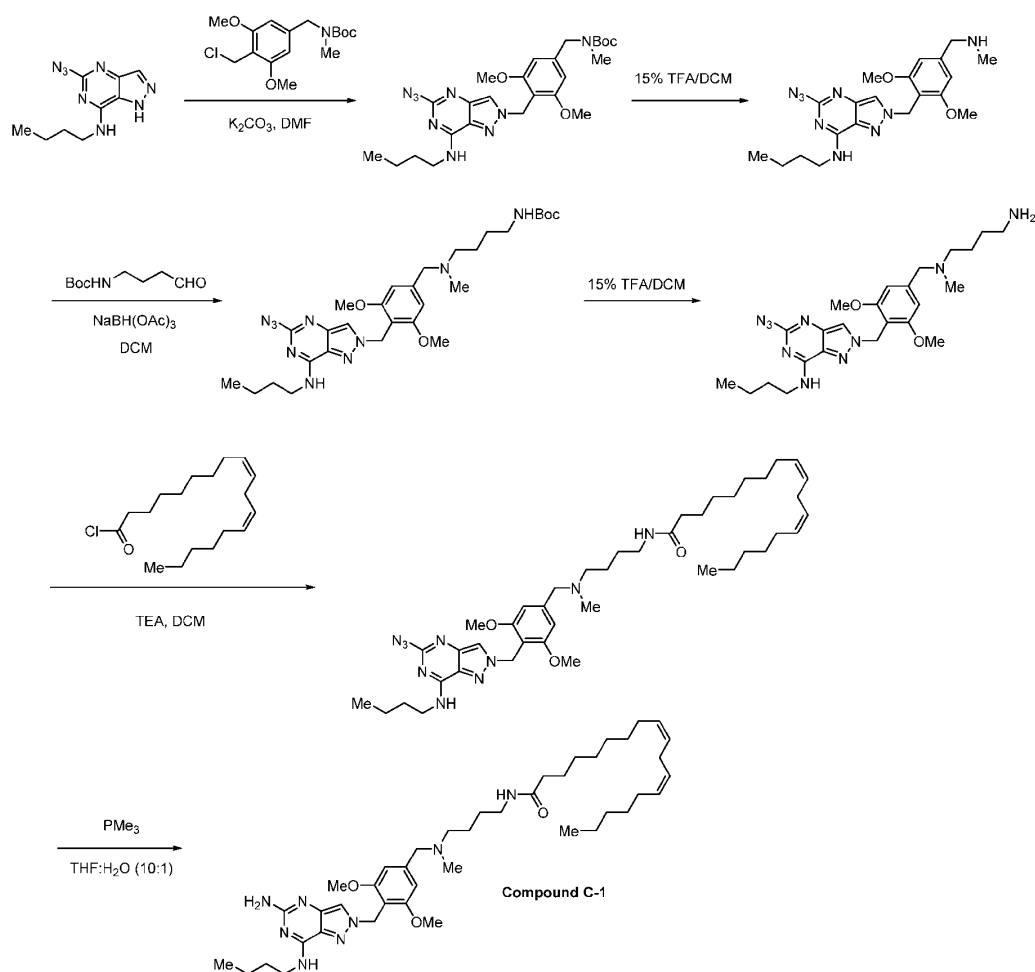
[0498] Utilizing the procedures described for **Compound B-1**, the following compounds were prepared substituting the appropriate reagents for 5-azido-*N*-butyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-7-amine in step 1.



Cmpd/ Ex. #	R	Name	Calc'd [M+H] ⁺	Observed [M+H] ⁺	Comments
B-18		<i>N</i> -(5-(4-(4-((5-amino-7-(butylamino)-3-methyl-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide	790.6	790.6	from Int. 2-3

EXAMPLE 5

Preparation of **Compound C-1**



(9*Z*,12*Z*)-*N*-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)octadeca-9,12-dienamide (Compound C-1)

*Step 1: tert-butyl 4-((5-azido-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl(methyl)carbamate*

[0499] To a mixture of *tert*-butyl 4-(chloromethyl)-3,5-dimethoxybenzyl(methyl)carbamate (**Int. 2-1**, 1 g, 3.03 mmol) in DMF (20 mL) was added 5-azido-*N*-butyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-7-amine (**Int. 4-1**, 0.634 g, 2.73 mmol) and K_2CO_3 (0.503 g, 3.64 mmol) at ambient temperature. After 2 h, the mixture was diluted with H₂O (20 mL) then extracted with EtOAc (3 x 30 mL). The combined organic extracts were washed with brine (2 x 30 mL) then dried (Na_2SO_4) then filtered then the filtrate was concentrated. The crude product was subjected to

silica gel chromatography (0-70% EtOAc/petroleum ether) to give the title compound. MS m/z ($M+H$)⁺: calculated 526.3, observed 526.3.

Step 2: 5-azido-N-butyl-2-(2,6-dimethoxy-4-((methylamino)methyl)benzyl)-2H-pyrazolo[4,3-d]pyrimidin-7-amine

[0500] To a solution of *tert*-butyl (4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)carbamate (1.2g, 2.283 mmol) in DCM (10 mL) was added TFA (2 mL) at ambient temperature. After 3 h, the mixture was concentrated to give the TFA salt of the title compound which was used directly in the next step. MS m/z ($M+H$)⁺: calculated 426.2, observed 426.3.

Step 3: tert-butyl (4-((4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)carbamate

[0501] To a mixture of crude 5-azido-N-butyl-2-(2,6-dimethoxy-4-((methylamino)methyl)benzyl)-2H-pyrazolo[4,3-d]pyrimidin-7-amine (TFA salt, 1.1 g, 2.163 mmol) and *tert*-butyl (4-oxobutyl)carbamate (**Int. 5-1**, 0.608 g, 3.24 mmol) in DCM (8 mL) was added NaBH(OAc)₃ (0.917 g, 4.33 mmol) at ambient temperature. After 3 h the mixture was concentrated. The crude product was subjected to silica gel chromatography (0-20% MeOH/DCM) to give the title compound. MS m/z ($M+H$)⁺: calculated 597.4, observed 597.5.

Step 4: N¹-(4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)-N¹-methylbutane-1,4-diamine

[0502] To a solution of *tert*-butyl (4-((4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)carbamate (790 mg, 1.324 mmol) in DCM (10 mL) was added TFA (2 mL) at room temperature. After 3 h the mixture was concentrated to give the TFA salt of the title compound which was used directly in the next step. MS m/z ($M+H$)⁺: calculated 497.3, observed 497.4.

Step 5: (9Z,12Z)-N-(4-((4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)octadeca-9,12-dienamide

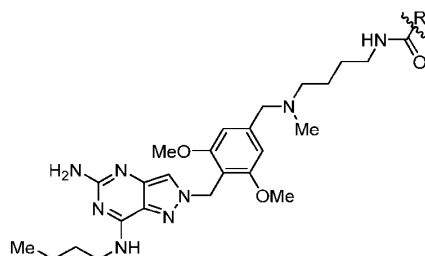
[0503] To a mixture of crude N¹-(4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)-N¹-methylbutane-1,4-diamine (TFA salt, 447 mg, 0.9 mmol) in DCM (6 mL) was added DIEA (0.314 mL, 1.800 mmol) and (9Z,12Z)-octadeca-9,12-dienoyl chloride (296 mg, 0.990 mmol) at 0°C. After the additions were complete the mixture was warmed to ambient temperature. After 2 h EtOAc (30 mL) was added then the mixture was washed with saturated NaHCO₃ (2 x 30 mL). The organic layer was dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography

(0-15% MeOH/DCM) to give the title compound. MS m/z (M+H)⁺: calculated 759.5, observed 759.6.

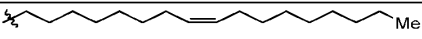
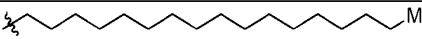
Step 6: (9Z,12Z)-N-(4-((4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)octadeca-9,12-dienamide

[0504] To a mixture of (9Z,12Z)-N-(4-((4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)octadeca-9,12-dienamide (430 mg, 0.566 mmol) in THF (5 mL) and water (0.5 mL) was added trimethylphosphane (1 M in THF, 1.699 mL, 1.699 mmol) then the mixture was heated to 50 °C. After 16 h the mixture was cooled to ambient temperature then diluted with EtOAc (30 mL). The resulting mixture was washed with water (30 mL) then dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was purified by prep-TLC developed using MeOH/DCM/ammonia (1/10/0.05) to give the title compound. **Compound C-1**: MS m/z (M+H)⁺: calculated 733.5, observed 733.5. ¹H-NMR (400 MHz, CD₃OD): δ 7.49 (s, 1H), 6.70 (s, 2H), 5.46 (s, 2H), 5.33 – 5.28 (m, 4H), 3.85 (s, 6H), 3.52 – 3.48 (m, 4H), 3.16 (t, J = 6.4 Hz, 2H), 2.74 (t, J = 6.2 Hz, 2H), 2.41 (t, J = 7.1 Hz, 2H), 2.22 (s, 3H), 2.14 (t, J = 7.5 Hz, 2H), 2.04 – 2.01 (m 4H), 1.66 – 1.41 (m, 10H), 1.36– 1.29 (m, 14H), 0.97 (t, J = 7.4 Hz, 3H), 0.88 (t, J = 6.8 Hz, 3H).

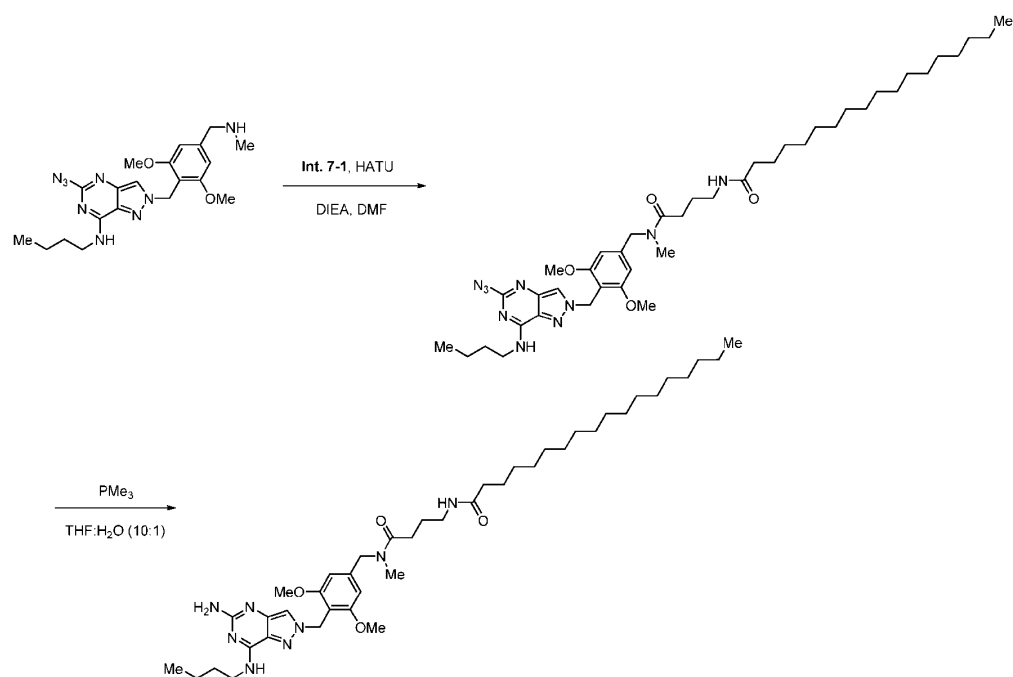
[0505] Utilizing the procedures described for **Compound C-1**, the following compounds were prepared substituting the appropriate reagents for (9Z,12Z)-octadeca-9,12-dienoyl chloride.



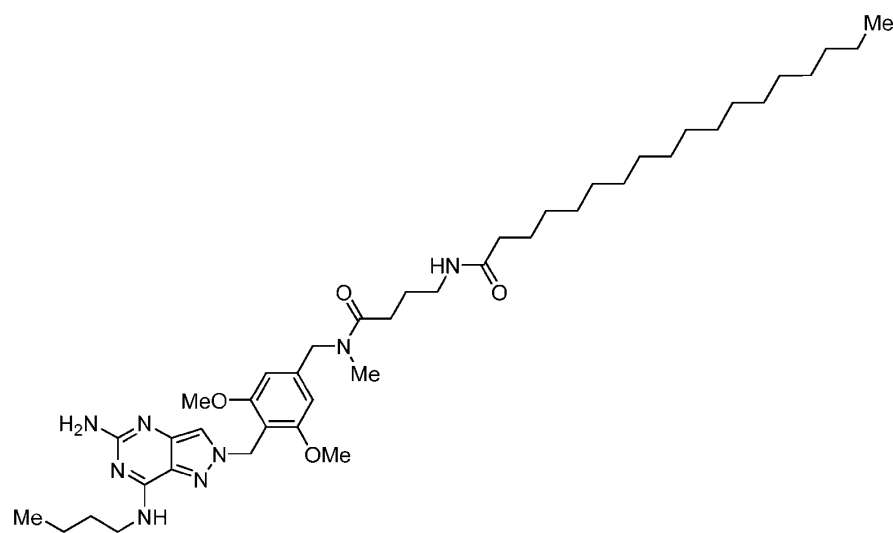
Cmpd.	R	Name	Calc'd [M+H] ⁺	Observed [M+H] ⁺
C-2		N-(4-((4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)octadeca-9,12-dienamide	681.5	681.7

		yl)(methyl)amino)butyl)tetradecanamide		
C-3		<i>N</i> -(4-((4-((5-amino-7-(butylamino)-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)oleamide	735.6	735.6
C-4		<i>N</i> -(4-((4-((5-amino-7-(butylamino)-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)stearamide	737.6	737.8

Preparation of Compound C-5



***N*-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)-4-oxobutyl)stearamide**



Step 1: N-(4-((4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)-4-oxobutyl)stearamide

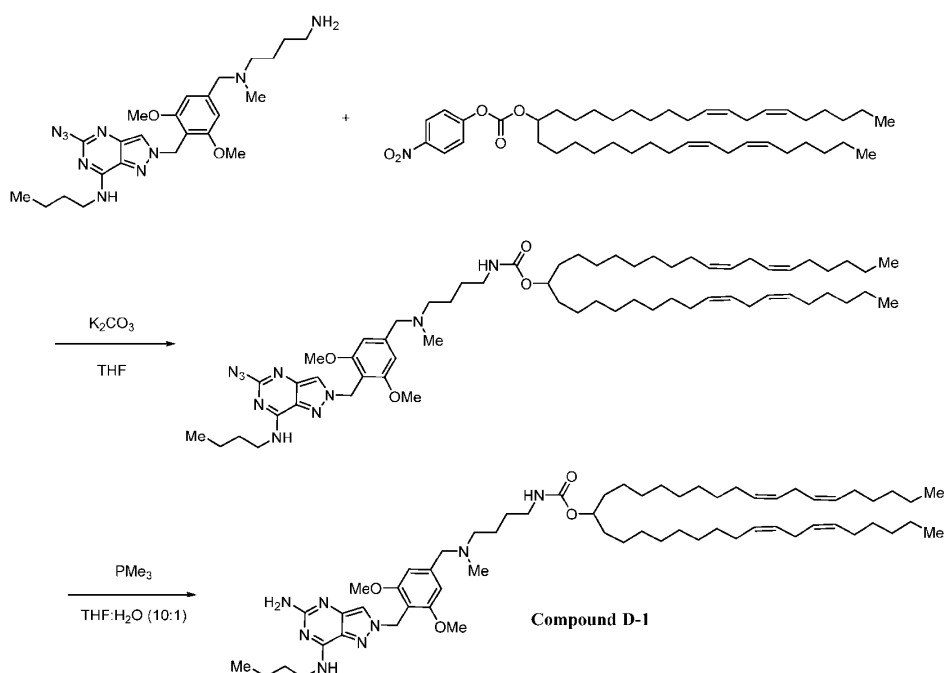
[0506] A mixture of 4-stearamidobutanoic acid (**Int. 7-1**, 132 mg, 0.357 mmol), 5-azido-*N*-butyl-2-(2,6-dimethoxy-4-((methylamino)methyl)benzyl)-2*H*-pyrazolo[4,3-*d*]pyrimidin-7-amine hydrochloride (**Compound C-1 step 2**, 110 mg, 0.238 mmol), HATU (181 mg, 0.476 mmol) and DIEA (0.208 mL, 1.191 mmol) in DMF (3 mL) was stirred at rt. After stirring overnight the mixture was purified directly by reverse-phase chromatography (CH₃CN/water with 0.05% FA modifier) to give the title compound. MS *m/z* (M+H)⁺: calculated 777.5, observed 777.6.

Step 2: N-(4-((4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)-4-oxobutyl)stearamide

[0507] To a solution of *N*-(4-((4-((5-azido-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)-4-oxobutyl)stearamide (160 mg, 0.206 mmol) in THF (3 mL):H₂O (0.60 mL) was added PMe₃ (1M in THF, 0.618 mL, 0.618 mmol) then the mixture was heated to 50 °C. After 5 hr the mixture was cooled to rt then quenched with MeOH (5 mL) then the resulting mixture was concentrated. The crude product was purified by reverse-phase chromatography (1:3 CH₃CN:MeOH/water) to give the title compound. **Compound C-5**: MS *m/z* (M+H)⁺: calculated 751.6, observed 751.6. ¹H-NMR (400 MHz, DMSO-*d*₆) δ 7.99 – 7.54 (m, 2H), 7.37 (d, *J* = 3.6 Hz, 1H), 6.54 (d, *J* = 19.6 Hz, 2H), 5.92 (s, 2H), 5.36 (s, 2H), 4.54 (d, *J* = 16.8 Hz, 2H), 3.79 (d, *J* = 8.4 Hz, 6H), 3.44 – 3.39 (m, 2H), 3.10 – 3.00 (m, 2H), 2.93 (s, 2H), 2.84 (s, 1H), 2.38 – 2.33 (m, 2H), 2.03 – 1.98 (m, 2H), 1.70 – 1.65 (m, 2H), 1.60 – 1.55 (m, 2H), 1.34 – 1.30 (m, 2H), 1.22 (d, *J* = 3.2 Hz, 30H), 0.90 – 0.82 (m, 6H).

EXAMPLE 6

Preparation of Compound D-1



(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(((4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)carbamate (Compound D-1)

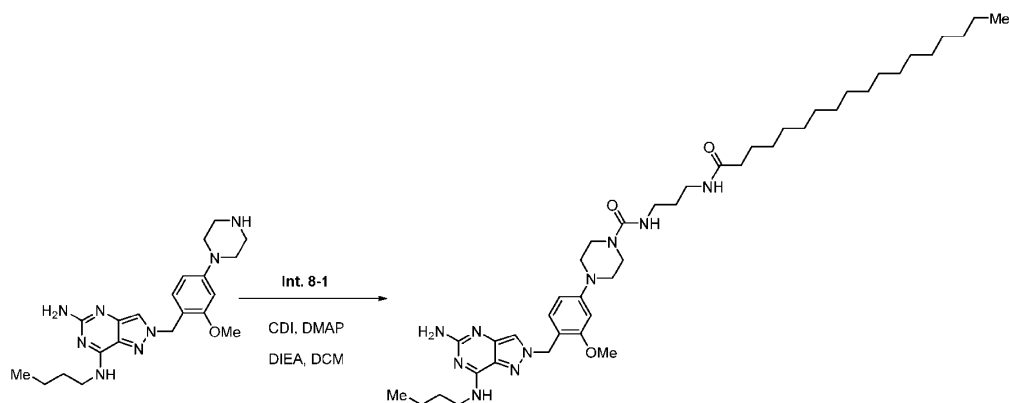
Step 1: (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(((4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)carbamate

[0508] To a mixture of (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-nitrophenyl carbonate (**Int. 6-1**, 355 mg, 0.511 mmol) and crude *N*¹-(4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)-*N*¹-methylbutane-1,4-diamine (TFA salt, 274 mg, 0.460 mmol) in THF (5 mL) was added K₂CO₃ (0.058 mL, 1.023 mmol) then the mixture was heated to 65 °C. After 15 h, the mixture was cooled to ambient temperature then concentrated. The crude product was subjected to silica gel chromatography (0-10% MeOH/DCM) to give the title compound. MS *m/z* (M+H)⁺: calculated 1051.8, observed 1052.0.

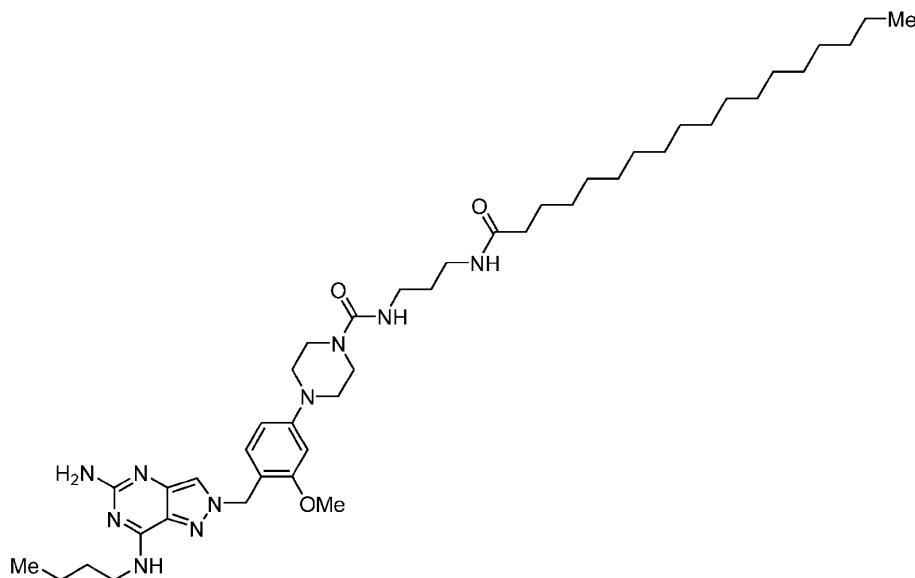
Step 2: (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(((4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)carbamate

[0509] To a mixture of (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-((4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)-amino)butyl)carbamate (500 mg, 0.475 mmol) in THF (10 mL) and H₂O (1.0 mL) was added trimethylphosphane (1 M in THF, 1.426 mL, 1.426 mmol) at ambient temperature. After 15 h the mixture was quenched with MeOH (1.42 mL) then concentrated. The crude product was purified by prep-TLC (DCM:MeOH = 10:1) to give the title compound. **Compound D-1**: MS *m/z* (M+H)⁺: calculated 1025.8, observed 1025.7. ¹H-NMR (300 MHz, CD₃OD): δ 7.65 (s, 1H), 6.71 (s, 2H), 5.49 (s, 2H), 5.35 – 5.32 (m, 8H), 4.86 – 4.85 (m, 1H), 3.86 (s, 6H), 3.56 – 3.55 (m, 4H), 3.12 – 3.10 (m, 2H), 2.77 – 2.75 (m, 4H), 2.45 – 2.43 (m, 2H), 2.25 (s, 3H), 2.05 – 2.03 (m, 8H), 1.68 – 1.66 (m, 6H), 1.46 – 1.44 (m, 6H), 1.32 – 1.27 (m, 36H), 0.97 – 0.94 (m, 9H).

Preparation of Compound D-2

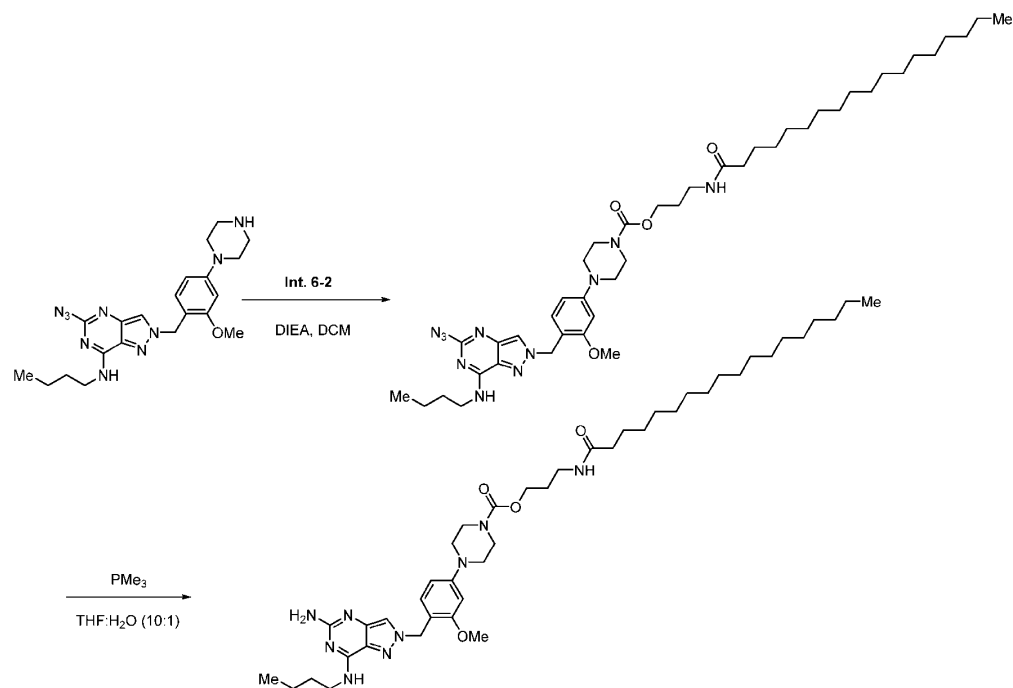


4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-N-(3-stearamidopropyl)piperazine-1-carboxamide

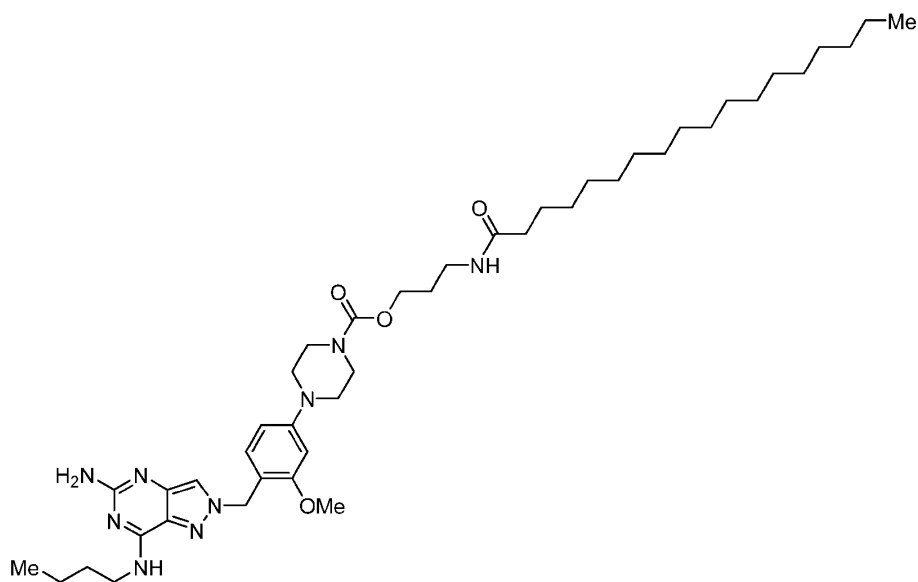


Step 1: 4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-N-(3-stearamidopropyl)piperazine-1-carboxamide

[0510] A mixture of *N*-(3-aminopropyl)stearamide (**Int. 8-1**, 457 mg, 1.342 mmol), CDI (136 mg, 0.839 mmol), DMAP (13.67 mg, 0.112 mmol) and DIEA (0.586 mL, 3.36 mmol) in DCM (10 mL) was stirred at 0 °C. After 30 minutes *N*⁷-butyl-2-(2-methoxy-4-(piperazin-1-yl)benzyl)-2H-pyrazolo[4,3-*d*]pyrimidine-5,7-diamine hydrochloride (500 mg, 1.119 mmol) was added. The mixture was warmed to rt then heated to 50 °C. After 3 hr the mixture was cooled to rt then concentrated. The crude product was purified by reverse-phase chromatography (CH₃CN/water with 0.1% TFA modifier) to give the title compound. **Compound D-2**: MS *m/z* (M+H)⁺: calculated 777.6, observed 777.6. ¹H-NMR (300 MHz, DMSO-*d*₆) δ 8.89 (s, 1H), 7.83 – 7.55 (m, 2H), 7.16 – 6.98 (m, 2H), 6.60 – 6.48 (m, 2H), 5.34 (s, 2H), 3.80 (s, 3H), 3.47 (s, 6H), 3.12 (s, 4H), 3.04 – 3.01 (m, 4H), 2.03 (t, *J* = 7.2 Hz, 2H), 1.60 – 1.48 (m, 6H), 1.22 (s, 28H), 0.96 – 0.77 (m, 6H).

Preparation of Compound D-3

3-stearamidopropyl 4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazine-1-carboxylate



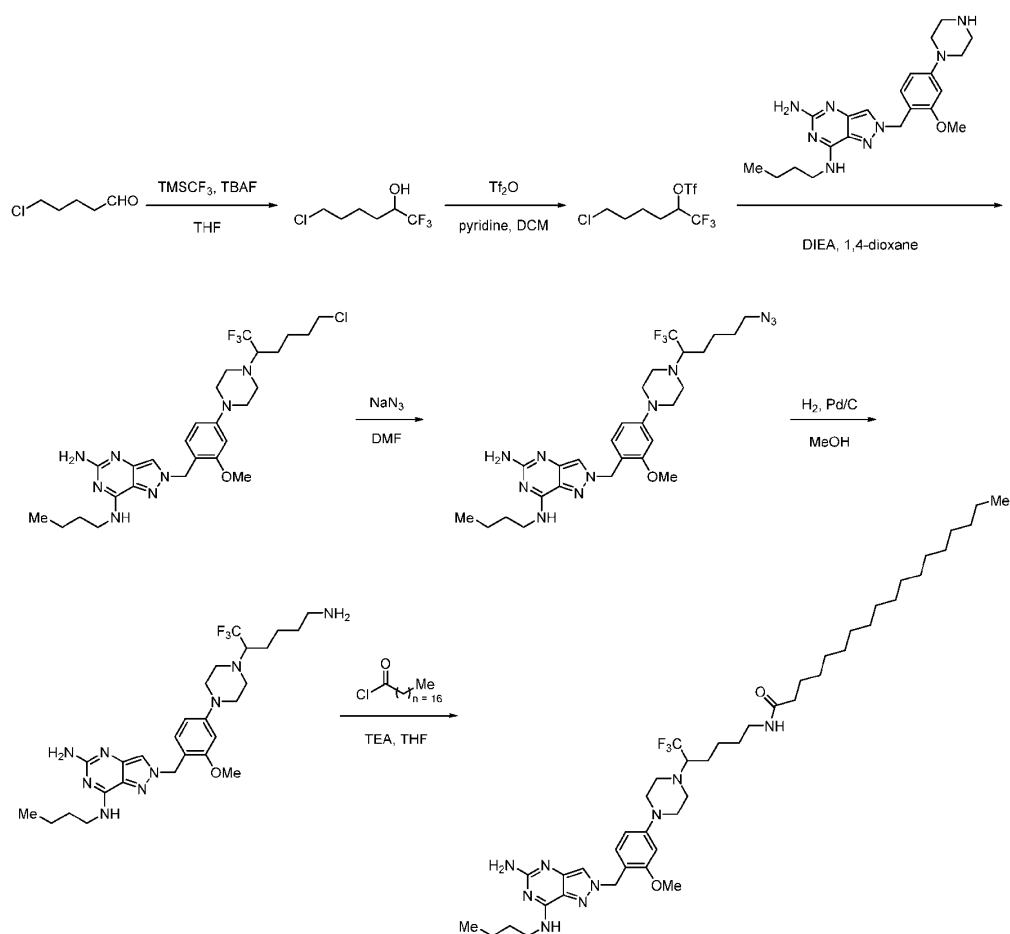
Step 1: 3-stearamidopropyl 4-(4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazine-1-carboxylate

[0511] A mixture of 4-nitrophenyl (3-stearamidopropyl) carbonate (**Int. 6-2**, 130 mg, 0.257 mmol), 5-azido-*N*-butyl-2-(2-methoxy-4-(piperazin-1-yl)benzyl)-2H-pyrazolo[4,3-*d*]pyrimidin-7-amine (**Compound B-1 step 3**, 56 mg, 0.128 mmol) and DIEA (0.045 mL, 0.257 mmol) in DCM (0.5 mL) was stirred at rt. After 1 hr the mixture was purified directly by prep-TLC (DCM/MeOH=15:1) to give the title compound. MS m/z (M+H)⁺: calculated 804.6, observed 804.7.

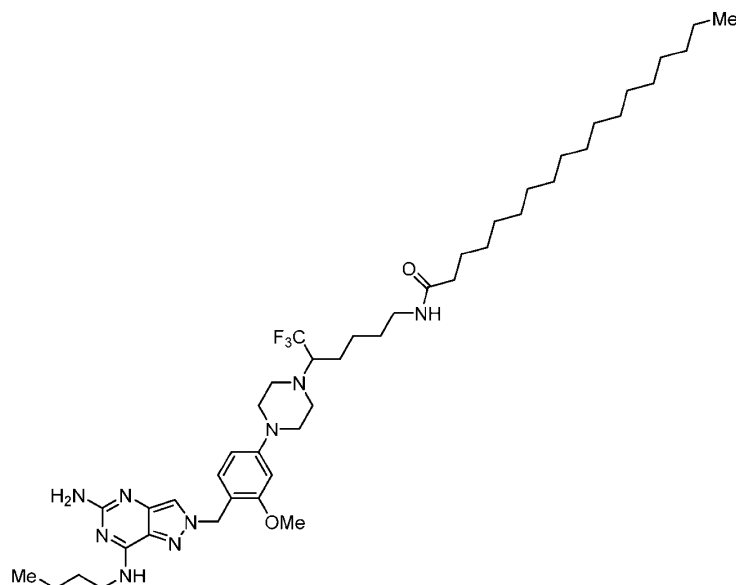
Step 2: 3-stearamidopropyl 4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazine-1-carboxylate

[0512] To a solution of 4-(4-((5-azido-7-(butylamino)-2H-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazine-1-carboxylate (80 mg, 0.099 mmol) in THF (2 mL):H₂O (0.5 mL) was added PMe₃ (1M in THF, 0.298 mL, 0.298 mmol) then the mixture was heated to 50 °C. After 5 hr the mixture was cooled to rt then quenched with MeOH (5 mL) then the resulting mixture was concentrated. The crude product was purified by prep-TLC(DCM:MeOH=10:1) to give the title compound. **Compound D-3**: MS m/z (M+H)⁺: calculated 778.6, observed 778.6. ¹H-NMR (400 MHz, DMSO-*d*₆) δ 7.80 (t, *J* = 5.6 Hz, 1H), 7.64 (s, 1H), 7.55 (s, 1H), 6.91 (d, *J* = 8.0 Hz, 1H), 6.60 (d, *J* = 2.0 Hz, 1H), 6.49 – 6.45 (m, 1H), 5.56 (s, 2H), 5.29 (s, 2H), 4.01 (t, *J* = 6.4 Hz, 2H), 3.80 (s, 3H), 3.49 - 3.38 (m, 6H), 3.14 – 3.11 (m, 6H), 2.03 (t, *J* = 7.2 Hz, 2H), 1.71 – 1.68 (m, 2H), 1.58 – 1.53 (m, 2H), 1.47 - 1.26 (m, 4H), 1.28 – 1.15 (m, 28H), 0.91 – 0.83 (m, 6H).

Preparation of **Compound D-4**



***N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-6,6,6-trifluorohexyl)stearamide**



Step 1: 6-chloro-1,1,1-trifluorohexan-2-ol

[0513] To a solution of 5-chloropentanal (4.5 g, 37.3 mmol) and trimethyl(trifluoromethyl)silane (6.37 g, 44.8 mmol) in THF (80 mL) was added tetrabutylammonium fluoride (0.485 mL, 0.485 mmol, 1M in THF) at 0 °C. After 1 hr the cooling bath was removed and the mixture warmed to rt. After 2 hr the mixture was quenched with 1N HCl (60 mL). After stirring for 2 hr the mixture was diluted with water (60 mL) then extracted with EtOAc (35 mL x 2). The combined organic layers were dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-5% EtOAc/pet. ether) to give the title compound. ¹H NMR (400 MHz, CDCl₃) δ 3.99 - 3.83 (m, 1H), 3.56 (t, *J* = 6.4 Hz, 2H), 2.85 - 2.66 (m, 1H), 1.90 - 1.79 (m, 2H), 1.78 - 1.68 (m, 2H), 1.67 - 1.54 (m, 2H).

Step 2: 6-chloro-1,1,1-trifluorohexan-2-yl trifluoromethanesulfonate

[0514] To a solution of 6-chloro-1,1,1-trifluorohexan-2-ol (1.6 g, 8.39 mmol) and pyridine (1.013 mL, 12.59 mmol) in DCM (25 mL) was added trifluoromethanesulfonic anhydride (1.702 mL, 10.07 mmol) dropwise at 0 °C. After 1 hr the cooling bath was removed and the mixture warmed to rt. After 2 hr the mixture was diluted with water (10 mL) then extracted with EtOAc (15 mL x 2). The combined organic layers were washed with 1 N HCl (20 mL) then saturated NaHCO₃ then dried (Na₂SO₄) then filtered then the filtrate was concentrated to give the title compound which was used in the next step without purification. ¹H NMR (400 MHz, CDCl₃) δ

5.11 - 4.96 (m, 1H), 3.58 (t, $J = 6.4$ Hz, 2H), 2.06 - 1.96 (m, 2H), 1.94 - 1.82 (m, 2H), 1.79 - 1.62 (m, 2H).

Step 3: N⁷-butyl-2-(4-(4-(6-chloro-1,1,1-trifluorohexan-2-yl)piperazin-1-yl)-2-methoxybenzyl)-2H-pyrazolo[4,3-d]pyrimidine-5,7-diamine

[0515] To a solution of *N⁷-butyl-2-(2-methoxy-4-(piperazin-1-yl)benzyl)-2H-pyrazolo[4,3-d]pyrimidine-5,7-diamine* (1.4 g, 3.41 mmol) in 1,4-dioxane (16 mL) was added DIEA (1.787 mL, 10.23 mmol) and 6-chloro-1,1,1-trifluorohexan-2-yl trifluoromethanesulfonate (1.650 g, 5.12 mmol) then the mixture was heated to 80 °C. After heating overnight the mixture was cooled to rt then concentrated. The crude product was purified by reverse-phase chromatography (CH₃CN/water with 10mM NH₄HCO₃ modifier) to give the title compound. MS m/z (M+H)⁺: calculated 583.3, observed 583.4. ¹H NMR (400 MHz, CD₃OD) δ 7.52 (s, 1H), 7.08 (d, $J = 8.4$ Hz, 1H), 6.57 (d, $J = 2.0$ Hz, 1H), 6.50 (dd, $J = 2.0, 8.4$ Hz, 1H), 5.32 (s, 2H), 3.81 (s, 3H), 3.58 (t, $J = 6.4$ Hz, 2H), 3.52 - 3.45 (m, 2H), 3.23 - 3.03 (m, 7H), 2.82 - 2.75 (m, 2H), 1.84 - 1.70 (m, 4H), 1.66 - 1.55 (m, 4H), 1.47 - 1.38 (m, 2H), 0.96 (t, $J = 7.2$ Hz, 3H).

Step 4: 2-(4-(4-(6-azido-1,1,1-trifluorohexan-2-yl)piperazin-1-yl)-2-methoxybenzyl)-N⁷-butyl-2H-pyrazolo[4,3-d]pyrimidine-5,7-diamine

[0516] To a solution of *N⁷-butyl-2-(4-(4-(6-chloro-1,1,1-trifluorohexan-2-yl)piperazin-1-yl)-2-methoxybenzyl)-2H-pyrazolo[4,3-d]pyrimidine-5,7-diamine* (160 mg, 0.274 mmol) in DMF (2 mL) was added sodium azide (80 mg, 1.231 mmol) then the mixture was heated to 100 °C. After 2 hr the mixture was cooled to rt then diluted with water (5 mL). The pH was adjusted to >9 with saturated NaHCO₃ (15 mL) then the mixture was extracted with EtOAc (15 mL x 2). The combined organic layers were dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was purified by prep-TLC (DCM/MeOH=10:1) to give the title compound. MS m/z (M+H)⁺: calculated 590.3, observed 590.3.

Step 5: 2-(4-(4-(6-amino-1,1,1-trifluorohexan-2-yl)piperazin-1-yl)-2-methoxybenzyl)-N⁷-butyl-2H-pyrazolo[4,3-d]pyrimidine-5,7-diamine

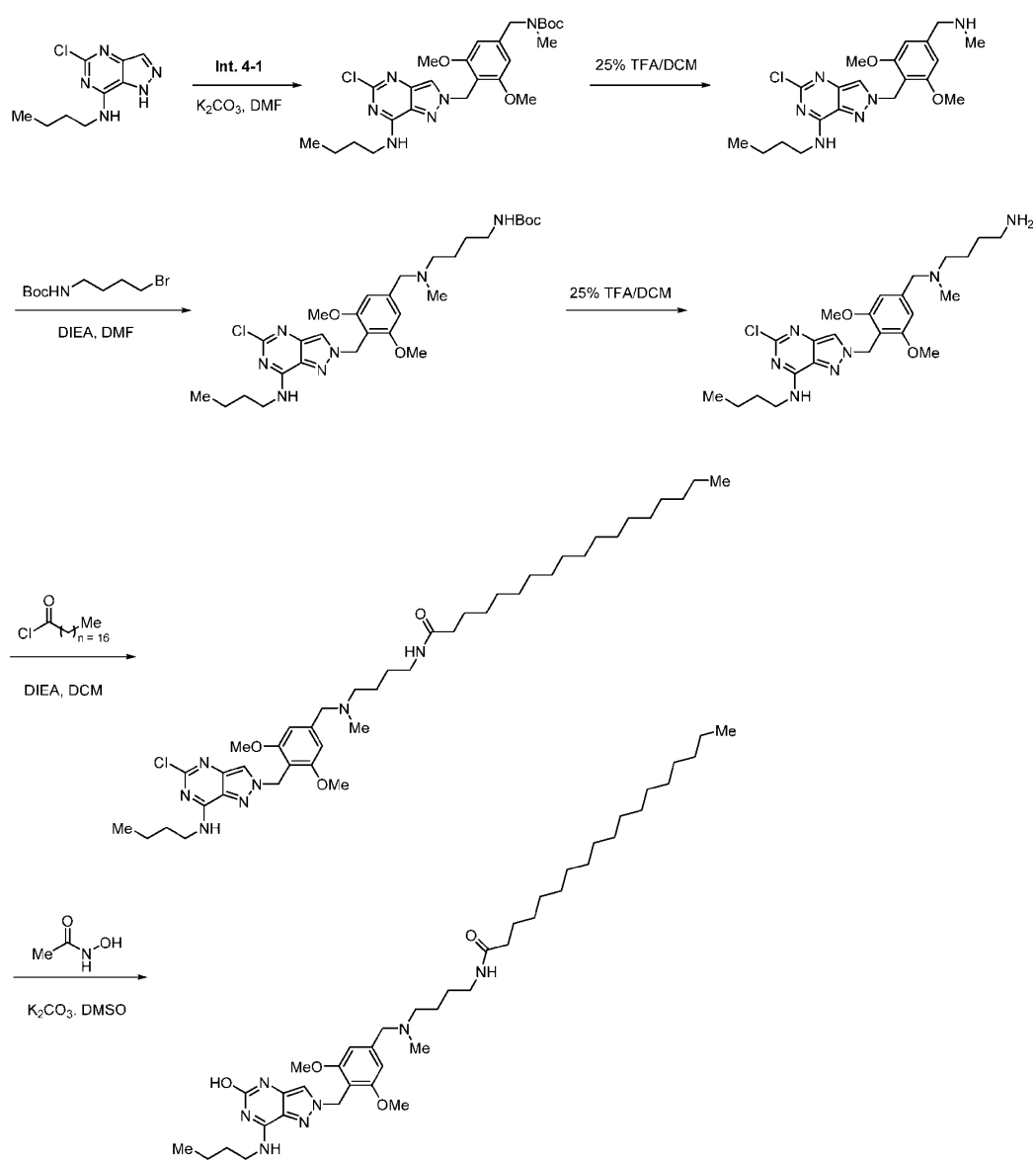
[0517] To a solution of *2-(4-(4-(6-azido-1,1,1-trifluorohexan-2-yl)piperazin-1-yl)-2-methoxybenzyl)-N⁷-butyl-2H-pyrazolo[4,3-d]pyrimidine-5,7-diamine* (100 mg, 0.170 mmol) in MeOH (2 mL) was added Pd/C (18.05 mg). The mixture was purged with H₂ (15 psi). After 2 hr the mixture was concentrated. The crude product was purified by prep-TLC (DCM/MeOH=10:1) to give the title compound. MS m/z (M+H)⁺: calculated 564.3, observed 564.3.

Step 6: N-(5-(4-(4-(5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-6,6,6-trifluorohexyl)stearamide

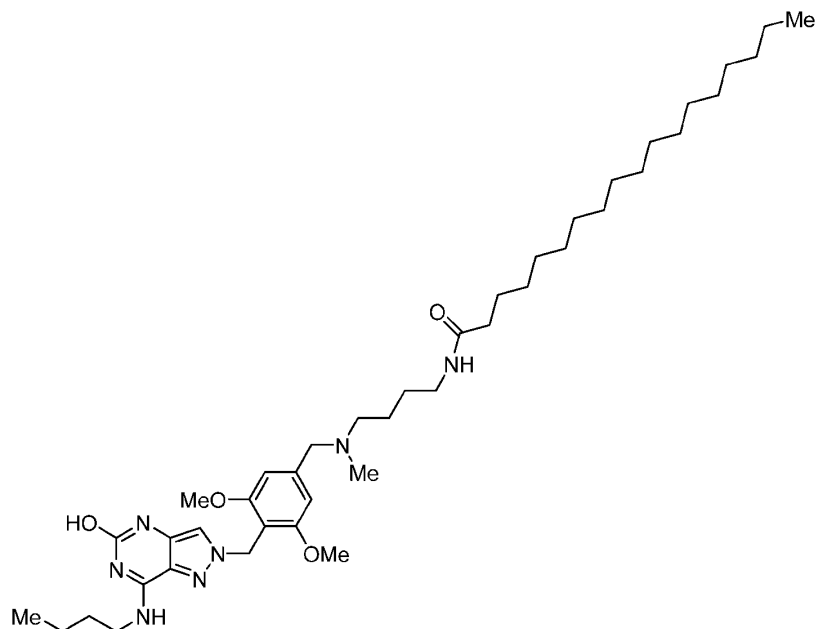
[0518] To a solution of 2-(4-(4-(6-amino-1,1,1-trifluorohexan-2-yl)piperazin-1-yl)-2-methoxybenzyl)-*N*⁷-butyl-2*H*-pyrazolo[4,3-*d*]pyrimidine-5,7-diamine (60 mg, 0.106 mmol) in DCM (1 mL) was added DIEA (0.056 mL, 0.319 mmol) and stearoyl chloride (32.2 mg, 0.106 mmol) at rt. After 2 hr the mixture was concentrated. The crude product was purified by reverse-phase chromatography (CH₃CN/water with 0.2% FA modifier) to give the title compound.

Compound D-4: MS *m/z* (M+H)⁺: calculated 830.6, observed 830.5. ¹H NMR (400 MHz, CD₃OD) δ 7.63 (s, 1H), 7.14 (d, *J* = 8.4 Hz, 1H), 6.58 (d, *J* = 2.0 Hz, 1H), 6.52 (dd, *J* = 2.0, 8.4 Hz, 1H), 5.37 (s, 2H), 3.83 (s, 3H), 3.58 (t, *J* = 7.2 Hz, 2H), 3.25 - 3.12 (m, 7H), 3.10 - 3.03 (m, 2H), 2.84 - 2.75 (m, 2H), 2.19 - 2.11 (m, 2H), 1.78 - 1.51 (m, 9H), 1.40 (s, 3H), 1.32 - 1.20 (m, 28H), 0.98 (t, *J* = 7.2 Hz, 3H), 0.89 (t, *J* = 6.8 Hz, 3H).

Preparation of Compound D-5



***N*-(4-((4-((7-(butylamino)-5-hydroxy-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)stearamide**



*Step 1: tert-butyl 4-((7-(butylamino)-5-chloro-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)carbamate*

[0519] To a solution of *N*-butyl-5-chloro-2*H*-pyrazolo[4,3-*d*]pyrimidin-7-amine (**Int. 2-1 step 1**, 800 mg, 3.54 mmol) in DMF (15 mL) was added *tert*-butyl 4-(chloromethyl)-3,5-dimethoxybenzyl)(methyl)carbamate (**Int. 4-1**, 1169 mg, 3.54 mmol) and K₂CO₃ (1470 mg, 10.63 mmol) at rt. After stirring overnight the mixture was diluted with water (80 mL) then extracted with EtOAc (15 mL x 3). The combined organic layers were washed with brine (20 mL x 2) then dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-40% EtOAc/pet. ether) to give the title compound. MS *m/z* (M+H)⁺: calculated 519.2, observed 519.3. ¹H NMR (400 MHz, CD₃OD) δ 7.88 (s, 1H), 6.59 (s, 2H), 5.57 (s, 2H), 4.44 (s, 2H), 3.85 (s, 6H), 3.55 (t, *J* = 7.2 Hz, 2H), 2.86 (s, 3H), 1.66 (quin, *J* = 7.2 Hz, 2H), 1.48 (br s, 9H), 1.45 - 1.39 (m, 2H), 0.98 (t, *J* = 7.6 Hz, 3H).

*Step 2: N-butyl-5-chloro-2-(2,6-dimethoxy-4-((methylamino)methyl)benzyl)-2*H*-pyrazolo[4,3-*d*]pyrimidin-7-amine*

[0520] To a solution of *tert*-butyl 4-((7-(butylamino)-5-chloro-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)carbamate (1 g, 1.927 mmol) in DCM (15 mL) was

added TFA (3 mL) at rt. After 3 hr the mixture was concentrated to give the TFA salt of the title compound which was used without purification. MS m/z (M+H)⁺: calculated 419.2, observed 419.3. ¹H NMR (400 MHz, CD₃OD) δ 7.99 (s, 1H), 6.85 (s, 2H), 5.65 (s, 2H), 4.19 (s, 2H), 3.91 (s, 6H), 3.63 (t, J = 7.2 Hz, 2H), 2.74 (s, 3H), 1.68 (quin, J = 7.2 Hz, 2H), 1.44 (qd, J = 7.6, 15.2 Hz, 2H), 0.98 (t, J = 7.6 Hz, 3H).

*Step 3: tert-butyl (4-((4-((7-(butylamino)-5-chloro-2H-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)carbamate*

[0521] To a solution of *N*-butyl-5-chloro-2-(2,6-dimethoxy-4-((methylamino)methyl)benzyl)-2H-pyrazolo[4,3-*d*]pyrimidin-7-amine (300 mg, 0.716 mmol) in DMF (10 mL) was added DIEA (0.375 mL, 2.148 mmol) and *tert*-butyl (4-bromobutyl)carbamate (217 mg, 0.859 mmol) then the mixture was heated to 40 °C. After heating overnight the mixture was cooled to rt then diluted with water (50 mL) then extracted with EtOAc (10 mL x 3). The combined organic layers were washed with brine (30 mL) then dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-5% MeOH/DCM) to give the title compound. MS m/z (M+H)⁺: calculated 590.3, observed 590.3.

*Step 4: N¹-(4-((4-((7-(butylamino)-5-chloro-2H-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)-N¹-methylbutane-1,4-diamine*

[0522] To a solution of *tert*-butyl (4-((4-((7-(butylamino)-5-chloro-2H-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)carbamate (250 mg, 0.424 mmol) in DCM (5 mL) was added TFA (1 mL) at rt. After stirring overnight the mixture was concentrated to give the TFA salt of the title compound which was used without purification. MS m/z (M+H)⁺: calculated 490.3, observed 490.3.

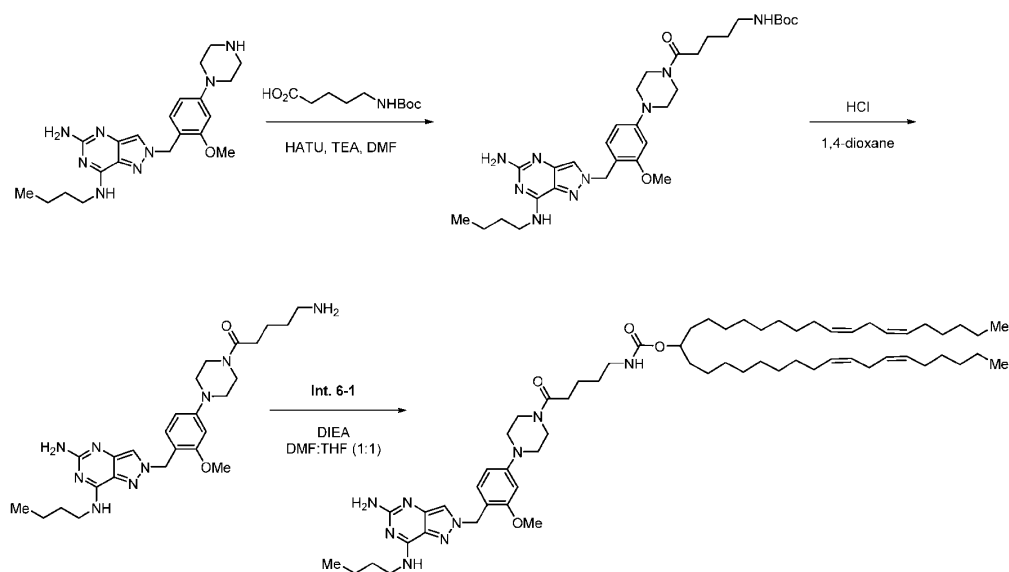
*Step 5: N-(4-((4-((7-(butylamino)-5-chloro-2H-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)stearamide*

[0523] To a solution of *N¹-(4-((4-((7-(butylamino)-5-chloro-2H-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)-N¹-methylbutane-1,4-diamine* (200 mg, 0.408 mmol) in DCM (5 mL) was added DIEA (0.214 mL, 1.224 mmol) then stearoyl chloride (124 mg, 0.408 mmol) at 0 °C. After the addition was complete the mixture was warmed to rt. After 2 hr the mixture was diluted with water (15 mL) then extracted with DCM (3 mL x 3). The combined organic layers were washed with brine (15 mL) then dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-5% MeOH/DCM) to give the title compound. MS m/z (M+H)⁺: calculated 756.5, observed 756.4.

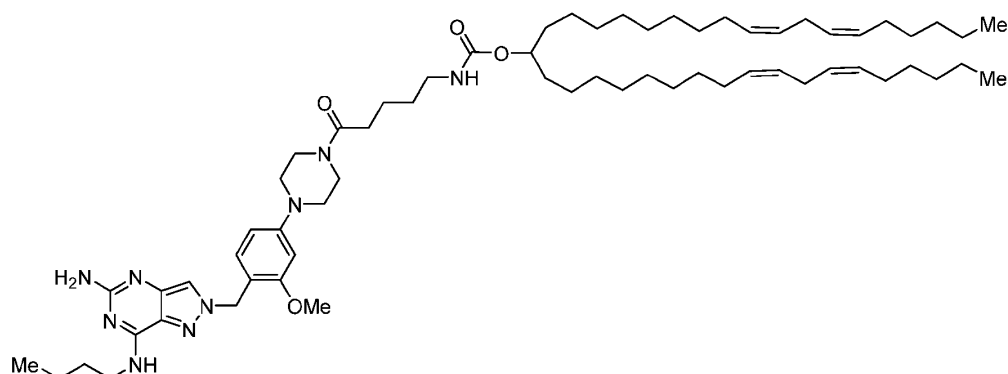
*Step 6: N-(4-((4-((7-(butylamino)-5-hydroxy-2H-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)stearamide*

[0524] To a solution of *N*-(4-((4-((7-(butylamino)-5-chloro-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)stearamide (90 mg, 0.119 mmol) in DMSO (7.5 mL) was added K₂CO₃ (49.3 mg, 0.357 mmol) and acetoxydroxamic acid (17.86 mg, 0.238 mmol) then the mixture was heated to 140 °C by microwave irradiation for 2 hr. Acetoxydroxamic acid (17.86 mg, 0.238 mmol) was added then the mixture was heated to 140 °C by microwave irradiation for 2 hr. The mixture was diluted with water (20 mL) then extracted with DCM (3 mL x 3). The combined organic layers were washed with brine (15 mL) then dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was purified by reverse-phase chromatography (CH₃CN/water with 0.2% FA modifier) to give the title compound. **Compound D-5**: MS *m/z* (M+H)⁺: calculated 738.6, observed 738.4. ¹H NMR (400 MHz, CD₃OD) δ 7.41 (s, 1H), 6.76 (s, 2H), 5.46 (s, 2H), 3.88 (s, 6H), 3.77 (br s, 2H), 3.53 (t, *J* = 7.2 Hz, 2H), 3.18 (t, *J* = 6.8 Hz, 2H), 2.71 - 2.61 (m, 2H), 2.41 (s, 3H), 2.16 (t, *J* = 7.2 Hz, 2H), 1.68 - 1.61 (m, 4H), 1.60 - 1.50 (m, 4H), 1.43 (dd, *J* = 7.2, 15.2 Hz, 2H), 1.27 (br s, 28H), 0.97 (t, *J* = 7.2 Hz, 3H), 0.92 - 0.86 (m, 3H).

Preparation of Compound D-6



(6*Z*,9*Z*,28*Z*,31*Z*)-heptatriaconta-6,9,28,31-tetraen-19-yl 5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)carbamate



Step 1: tert-butyl (5-(4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)carbamate

[0525] To a solution of 5-((*tert*-butoxycarbonyl)amino)pentanoic acid (0.699 g, 3.22 mmol) in DMF (20 ml) was added TEA (0.678 g, 6.70 mmol) and HATU (1.528 g, 4.02 mmol) at rt. After 20 minutes *N*⁷-butyl-2-(2-methoxy-4-(piperazin-1-yl)benzyl)-2*H*-pyrazolo[4,3-*d*]pyrimidine-5,7-diamine (1.1 g, 2.68 mmol) was added. After stirring overnight the mixture was filtered then purified directly by reverse-phase chromatography (CH₃CN/water with 10mM NH₄HCO₃ modifier) to give the title compound. MS *m/z* (M+H)⁺: calculated 610.4, observed 610.5. ¹H NMR (400 MHz, CD₃OD) δ 7.53 (s, 1H), 7.11 (d, *J* = 8.4 Hz, 1H), 6.60 (d, *J* = 2.0 Hz, 1H), 6.53 (dd, *J* = 2.0, 8.4 Hz, 1H), 5.34 (s, 2H), 3.83 (s, 3H), 3.75 - 3.65 (m, 4H), 3.50 (t, *J* = 7.2 Hz, 2H), 3.25 - 3.21 (m, 2H), 3.20 - 3.16 (m, 2H), 3.06 (t, *J* = 6.8 Hz, 2H), 2.45 (t, *J* = 7.6 Hz, 2H), 1.68 - 1.59 (m, 4H), 1.57 - 1.49 (m, 2H), 1.48 - 1.43 (m, 2H), 1.41 (s, 9H), 0.97 (t, *J* = 7.2 Hz, 3H).

Step 2: 5-amino-1-(4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)pentan-1-one

[0526] To a solution of *tert*-butyl (5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)carbamate (800 mg, 1.312 mmol) in DCM (10 ml) was added TFA (2 ml) at rt. After 1 hr the mixture was concentrated to give the TFA salt of the title compound which was used without purification. MS *m/z* (M+H)⁺: calculated 510.3, observed 510.3.

Step 3: (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl (5-(4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)carbamate

[0527] To a solution of 5-amino-1-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)pentan-1-one (660 mg, 1.295 mmol)

in DMF (8 ml) and THF (8 ml) was added DIEA (4.52 ml, 25.9 mmol) and (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl (4-nitrophenyl) carbonate (**Int. 6-1**, 1168 mg, 1.683 mmol) at rt. After 30 minutes the mixture was diluted with water (20 mL) then extracted with EtOAc (20 mL x 2). The combined organic layers were dried (Na₂SO₄) then filtered then the filtrate was concentrated. The crude product was subjected to silica gel chromatography (0-10% MeOH/DCM) to give the title compound with impurities. The compound was further purified by reverse-phase chromatography (CH₃CN/water with 0.1% TFA modifier) to give the title compound as the TFA salt. The salt was taken up in saturated NaHCO₃ (20 mL) then extracted with EtOAc (20 mL x 2). The combined organic layers were dried (Na₂SO₄) then filtered then the filtrate was concentrated to give the title compound. **Compound D-6**: MS *m/z* (M+H)⁺: calculated 1064.8, observed 1064.8. ¹H NMR (400 MHz, CD₃OD) δ 7.61 (s, 1H), 7.14 (d, *J* = 8.4 Hz, 1H), 6.60 (d, *J* = 1.6 Hz, 1H), 6.53 (dd, *J* = 2.0, 8.4 Hz, 1H), 5.39 - 5.26 (m, 10H), 4.67 (td, *J* = 6.0, 12.0 Hz, 1H), 3.84 (s, 3H), 3.74 - 3.66 (m, 4H), 3.55 (t, *J* = 7.2 Hz, 2H), 3.25 - 3.21 (m, 2H), 3.20 - 3.16 (m, 2H), 3.13 (br t, *J* = 6.4 Hz, 2H), 2.76 (t, *J* = 6.0 Hz, 4H), 2.46 (br t, *J* = 7.2 Hz, 2H), 2.09 - 2.00 (m, 8H), 1.70 - 1.61 (m, 4H), 1.56 - 1.47 (m, 5H), 1.45 - 1.39 (m, 2H), 1.35 - 1.25 (m, 36H), 0.97 (t, *J* = 7.2 Hz, 3H), 0.90 (t, *J* = 6.8 Hz, 6H).

EXAMPLE 7

Preparation of Pneumococcal Polysaccharide-Carrier Protein Conjugates

[0528] Polysaccharide(s) (as highlighted below and in the Tables and Examples) was dissolved, sized to a target molecular mass, chemically activated and buffer-exchanged by ultrafiltration. Activated polysaccharide and purified CRM197 (a carrier protein) were individually lyophilized and re-dissolved in DMSO. Re-dissolved polysaccharide and CRM197 solutions were then combined and conjugated as described below. The resulting conjugate was purified by ultrafiltration prior to a final 0.2-micron filtration. Several process parameters within each step, such as pH, temperature, concentration, and time were controlled to yield conjugates with desired attributes.

Polysaccharide size reduction

[0529] Purified pneumococcal capsular polysaccharide (otherwise termed "Ps") powder was dissolved in water. With the exception of ST-19A, (serotype is otherwise termed "ST") which is not sized reduced, dissolved polysaccharide was 0.45-micron filtered and either homogenized or acid hydrolyzed to reduce the molecular mass of the Ps. Target Ps size was achieved for homogenization by controlling the pressure and number of passes. Target Ps size was achieved for acid hydrolysis by controlling the temperature and time. Polysaccharide was then 0.2-micron

filtered and concentrated and diafiltered against water using a 5 or 10 kDa NMWCO tangential flow ultrafiltration membrane.

De-O-acetylation (ST-15B only)

[0530] Size reduced ST-15B Ps solution was heated to 60°C and sodium bicarbonate pH 9.4 buffer was added to a final concentration of 50 mM. The batch was incubated at 60°C to release O-acetyl groups. Potassium phosphate pH 6 buffer was added to neutralize pH and the solution was cooled to ambient temperature. The solution was then concentrated and diafiltered against water using a 5 or 10 kDa NMWCO tangential flow ultrafiltration membrane.

Deketalization (ST-4 only)

[0531] Sized reduced ST-4 Ps solution was adjusted to 50°C and pH 4.1 with a sodium acetate buffer to partially deketalize the polysaccharide. The polysaccharide solution was then cooled to 22°C prior to activation.

Polysaccharide oxidation

[0532] The polysaccharide solution was adjusted to 22°C for all serotypes, except for ST-5, 7F and 19F, which were adjusted to 4°C. The solution was also adjusted to pH 4-5 with a sodium acetate buffer to minimize polysaccharide size reduction due to activation. Polysaccharide activation was initiated with the addition of a sodium metaperiodate solution. The amount of sodium metaperiodate added was controlled to achieve a target level of polysaccharide activation (moles aldehyde per mole of polysaccharide repeating unit).

[0533] The activated product for all serotypes except ST-5 was diafiltered against 10 mM potassium phosphate, pH 6.4 followed by diafiltration against water using a 5 or 10 kDa NMWCO tangential flow ultrafiltration membrane. For ST-5, the activated product was diafiltered against 10 mM sodium acetate, pH 4.1 followed by diafiltration against water using a 5 kDa NMWCO tangential flow ultrafiltration membrane. Ultrafiltration was conducted at 2-8°C for all serotypes.

Polysaccharide conjugation to CRM197

[0534] Purified CRM197, obtained through expression in *Pseudomonas fluorescens* as previously described (WO 2012/173876 A1), was diafiltered against 2 mM phosphate, pH 7.2 buffer using a 5 kDa NMWCO tangential flow ultrafiltration membrane and 0.2-micron filtered. Activated polysaccharides were formulated for lyophilization with water and sucrose. CRM197 was formulated for lyophilization at 6 mg Pr/mL (the CRM197 protein is otherwise referred to as "Pr") with sucrose concentration of 1% w/v. Formulated Ps and CRM197 solutions were individually lyophilized. Lyophilized Ps and CRM197 materials were re-dissolved individually in equal volumes of DMSO. Additives such as salt were spiked into the Ps-DMSO for some

serotypes. The polysaccharide and CRM197 solutions were blended to achieve a target polysaccharide concentration and polysaccharide to CRM197 mass ratio. The mass ratio was selected to control the polysaccharide to CRM197 ratio in the resulting conjugate. A reducing agent such as sodium cyanoborohydride was added for most serotypes and conjugation proceeded at 22°C.

Final Reduction

[0535] A reducing agent such as sodium borohydride was added following the conjugation reaction and incubated at 22°C for all serotypes. The batch was diluted into 150 mM sodium chloride, with approximately 0.025% (w/v) polysorbate 20, at approximately 4°C. Potassium phosphate buffer was then added to neutralize the pH. Some lots were concentrated and diafiltered at approximately 4°C against 150 mM sodium chloride, 25 mM potassium phosphate pH 7, using a 30 kDa NMWCO tangential flow ultrafiltration membrane.

Final filtration and product storage

[0536] Individual batches were then concentrated and diafiltered against 10 mM histidine in 150 mM sodium chloride, pH 7.0, with 0.015% (w/v) PS-20, at 4°C using a 300 kDa NMWCO tangential flow ultrafiltration membrane. Specifically, for ST-5, halfway through the diafiltration step, ST-5 conjugate was harvested and incubated with 50 mM sodium bicarbonate, pH 9.3 for 3 hours. The ST-5 solution was neutralized with 1.5 M potassium phosphate, pH 6.0 prior to completing diafiltration.

[0537] The individual retentate batches were 0.2-micron filtered (with 0.5-micron prefilter) then diluted with additional 10 mM histidine in 150 mM sodium chloride, pH 7.0 with 0.015% (w/v) PS-20, dispensed into aliquots and frozen at $\leq -60^{\circ}\text{C}$. Serotype specific conjugate details can be found as previously described (WO2011/100151, WO2019/139692 and WO2020/131763).

EXAMPLE 8

Formulation of Pneumococcal Conjugate Compositions

[0538] Individual pneumococcal polysaccharide-carrier protein conjugates prepared utilizing different chemistries as described in Example 7 were used for the formulation of a 1- or 24-valent pneumococcal conjugate composition referred to as PCV1 or PCV24, respectively.

[0539] The PCV1 formulation, to be added to the Compound A-1-SNE, Compound B-1-SNE or Compound D-1-SNE, contained serotype 6B conjugated using reductive amination as described in Examples, supra, and formulated in 20 mM L-Histidine pH 5.8, 150 mM NaCl and 0.1% (w/v) PS-20 for a final concentration of 0.4 $\mu\text{g/mL}$ (w/v) pneumococcal polysaccharide

(PnPs) in the vaccine. The PCV1 vaccine formulation, prepared with APA and serotype 6B conjugated using reductive amination in an aprotic (DMSO) solvent, as described in Example 7, was formulated in 20 mM L-Histidine pH 5.8, 150 mM NaCl and 0.2% (w/v) PS-20 and 250 µg (Al)/mL in the form of APA for a final concentration of 0.4 µg/mL (w/v) pneumococcal polysaccharide (PnPs) in the vaccine.

[0540] The PCV24 formulation, to be added to Compound A-1-SNE for the IRM study, contained serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, de-O-Ac15B, 18C, 19A, 19F, 22F, 23B, 23F, 24F, 33F and 35B, all conjugated to CRM197 using reductive amination and formulated in 20 mM L-Histidine pH 5.8 150 mM NaCl and 0.1% PS-20. Each polysaccharide-carrier protein conjugate was formulated at 0.8 µg/mL (w/v) pneumococcal polysaccharide (PnPs) for a final concentration of 19.2 µg/mL PnPs in the vaccine.

[0541] The PCV24 formulation, either with or without APA for the IRM study, contained serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, de-O-Ac15B, 18C, 19A, 19F, 22F, 23B, 23F, 24F, 33F and 35B, all conjugated to CRM197 as described in Examples, supra using reductive amination and formulated in 20 mM L-Histidine pH 5.8 150 mM NaCl and 0.2% PS-20 and 250 µg (Al)/mL in the form of APA (if needed). Each polysaccharide-carrier protein conjugate was formulated at 4.0 µg/mL (w/v) pneumococcal polysaccharide (PnPs) for a final concentration of 96 µg /mL PnPs in the vaccine.

[0542] PCV20, which contains the following serotypes (1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F), all conjugated to CRM197, was purchased from Myonex.

[0543] The PCV26 formulation, to be added to Compound B-1-SNE for the mouse study, contained serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, de-O-Ac15B, 16F, 18C, 19A, 19F, 22F, 23A, 23B, 23F, 24F, 33F and 35B, all conjugated to CRM197 using reductive amination and formulated in 20 mM L-Histidine pH 5.8 150 mM NaCl and 0.1% PS-20. Each polysaccharide-carrier protein conjugate was formulated at 0.8 µg/mL (w/v) pneumococcal polysaccharide (PnPs) for a final concentration of 19.2 µg/mL PnPs in the vaccine.

[0544] To prepare the PCV formulations, the required volumes of monovalent bulk conjugates needed to obtain the indicated final concentration of (w/v) pneumococcal polysaccharide (also referred to as PnPs) were calculated based on the batch volume or mass and the bulk polysaccharide concentration.

[0545] The formulation process consisted of a conjugate bulk blend preparation at 2X the final concentration of PnPs blends in 20 mM Histidine, 0.05 to 0.15% (w/v) PS-20, and 150 mM sodium chloride, pH 5.8.

[0546] Histidine pH 5.8, PS-20 and sodium chloride solutions were prepared and added to the formulation vessel. The individual pneumococcal polysaccharide-carrier protein conjugates, stored frozen, were thawed at 2-8°C and then added to the formulation vessel. During the addition of polysaccharide-carrier protein conjugate to the formulation buffer (conjugate blend), the vessel was mixed to ensure homogeneity using a magnetic stir bar or magnetic impeller. After all additions were made and the solution was stirred, the conjugate blend was passed through sterilizing filters and collected in a vessel with or without APA. In some cases, the sterilizing filters were chased with 150 mM sodium chloride to adjust the batch to target concentration.

[0547] The formulations were filled into plastic syringes, glass syringes, or vials.

EXAMPLE 9

Preparation of a Stable Nanoemulsion (SNE) Adjuvant System With and Without Compounds

[0548] SNE adjuvant formulations are prepared as described in the examples supra, using the following compounds:

(*N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide (Compound A-1);

(*S*)-*N*-(5-(4-(4-((5-amino-7-((1-hydroxypentan-2-yl)amino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide (Compound A-2);

(*S*)-1-(4-(4-((5-amino-7-((1-hydroxypentan-2-yl)amino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)octadecan-1-one (Compound A-3);

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide (Compound B-1);

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)tetradecanamide (Compound B-2);

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)oleamide (Compound B-3);

(9*Z*,12*Z*)-*N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)octadeca-9,12-dienamide (Compound B-4);

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-1,4-diazepan-1-yl)-5-oxopentyl)stearamide (Compound B-5);

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperidin-1-yl)-5-oxopentyl)stearamide (Compound B-6);

N-(5-(3-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)azetidin-1-yl)-5-oxopentyl)stearamide (Compound B-7);

1-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-*N*-(3-stearamidopropyl)piperidine-4-carboxamide (Compound B-8);

(1*s*,3*s*)-3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-*N*-octadecylcyclobutane-1-carboxamide (Compound B-9);

(1*s*,3*s*)-3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-*N*-hexadecylcyclobutane-1-carboxamide (Compound B-10);

N-(3-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-3-oxopropyl)stearamide (Compound B-11);

N-(7-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-7-oxoheptyl)stearamide (Compound B-12);

N-(3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)cyclobutyl)stearamide (Compound B-13);

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-4,4-dimethyl-5-oxopentyl)stearamide (Compound B-14);

N-(6-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-methyl-6-oxohexan-2-yl)stearamide (Compound B-15);

1-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-(octadecyloxy)pentan-1-one (Compound B-16);

1-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-(octadecylamino)pentan-1-one (Compound B-17);

N-(5-(4-(4-((5-amino-7-(butylamino)-3-methyl-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide (Compound B-18);

(9*Z*,12*Z*)-*N*-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)octadeca-9,12-dienamide (Compound C-1);

N-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)tetradecanamide (Compound C-2);

N-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)oleamide (Compound C-3);

N-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)stearamide (Compound C-4);

N-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)-4-oxobutyl)stearamide (Compound C-5);

(6*Z*,9*Z*,28*Z*,31*Z*)-heptatriaconta-6,9,28,31-tetraen-19-yl (4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)carbamate (Compound D-1);

4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-*N*-(3-stearamidopropyl)piperazine-1-carboxamide (Compound D-2);

3-stearamidopropyl 4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazine-1-carboxylate (Compound D-3);

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-6,6,6-trifluorohexyl)stearamide (Compound D-4);

N-(4-((4-((7-(butylamino)-5-hydroxy-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl)stearamide (Compound D-5); and

(6*Z*,9*Z*,28*Z*,31*Z*)-heptatriaconta-6,9,28,31-tetraen-19-yl (5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)carbamate (Compound D-6).

[0549] The SNE is a multi-component emulsion formulation consisting of 3 stabilizing ingredients; SPAN-85, PS-20 and squalene with a compound, for example, Compound A-1 (referred to as Compound A-1-SNE, see Table 1) and without a compound (referred to as SNE, see Table 2). This formulation is prepared by combining and mixing the compound, SPAN-85, PS-20 and squalene components after dissolving in 100% EtOH together (Table 1).

[0550] The process of making the compound-stable nanoemulsion (Compound-SNE) consists of 5 steps: 1) solution preparation of a component mixture including the 3 stabilizing ingredients; SPAN-85, PS-20 and squalene, with a compound 2) SNE formation by means of T-mixing; 3) ultra-filtration; 4) bioburden reduced filtration; and 5) sterile filtration and vial filling.

Solution preparation of stabilizer/compound mixture

[0551] The stabilizer/compound components were weighed and combined before being dissolved in ethanol before being sterile filtered to form the component mixture. Histidine buffer (20 mM Histidine pH 5.8) was prepared.

SNE formation by means of T-mixing

[0552] The component mixture and Histidine buffer were then mixed together at adjacent ends of a T-tube mixer. The stream exiting the T-mix apparatus was immediately diluted 1:1 with 20 mM Histidine, 0.05% PS-20 and 75 mM NaCl, and then collected as formed SNE.

Ultra-filtration

[0553] The SNE intermediate was then subjected to Dialysis or ultra-filtration with a 500 kDA NMWCO in order to both concentrate the material approximately 10-fold as well as buffer exchange the material against 20 mM Histidine, 0.05% (w/v) PS-20 and 75 mM NaCl, pH 5.8. or 20 mM Histidine pH 5.8 or 20 mM Histidine 0.05% PS-20 pH 5.8. After the dialysis or diafiltration, there was a final concentration step performed in order to achieve final target concentration.

Bioburden reduced filtration

[0554] The adjuvant bulk was then pre-filtered with a 0.45 µm cellulose acetate (CA) filter followed by a 0.2 µm CA bioburden-reducing filter, and stored liquid at 4°C.

Sterile filtration and vial filling

[0555] The adjuvant bulk was passed through a 0.45 µm polyvinylidene fluoride (PVDF) bioburden reducing filter and a 0.22 µm PVDF sterilizing grad filter and received. The filtered adjuvant bulk was then diluted with 20 mM Histidine 0.05% (w/v) PS-20, and 75 mM NaCl pH 5.8 to the target SNE adjuvant concentration. This diluted final bulk adjuvant was then filled into glass vials and stored at 4°C.

Table 1: Composition of Compound A-1-SNE Adjuvant

Component	Description	Content of Each Component (Mole %)	Molecular Weight (g/mol)	Content of Each Component (Mass %)
Compound A-1	<i>N</i> -(5-(4-(4-((5-amino-7-(butylamino)-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide	2.42-2.71	599.3	2.44-3.03
Squalene	Squalene	70.62-79.16	410.72	48.780-60.61
SPAN-85	sorbitan trioleate	10.19-15.15	957.5	18.18-24.39
PS-20	polysorbate-20	7.94-11.81	1228	18.18-24.39
Buffer Matrix	20 mM Histidine, 0.05% PS-20, 75 mM NaCl, pH 5.8	N/A		

Table 2: Composition of Compound B-1-SNE Adjuvant

Component	Description	Content of Each Component (Mole %)	Molecular Weight (g/mol)	Content of Each Component (Mass %)
Compound B-1	<i>N</i> -(5-(4-(4-((5-amino-7-(butylamino)-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide	1.67-3.73	431.3	1.22-3.03
Squalene	Squalene	78.33-141.1	410.72	60.61-97.6
SPAN-85	sorbitan trioleate	2.02-15.1	957.5	4.44-24.4
PS-20	polysorbate-20	1.57-11.7	1228	4.44-24.4
Buffer Matrix	20 mM Histidine, 0-0.05% PS-20, 0-75 mM NaCl, pH 5.8-7.0	N/A		

Table 3: Composition of Compound C-4-SNE Adjuvant

Component	Description	Content of Each Component (Mole %)	Molecular Weight (g/mol)	Content of Each Component (Mass %)
Compound C-4	<i>N</i> -(4-((4-((5-amino-7-(butylamino)-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methyl)amino)butyl) stearamide	2.22	737.6	3.03
Squalene	Squalene	79.56	410.72	60.61
SPAN-85	sorbitan trioleate	10.24	957.5	18.18
PS-20	polysorbate-20	7.98	1228	18.18
Buffer Matrix	20 mM Histidine, 0.05% PS-20, 75 mM NaCl, pH 5.8	N/A		

Table 4: Composition of SNE Adjuvant

Component	Description	Content of Each Component (Mole %)	Molecular Weight (g/mol)	Content of Each Component (Mass %)
Squalene	Squalene	83.33	410.72	92.91
SPAN-85	sorbitan trioleate	8.33	957.5	3.99
PS-20	polysorbate-20	8.33	1228	3.11
Buffer Matrix	20 mM Histidine, 0.05% PS-20, 75 mM NaCl, pH 5.8	N/A		

EXAMPLE 9A

Alternate Preparation of a Stable Nanoemulsion (Compound B-1-SNE) Adjuvant System

[0556] The Compound B-1-SNE is a multi-component emulsion formulation consisting of 3 stabilizing ingredients: SPAN-85, PS-20 and squalene and the Compound B-1 (see Table 5). This formulation is prepared by combining and mixing the Compound B-1, SPAN-85, PS-20 and squalene components after dissolving in 100% EtOH together.

[0557] The process of making the compound-stable nanoemulsion (Compound B-1-SNE) consists of 5 steps: 1) solution preparation of a component mixture that includes the 3 stabilizing ingredients: SPAN-85, PS-20 and squalene and Compound B-1; 2) SNE formation by means of T-mixing or controlled precipitation process utilizing mixing systems to drive self-assembly of the nanoemulsion; 3) ultra-filtration; 4) bioburden reduced filtration; and 5) sterile filtration and vial filling.

Solution preparation of stabilizer/compound mixture

[0558] The stabilizer/compound components were weighed and combined before being dissolved in ethanol before being heated at 40°C for 30 min and sterile filtered to form the component mixture.

SNE formation by means of T-mixing or controlled precipitation process utilizing mixing systems to drive self-assembly of the nanoemulsion

[0559] The component mixture and Histidine buffer were then mixed together at adjacent ends of a T-tube mixer or fluidic assembly. The stream exiting the apparatus was immediately diluted 1:1 with 20 Histidine pH 5.8, and then collected as the formed SNE.

Ultra-filtration

[0560] The SNE intermediate was then subjected to Dialysis or ultra-filtration with a 500 kDA NMWCO in order to both concentrate the material approximately 10-fold as well as buffer exchange the material against 20 mM Histidine, 0.05% (w/v) PS-20 and 75 mM NaCl, pH 5.8 or 20 mM Histidine pH 5.8 or 20 mM Histidine 0.05% PS-20 pH 5.8. After the dialysis or diafiltration, there was a final concentration step performed in order to achieve final target concentration.

[0561] Histidine pH 5.8, PS-20, sodium chloride, L-met, and EDTA solutions were prepared and added to a formulation vessel. The adjuvant bulk SNE was added to the formulation vessel. During the addition of the SNE to the formulation buffer, the vessel was mixed to ensure homogeneity using a magnetic stir bar or magnetic impeller. After all additions were made and the solution was stirred the formulations were filled into plastic syringes, glass syringes, or vials.

Table 5: Composition of Compound B-1-SNE Adjuvant

Component	Description	Content of Each Component (Mole %)	Molecular Weight (g/mol)	Content of Each Component (Mass %)
Compound B-1	<i>N</i> -(5-(4-(4-((5-amino-7-(butylamino)-2 <i>H</i> -pyrazolo[4,3- <i>d</i>]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide	0.01-0.1	431.3	0.30-3.03
Squalene	Squalene	80.0-98.0	410.72	61.5-97.0
SPAN-85	sorbitan trioleate	1.5-15.0	957.5	3.1-25.0
PS-20	polysorbate-20	1.0-10.0	1228	1.2-24.0
Buffer Matrix	5-25 mM Histidine, pH 5.8, 0.05% PS-20, 0-75 mM NaCl, 5-12 mM L-met, 5-10 mM EDTA	N/A		

EXAMPLE 10

Cell-Based TLR 7/8 Assay to Evaluate Compound-SNE Activity

[0562] As shown in Figure 1, a cell-based activation system was used to assess activity of the Compound-formulated nanoemulsion systems (Compound B-1-SNE), prepared as described in examples, supra. HEK-Blue™ TLR7 and TLR8 cells (InvivoGen) were designed to assess the stimulation of TLR7 and TLR8 by monitoring the activation of NF-κB and AP-1. Compound B-1-SNE samples were diluted 1:2 across an 8-point titration in diluent buffer histidine/PS-20 in a sterile V-bottom plate. 20 μL of samples were transferred to corresponding wells of two flat bottom assay plates. Media was aspirated from HEK-Blue™ human TLR 7 and TLR 8 cells (T-75 flasks) and gently rinsed with pre-warmed (37°C) DPBS for a total volume of 5 mL/flask. The DPBS was gently removed from the cells and cells were gently manually dislodged and resuspended in 2 mL of DPBS. Cell assay suspensions were then prepared at approximately 2.2 x 10⁵ cells per mL of HEK-Blue™ detection medium (InvivoGen). 180 μL of cell suspension in detection medium was added to each well of the two flat bottom assay plates containing the diluted Compound B-1-SNE samples for a final dilution of sample equaling 1:10 (180 μL of cell suspension in detection medium and 20 μL of sample). The cells were then incubated at 37°C in 5% CO₂ for 20 hrs. The reporter gene, NF-Kb, induces the production of secreted embryonic alkaline phosphatase (SEAP) when stimulated and can be monitored using the vendor supplied SEAP detection kit. SEAP colorimetric changes were read at an absorbance of 640 nm using a SpectroMax Stakmax plate reader. As shown in Figure 1, Compound B-1-SNE activates both

TLR7 and TLR8 receptors with an EC₅₀ of 65.4 µg/mL for TLR7 and 82.3 µg/mL for TLR8. EC₅₀ values were calculated based on total Compound B-1-SNE concentration using GraphPad Prism Software and analyzed using agonist vs. response variable slope (four parameters) least squares fit.

EXAMPLE 10A

Further Cell-Based TLR 7/8 Assay Information to Evaluate Compound-SNE Activity

[0563] Further to the description in Example 10, a cell-based activation system was used to assess activity of the Compound-formulated nanoemulsion systems, prepared as described in examples, supra. HEK-Blue™ TLR7 and TLR8 cells (InvivoGen) were designed to assess the stimulation of TLR7 and TLR8 by monitoring the activation of NF-κB and AP-1. Compound-SNE samples were diluted 1:2 across an 8-point titration in diluent buffer histidine/PS-20 in a sterile V-bottom plate. 20 µL of samples were transferred to corresponding wells of two flat bottom assay plates. Media was aspirated from HEK-Blue™ human TLR 7 and TLR 8 cells (T-75 flasks) and gently rinsed with pre-warmed (37°C) DPBS for a total volume of 5 mL/flask. The DPBS was gently removed from the cells and cells were gently manually dislodged and resuspended in 2 mL of DPBS. Cell assay suspensions were then prepared at approximately 2.2 x 10⁵ cells per mL of HEK-Blue™ detection medium (InvivoGen). 180 µL of cell suspension in detection medium was added to each well of the two flat bottom assay plates containing the diluted Compound-SNE samples for a final dilution of sample equaling 1:10 (180 µL of cell suspension in detection medium and 20 µL of sample). The cells were then incubated at 37°C in 5% CO₂ for 20 hrs. The reporter gene, NF-Kb, induces the production of secreted embryonic alkaline phosphatase (SEAP) when stimulated and can be monitored using the vendor supplied SEAP detection kit. SEAP colorimetric changes were read at an absorbance of 640 nm using a SpectroMax Stakmax plate reader. EC₅₀s were calculated based on Compound concentrations only, not total Compound-SNE concentration (Table 6) using GraphPad Prism Software and analyzed using agonist vs. response variable slope (four parameters) least squares fit. As shown in Table 6, Compounds formulated as an SNE activate both TLR7 and TLR8 receptors.

Table 6: EC50 values, based on Compound concentration, for Compounds formulated as SNEs

Compound Number	huTLR7 EC ₅₀ (µg/mL)	huTLR8 EC ₅₀ (µg/mL)	Compound Number	huTLR7 EC ₅₀ (µg/mL)	huTLR8 EC ₅₀ (µg/mL)
B-1	0.65	0.43	B-13	67.68	2.38
B-2	0.15	0.22	C-5	0.48	0.07
B-3	0.12	0.1	B-11	2.3	0.3
B-4	0.3	0.3	B-6	3.25	2.01
A-1	12.01	4.33	B-10	90.94	56.99
C-4	3.65	0.86	D-2	13.76	4.52
C-2	0.23	0.12	B-8	50.18	41.9
C-3	0.37	0.12	B-5	10.59	3.33
C-1	0.19	0.07	B-7	6.3	2.53
D-1	17.63	159.2	B-14	108.3	9.53
B-12	18.8	20.53	A-2	6.3	0.38
D-3	18.46	10.77	B-18	0.25	0.17

EXAMPLE 11**PCV1 Immunogenicity in Mice: Evaluation of Adjuvant Systems**

[0564] Young female Balb/C mice (6-8 weeks old, n=10/group) were intramuscularly (IM) immunized with 0.2 mL of PCV1 formulated with different adjuvants (Table 7) on day 0, day 28, and day 56. PCV1 was dosed at 0.08, µg PnPs (6B conjugated to CRM197) per immunization. Mice were observed at least daily by trained animal care staff for any signs of illness or distress. The vaccine formulations in mice were deemed to be safe and well tolerated, as no vaccine-related adverse events were noted. All animal experiments were performed in strict accordance with the recommendations in the Guide for Care and Use of Laboratory Animals of the National Institutes of Health. The mouse experimental protocol was approved by the Institutional Animal Care and Use Committee at Merck & Co., Inc. (Rahway, NJ, USA).

Table 7: Formulations Assessed in PCV1 Immunogenicity Study in Mice

Formulation
ST-6B-CRM197; 0.08 µg PnPs/mL 20 mM L-histidine, 150 mM NaCl, 0.1% w/v PS-20, pH 5.8 [25 µg/dose APA]

ST-6B-CRM197; 0.08 µg PnPs/mL; 20 mM L-histidine, 150 mM NaCl, 0.1% w/v PS-20, pH 5.8 [15 µg/dose Compound A-1-SNE]
ST-6B-CRM197; 0.08 µg PnPs/mL; 20 mM L-histidine, 150 mM NaCl, 0.1% w/v PS-20, pH 5.8 [1.5 µg/dose Compound A-1-SNE]
ST-6B-CRM197; 0.08 µg PnPs/mL; 20 mM L-histidine, 150 mM NaCl, 0.1% w/v PS-20, pH 5.8 [0.15 µg/dose Compound A-1-SNE]
ST-6B-CRM197; 0.8 µg PnPs/mL; 20 mM L-histidine, 150 mM NaCl, 0.1% w/v PS-20, pH 5.8 [30 µg/dose Compound B-1-SNE]
ST-6B-CRM197; 0.08 µg PnPs/mL; 20 mM L-histidine, 150 mM NaCl, 0.1% w/v PS-20, pH 5.8 [3 µg/dose Compound B-1-SNE]
ST-6B-CRM197; 0.08 µg PnPs/mL; 20 mM L-histidine, 150 mM NaCl, 0.1% w/v PS-20, pH 5.8 [0.3 µg/dose Compound B-1-SNE]
ST-6B-CRM197; 0.08 µg PnPs/mL; 20 mM L-histidine, 150 mM NaCl, 0.1% w/v PS-20, pH 5.8 [30 µg/dose Compound C-4-SNE]
ST-6B-CRM197; 0.08 µg PnPs/mL; 20 mM L-histidine, 150 mM NaCl, 0.1% w/v PS-20, pH 5.8 [3 µg/dose Compound C-4-SNE]
ST-6B-CRM197; 0.08 µg PnPs/mL; 20 mM L-histidine, 150 mM NaCl, 0.1% w/v PS-20, pH 5.8 [0.3 µg/dose Compound C-4-SNE]

[0565] Mouse sera were evaluated for serotype 6B (ST-6B)-specific IgG antibody titers using an ELISA immunoassay (Figure 2). PCV1 immunization of Balb/C mice generated ST-6B-specific IgG antibody titers when formulated with APA, Compound A-1-SNE (at either 0.15, 1.5 or 15 µg Compound A-1 per dose), Compound B-1-SNE (at either 0.3, 3 or 30 µg Compound B-1 per dose), or Compound C-4-SNE (0.3, 3 or 30 µg Compound C-4 per dose). All formulations were found to be immunogenic, resulted in increased antibody titers after boosting, and, in general, demonstrated a dose-dependent response profile.

[0566] Functional antibody titers were determined through opsonophagocytic assays (OPA, Figure 3) based on previously described protocols at www.vaccine.uab.edu and Opsotiter® 3 software owned by and licensed from University of Alabama (UAB) Research Foundation (See Caro-Aguilar I. et al., Vaccine (2017) 35(6):865-72 and Burton R.L. and Nahm M.H. Clin. Vaccine Immunol. (2006) 13(9):1004-9). ST-6B-specific functional antibody titers were generated in Balb/C mice, further confirming all formulations were immunogenic.

EXAMPLE 12

PCV24 Immunogenicity Study in Infant Rhesus Monkey (IRM)

[0567] PCV24 (serotypes- 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15C, 18C, 19A, 19F, 22F, 23B, 23F, 24F, 33F and 35B each individually conjugated to CRM197) and adjuvant formulations were prepared or obtained (PCV20) as described in Examples, supra. Serotype “15C” is a de-O-acetylated 15B serotype. The PCV24 serotypes can also be defined as (serotypes- 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, de-O-acetylated 15B, 18C, 19A, 19F, 22F, 23B, 23F, 24F, 33F and 35B). IRMs (Infant Rhesus Monkeys, n=5/group) were intramuscularly immunized with either 0.1 mL (No SNE Adjuvant or Aluminum Adjuvant) or 0.5 mL (Compound B-1-SNE) vaccine, as described in Table 8, below, on days 0, 28 and 56. Sera were collected prior to study start (pre) and on days 14 (PD1), 42 (PD2), 70 (PD3). IRMs were observed at least once daily by trained animal care staff for any signs of illness or distress. The vaccine formulations in IRMs were deemed to be safe and well tolerated, and no vaccine-related adverse events were noted.

Table 8: PCV24 Formulations Evaluated in an Infant Rhesus Macaque Immunogenicity Model

Formulation
PCV24 in 20 mM L-histidine, 150 mM NaCl, 0.2% w/v PS-20, pH 5.8 [No Adjuvant]
PCV24 in 20 mM L-histidine, 150 mM NaCl, 0.2% w/v PS-20, pH 5.8, 250 µg/mL APA [Aluminum Phosphate Adjuvant]
PCV24 in 20 mM L-histidine, 75 mM NaCl, 0.05 % w/v PS-20, pH 5.8 [300 µg/mL Compound B-1-SNE (6.0 mg/mL of squalene; 1.8 mg/mL of PS-20; 1.8 mg/mL of SPAN-85)]
PCV24 in 20 mM L-histidine, 75 mM NaCl, 0.05 % w/v PS-20, pH 5.8 [100 µg/mL Compound B-1-SNE (2.0 mg/mL of squalene; 0.6 mg/mL of PS-20; 0.6 mg/mL of SPAN-85)]
PCV24 in 20 mM L-histidine, 75 mM NaCl, 0.05 % w/v PS-20, pH 5.8 [30 µg/mL Compound B-1-SNE (0.6 mg/mL of squalene; 0.18 mg/mL of PS-20; 0.18 mg/mL of SPAN-85)]

[0568] Serotype-specific IgG responses were evaluated using a multiplexed electrochemiluminescence (ECL) assay developed for use with rhesus monkey serum and based on the human assay described in Marchese et al., Clin Vaccine Immunol (2009) 16(3):387-96.

[0569] At post-dose 3 (day 70), PCV24 formulated with Compound B-1-SNE at all dose levels showed significantly higher antibody titers when compared to PCV24 without adjuvant (Figure

4). Statistical significance was defined as having a GMT ratio with lower 95% CI exceeding 1.0. As shown in Figure 5, PCV24 formulated with Compound B-1-SNE, at all dose levels, showed significantly higher antibody titers when compared to PCV24 formulated with APA for the majority of serotypes at post-dose 3. PCV24 formulated with Compound B-1-SNE, at all dose levels, showed significantly higher antibody titers when compared to PCV20 for the majority of shared serotypes at post-dose 3 (Figure 6).

EXAMPLE 13

PCV24 Immunogenicity Study in Infant Rhesus Monkey (IRM)

[0570] PCV24 (serotypes- 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15C, 18C, 19A, 19F, 22F, 23B, 23F, 24F, 33F and 35B each individually conjugated to CRM197) and adjuvant formulations were prepared or obtained (PCV20) as described in Examples, supra. Serotype “15C” is a de-O-acetylated 15B serotype. The PCV24 serotypes can also be defined as (serotypes- 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, de-O-acetylated 15B, 18C, 19A, 19F, 22F, 23B, 23F, 24F, 33F and 35B). IRMs (Infant Rhesus Monkeys, n=5/group) were intramuscularly immunized with 0.1 mL PCV20 (Aluminum Adjuvant) or 0.5 mL PCV24 adjuvanted with Compound B-1-SNE, as described in Table 9, below, on days 0, 28 and 56. Sera were collected prior to study start (pre) and on days 14 (PD1), 42 (PD2), 70 (PD3). IRMs were observed at least once daily by trained animal care staff for any signs of illness or distress. The vaccine formulations in IRMs were deemed to be safe and well tolerated, and no vaccine-related adverse events were noted.

Table 9: PCV24 Formulations Evaluated in an Infant Rhesus Macaque Immunogenicity Model

Formulation
PCV20 [Aluminum Phosphate Adjuvant], PREVNAR 20™
PCV24 in 20 mM L-histidine, 150 mM NaCl, 0.1 % w/v PS-20, pH 5.8 [200 µg/mL Compound B-1-SNE (8.0 mg/mL of squalene; 2 mg/mL of PS-20; 2 mg/mL of SPAN-85)]
PCV24 in 20 mM L-histidine, 150 mM NaCl, 0.1 % w/v PS-20, pH 5.8 [20 µg/mL Compound B-1-SNE (0.8 mg/mL of squalene; 0.2 mg/mL of PS-20; 0.2 mg/mL of SPAN-85)]
PCV24 in 20 mM L-histidine, 150 mM NaCl, 0.1 % w/v PS-20, pH 5.8 [2 µg/mL Compound B-1-SNE (0.08 mg/mL of squalene; 0.02 mg/mL of PS-20; 0.02 mg/mL of SPAN-85)]
PCV24 in 20 mM L-histidine, 150 mM NaCl, 0.1 % w/v PS-20, pH 5.8

[0.2 µg/mL Compound B-1-SNE (0.008 mg/mL of squalene; 0.002 mg/mL of PS-20; 0.002 mg/mL of SPAN-85)]

[0571] Serotype-specific IgG responses were evaluated using a multiplexed electrochemiluminescence (ECL) assay developed for use with rhesus monkey serum and based on the human assay described in Marchese et al., Clin Vaccine Immunol (2009) 16(3):387-96.

[0572] At post-dose 3 (day 70), PCV24 formulated with Compound B-1-SNE at the 100 µg dose level showed significantly higher antibody titers for all shared serotypes when compared to PCV20 (Figure 7). Statistically higher antibody titers were observed for 17 of 19 shared serotypes at the 10 µg dose level, 7 of 19 shared serotypes at the 1 µg dose level, 1 of 19 shared serotypes at the 0.1 µg dose level when compared to PCV20. All other titers were statistically comparable between the two groups. Statistical significance was defined as having a GMT ratio with lower 95% CI exceeding 1.0.

EXAMPLE 14

PCV26 Immunogenicity and Functional Antibody Responses in Mice

[0573] Female Balb/c mice (6-8 weeks old, n=15/group) were intramuscularly immunized with 0.1 mL of 26-valent pneumococcal conjugate vaccine (PCV) on day 0, day 28, and day 56. PCV26 was administered at 0.4 µg of each pneumococcal polysaccharide (1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, de-O-acetylated 15B, 16F, 18C, 19A, 19F, 22F, 23A, 23B, 23F, 24F, 33F, 35B) all individually conjugated to CRM197 and adjuvanted with aluminum phosphate adjuvant (APA) or Compound B-1-SNE. Adjuvants were utilized at concentrations as described in Table 10. Mice were observed at least daily by trained animal care staff for any signs of illness or distress.

Table 10: PCV26 Formulations

Formulation
PCV26 (104 µg (PnPs)/mL) in 18.1 mM L-histidine, 150 mM NaCl, 0.09% w/v PS-20, pH 5.8 250 µg/mL APA
PCV26 (104 µg (PnPs)/mL) in 13.3 mM L-histidine, 75 mM NaCl, 0.09 % w/v PS-20, pH 5.8 6.5 mg/mL Compound B-1-SNE (0.1 mg/mL of Compound B-1, 4 mg/mL of squalene; 1.2 mg/mL of SPAN-85; and 1.2 mg/mL of PS-20)

[0574] Mouse sera were collected prior to study start (pre-immune, day 0) and on days 14 (PD1), 42 (PD2), and 70 (PD3). Sera were evaluated for IgG immunogenicity using a multiplexed electrochemiluminescence (ECL) assay. This assay was developed for use with

mouse serum based on the human assay described by Marchese et al. (Marchese R. D., et al., Clin. Vaccine Immunol. (2009) 16(3):387-396) using technology developed by MesoScale Discovery (a division of MesoScale Diagnostics, LLC, Gaithersburg, MD) which utilizes a SULFO-TAG™ label that emits light upon electrochemical stimulation. SULFO-TAG™-labeled anti-mouse IgG was used as the secondary antibody for testing mouse serum samples. Functional antibody titers were determined through multiplexed opsonophagocytic assays (MOPA) based on previously described protocols at www.vaccine.uab.edu and Opsotiter® 3 software owned by and licensed from University of Alabama (UAB) Research Foundation (Caro-Aguilar, I. et al., Vaccine (2017) 35(6):865-872; and Burton R. L., and Nahm M. H., Clin. Vaccine Immunol. (2006) 19(9):1004-1009).

[0575] Mouse sera were tested individually in the ECL assay to determine antibody titers and generated antibody titers for all serotypes in the vaccine. Day 0 sera was pooled by group prior to testing in the ECL assay. Antibody titers in mice immunized with PCV26/Compound B-1-SNE were comparable to or higher than titers in mice immunized with PCV26/APA and the maximal difference was observed by day 42 (Figure 8).

[0576] It is also of note that PCV26, which contains polysaccharide conjugates 15A-CRM197, deOAc15B-CRM197, 6A-CRM197, and 6B-CRM197 also provided cross-reactivity to 15B and 6C and these titers in mice immunized with PCV26/Compound B-1-SNE were comparable to or higher than those in mice immunized with PCV26/APA (Figure 8).

[0577] For all timepoints, mouse sera was pooled by group prior to testing in the MOPA to determine functional antibody titers and PCV26 generated functional antibody titers in mice which killed vaccine-type bacterial serotypes. Similar to results from the ECL assay, functional antibody titers in mice immunized with PCV26/Compound B-1-SNE were comparable to or trended higher than titers in mice immunized with PCV26/APA and the maximal difference was observed by day 42 (PD2).

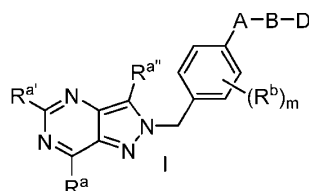
[0578] Antibody subclassing was performed to determine if the immune response was driven towards Th1 or Th2. Mouse sera from day 70 (PD3) were tested individually in an IgG subclassing assay to quantify IgG2a and IgG1 antibody titers and the ratio of IgG2a/IgG1 was calculated. A higher ratio indicates immunity driven more towards a Th1 response while a lower ratio indicates immunity driven more towards a Th2 response. Data from three representative serotypes (18C, 19F, and 24F) indicate PCV26/Compound B-1-SNE immunized mice have a more Th1-like response while PCV26/APA immunized mice have a more Th2-like response (Figure 9). Unpaired T test showed a significant difference in the IgG2a/IgG1 ratio between the PCV26/APA and PCV26/Compound B-1-SNE immunized groups across all three representative

serotypes ($P < 0.0001$). The addition of Compound B-1-SNE to a pneumococcal antigen composition is beneficial to not only the response but also to the quality of the response. This data indicates there is a difference in the quality of the immune response induced by Compound B-1-SNE adjuvant and APA adjuvant.

[0579] On day 77, 10 mice per group were intratracheally challenged with 10^5 cfu of *S. pneumoniae* serotype 24F bacteria in 0.1 mL of PBS. After challenge, mice were weighed daily and blood was collected at 24 hours, 48 hours and 72 hours to assess bacteremia. Mice were monitored by trained animal care staff for any sign of illness or distress in accordance with a schedule approved by the Institutional Animal Care and Use Committee and euthanized if defined criterion are met. All animal experiments were performed in strict accordance with the recommendations in the Guide for Care and Use of Laboratory Animals of the National Institutes of Health. The mouse experimental protocol was approved by the Institutional Animal Care and Use Committee at Merck & Co., Inc. (Rahway, NJ, USA). All PCV26 immunized mice were protected from bacteremia and showed 100% survival after intratracheal challenge with *S. pneumoniae* serotype 24F (Figure 10). One-Way ANOVA with Dunnett Multiple comparisons test indicated that PCV26/APA and PCV26/Compound B-1-SNE immunized groups were significantly protected from bacteremia when compared to the naïve control group ($P < 0.0001$) (Figure 10A). Mantel-Cox log-rank test of survival curves indicated that PCV26/APA and PCV26/Compound B-1-SNE immunized groups were significantly protected from challenge when compared to the naïve control group ($P < 0.0001$) (Figure 10B).

WHAT IS CLAIMED IS:

1. A compound having the structure set forth in Formula I:



wherein:

R^a is selected from H, -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, and $NR^3R^{3'}$, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, and -O(C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

$R^{a'}$ is selected from H, -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, and $-NR^3R^{3'}$, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, and -O(C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

$R^{a''}$ is selected from H, -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, and $-NR^3R^{3'}$, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, and -O(C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

R^3 and $R^{3'}$ are independently selected from H, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, and (C₁-C₆)alkynyl, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl and (C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine, or R^3 and $R^{3'}$, together with the nitrogen to which they are attached, join together to form a (C₃-

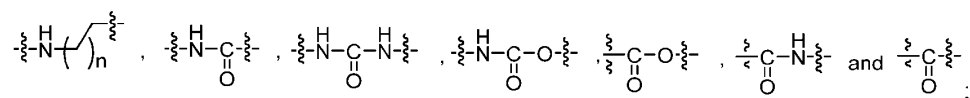
C₆)heterocycloalkyl, wherein said (C₃-C₆)heterocycloalkyl is optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

each occurrence of R^b is independently selected from H, -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, or NR[']R['], wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, and -O(C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

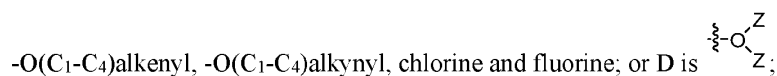
A is a carbon- or nitrogen-linked spacer selected from (C₁-C₆)alkyl, heterocycloalkyl, heterocycloalkyl-C(O)-R^z-, (C₁-C₄)alkyl-N(R^z)-R^z-, aryl, and heteroaryl, wherein said (C₁-C₆)alkyl, heterocycloalkyl, aryl and heteroaryl are optionally substituted with one to six substituents, independently selected from the group consisting of -OH, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, -O(C₁-C₆)alkynyl, chlorine, fluorine, and NR[']R['], wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl, (C₁-C₆)alkynyl, -O(C₁-C₆)alkyl, -O(C₁-C₆)alkenyl, and -O(C₁-C₆)alkynyl are optionally substituted with one to six substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

each occurrence of R^z is independently H or (C₁-C₆)alkyl;

B is a functional group selected from



D is a lipid selected from (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl, wherein said (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl, and (C₆-C₂₀)alkynyl are optionally substituted with one to six substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine; or D is



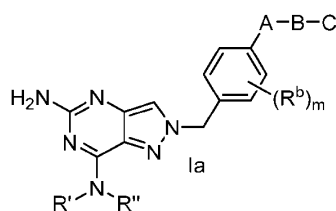
each occurrence of Z is independently selected from (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl, and (C₆-C₂₀)alkynyl, wherein said (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl, and (C₆-C₂₀)alkynyl are optionally substituted with one to six substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

m is 0, 1, 2, 3, 4 or 5; and

n is 0, 1, 2, 3, 4 or 5;

or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1 having the structure set forth in Formula Ia:

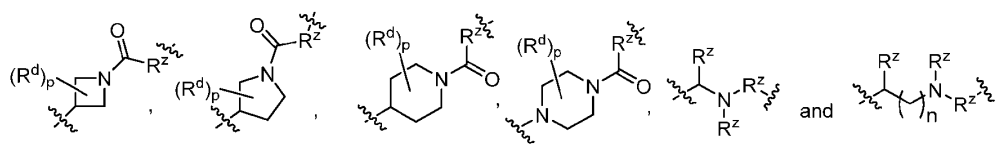


wherein:

R' and R'' are independently selected from H, (C₁-C₆)alkyl, (C₁-C₆)alkenyl, and (C₁-C₆)alkynyl, wherein said (C₁-C₆)alkyl, (C₁-C₆)alkenyl and (C₁-C₆)alkynyl are optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine, or R' and R'', together with the nitrogen to which they are attached, join together to form a (C₃-C₆)heterocycloalkyl, wherein said (C₃-C₆)heterocycloalkyl is optionally substituted with one to four substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

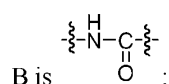
each occurrence of R^b is -O(C₁-C₄)alkyl, wherein said -O(C₁-C₄)alkyl is optionally substituted with one or two substituents, independently selected from the group consisting of -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

A is selected from



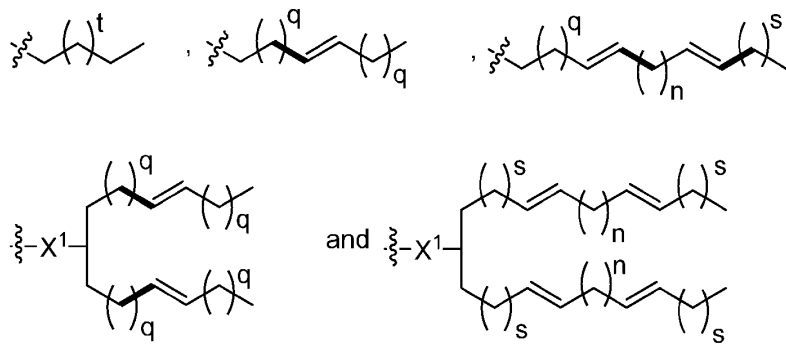
each occurrence of R^Z is independently H or (C₁-C₆)alkyl;

each occurrence of R^d is independently selected from -OH, (C₁-C₄)alkyl, -O(C₁-C₄)alkyl, chlorine and fluorine;



B is

D is a lipid chain selected from:



wherein any carbon on the lipid chain is optionally substituted with -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine,

wherein  is cis or trans stereochemistry,

X¹ is -O-, -C(R)²-, or -NR-, and

each occurrence of R is independently selected from H, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine;

m is 0, 1 or 2;

n is 0, 1, 2 or 3;

p is 0, 1 or 2;

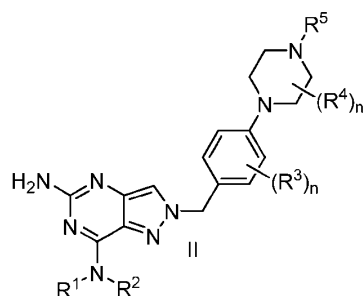
q is 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9;

s is, 1, 2, 3, 4, 5, 6, 7 or 8; and

t is 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18;

or a pharmaceutically acceptable salt thereof.

3. The compound of claim 1 having the structure set forth in Formula II:



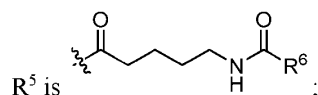
wherein:

R^1 is (C₁-C₆)alkyl, wherein said (C₁-C₆)alkyl is optionally substituted with one to four substituents independently selected from -OH and -O(CH₃);

R^2 is H, methyl or -O(CH₃);

each occurrence of R^3 is independently H, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl or -O(C₁-C₄)alkyl wherein said (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl or -O(C₁-C₄)alkyl are optionally substituted with one or two substituents independently selected from -OH and -O(CH₃);

each occurrence of R^4 is independently H, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl or -O(C₁-C₄)alkyl wherein said (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl or -O(C₁-C₄)alkyl are optionally substituted with one or two substituents independently selected from -OH and -O(CH₃);

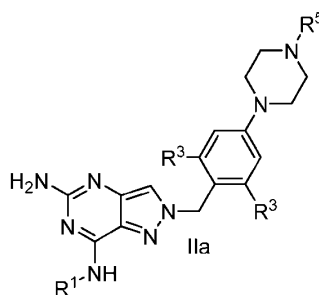


R^6 is selected from (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl, wherein said (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl, and (C₆-C₂₀)alkynyl are optionally substituted with one to six substituents independently selected from -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine; and

each occurrence of n is 4;

or a pharmaceutically acceptable salt thereof.

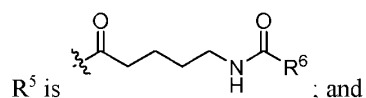
4. The compound of claim 3 having the structure set forth in Formula IIa:



wherein:

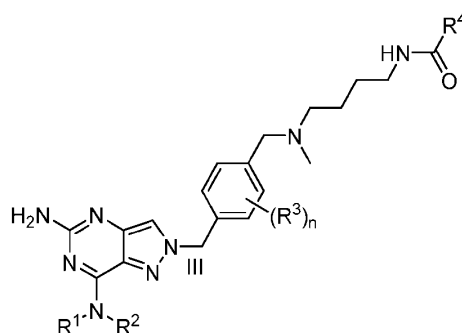
R^1 is butyl, wherein said butyl is optionally substituted with one or two -OH;

each occurrence of R^3 is independently H or -O(CH₃); and



R^6 is selected from (C₁₀-C₂₀)alkyl, (C₁₀-C₂₀)alkenyl and (C₁₀-C₂₀)alkynyl;
or a pharmaceutically acceptable salt thereof.

5. The compound of claim 1 having the structure set forth in Formula III:



wherein:

R^1 is (C₁-C₆)alkyl, wherein said (C₁-C₆)alkyl is optionally substituted with one to four substituents independently selected from -OH and -O(CH₃);

R^2 is H, methyl or -O(CH₃);

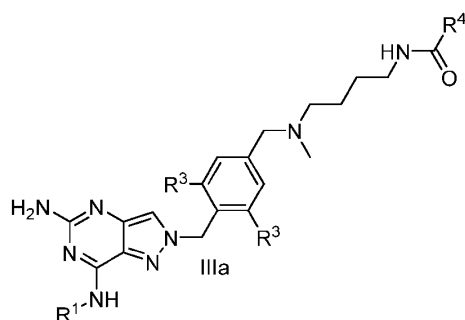
each occurrence of R^3 is independently H, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl or -O(C₁-C₄)alkyl wherein said (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl or -O(C₁-C₄)alkyl are optionally substituted with one or two substituents independently selected from -OH and -O(CH₃);

R^4 is selected from (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl, wherein said (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl are optionally substituted with one to six substituents selected from -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine and fluorine; and

n is 4;

or a pharmaceutically acceptable salt thereof.

6. The compound of claim 5 having the structure set forth in Formula IIIa:



wherein:

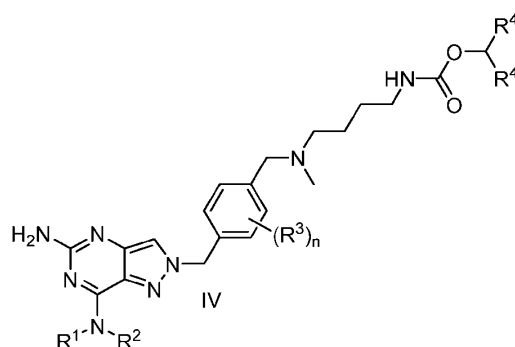
R^1 is butyl, wherein said butyl is optionally substituted with one or two -OH;

each occurrence of R^3 is independently H or -O(CH₃); and

R^4 is selected from (C₁₀-C₂₀)alkyl, (C₁₀-C₂₀)alkenyl and (C₁₀-C₂₀)alkynyl;

or a pharmaceutically acceptable salt thereof.

7. The compound of claim 1 having the structure set forth in Formula IV:



wherein:

R^1 is (C₁-C₆)alkyl, wherein said (C₁-C₆)alkyl is optionally substituted with one to four substituents selected from -OH and -O(CH₃);

R^2 is H, methyl or -O(CH₃);

each occurrence of R^3 is independently H, (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, or -O(C₁-C₄)alkyl wherein said (C₁-C₄)alkyl, (C₁-C₄)alkenyl, (C₁-C₄)alkynyl, and -O(C₁-C₄)alkyl are optionally substituted with one or two substituents independently selected from -OH and -O(CH₃);

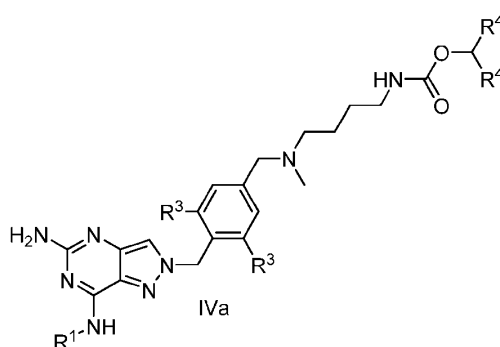
each occurrence of R^4 is independently selected from (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl, wherein said (C₆-C₂₀)alkyl, (C₆-C₂₀)alkenyl and (C₆-C₂₀)alkynyl

are optionally substituted with one to six substituents independently selected from -OH, -O(C₁-C₄)alkyl, -O(C₁-C₄)alkenyl, -O(C₁-C₄)alkynyl, chlorine or fluorine; and

n is 4:

or a pharmaceutically acceptable salt thereof.

8. The compound of claim 7 having the structure set forth in Formula IVa:



wherein:

R¹ is butyl, wherein said butyl is optionally substituted with one or two -OH;

each occurrence of R³ is independently H or -O(CH₃); and

each occurrence of R⁴ is independently selected from (C₁₀-C₂₀)alkyl, (C₁₀-

C₂₀)alkenyl and (C₁₀-C₂₀)alkynyl;

or a pharmaceutically acceptable salt thereof.

9. The compound of claim 1 selected from:

(*N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide;

(S)-N-(5-(4-(4-((5-amino-7-((1-hydroxypentan-2-yl)amino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3,5-dimethoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)tetradecanamide;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)oleamide;

(9Z,12Z)-N-(5-(4-(4-((5-amino-7-(butylamino)-2H-pyrazolo[4,3-d]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)octadeca-9,12-dienamide;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-1,4-diazepan-1-yl)-5-oxopentyl)stearamide;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperidin-1-yl)-5-oxopentyl)stearamide;

N-(5-(3-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)azetidin-1-yl)-5-oxopentyl)stearamide;

1-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-*N*-(3-stearamidopropyl)piperidine-4-carboxamide;

(1*s*,3*s*)-3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-*N*-hexadecylcyclobutane-1-carboxamide;

N-(3-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-3-oxopropyl)stearamide;

N-(7-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-7-oxoheptyl)stearamide;

N-(3-(2-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-2-oxoethyl)cyclobutyl)stearamide;

N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-4,4-dimethyl-5-oxopentyl)stearamide;

N-(5-(4-(4-((5-amino-7-(butylamino)-3-methyl-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide;

(9*Z*,12*Z*)-*N*-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)octadeca-9,12-dienamide;

N-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)tetradecanamide;

N-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)oleamide;

N-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)stearamide;

N-(4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)-4-oxobutyl)stearamide;

(6*Z*,9*Z*,28*Z*,31*Z*)-heptatriaconta-6,9,28,31-tetraen-19-yl (4-((4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3,5-dimethoxybenzyl)(methylamino)butyl)carbamate;

4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)-*N*-(3-stearamidopropyl)piperazine-1-carboxamide; and

3-stearamidopropyl 4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazine-1-carboxylate;
or a pharmaceutically acceptable salt thereof.

10. A compound which is:
N-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide;
or a pharmaceutically acceptable salt thereof.

11. A formulation comprising: (i) a compound of any one of claims 1-10 or a pharmaceutically acceptable salt thereof; (ii) one or more emulsifiers; and (iii) a terpene.

12. A formulation comprising: (i) a compound of any one of claims 1-10 or a pharmaceutically acceptable salt thereof; (ii) sorbitan trioleate (SPAN-85); (iii) polysorbate-20 (PS-20) or polysorbate-80 (PS-80); and (iv) squalene.

13. The formulation of claim 12 wherein the compound is *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, or a pharmaceutically acceptable salt thereof.

14. A pharmaceutical composition comprising: (i) at least one antigen; (ii) a compound of any one of claims 1-10 or a pharmaceutically acceptable salt thereof; (iii) one or more emulsifiers; (iv) a terpene; and (v) a pharmaceutically acceptable carrier.

15. A pharmaceutical composition comprising: (i) at least one antigen; (ii) a compound of any one of claims 1-10 or a pharmaceutically acceptable salt thereof; (iii) sorbitan trioleate (SPAN-85); (iv) polysorbate-20 (PS-20) or polysorbate-80 (PS-80); (v) squalene; and (vi) a pharmaceutically acceptable carrier.

16. The pharmaceutical composition of claim 15, wherein the compound is *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, or a pharmaceutically acceptable salt thereof.

17. An immunogenic composition comprising: (i) at least one antigen; (ii) a compound of any one of claims 1-10 or a pharmaceutically acceptable salt thereof; (iii) one or more emulsifiers; (iv) a terpene; and (v) a pharmaceutically acceptable carrier.

18. An immunogenic composition comprising: (i) at least one antigen; (ii) a compound of any one of claims 1-10 or a pharmaceutically acceptable salt thereof; (iii) sorbitan trioleate (SPAN-85); (iv) polysorbate-20 (PS-20) or polysorbate-80 (PS-80); (v) squalene; and (vi) a pharmaceutically acceptable carrier.

19. The immunogenic composition of claim 18, wherein the compound is *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, or a pharmaceutically acceptable salt thereof.

20. A single-dose vaccine composition comprising: (i) at least one antigen; (ii) a compound of any one of claims 1-10 or a pharmaceutically acceptable salt thereof; (iii) one or more emulsifiers; (iv) a terpene; and (v) a pharmaceutically acceptable carrier, wherein a single dose of the vaccine composition is sufficient to elicit a desired immune response against the at least one antigen.

21. A single-dose vaccine composition comprising: (i) at least one antigen; (ii) a compound of any one of claims 1-10 or a pharmaceutically acceptable salt thereof; (iii) sorbitan trioleate (SPAN-85); (iv) polysorbate-20 (PS-20) or polysorbate-80 (PS-80); (v) squalene; and (vi) a pharmaceutically acceptable carrier, wherein a single dose of the vaccine composition is sufficient to elicit a desired immune response against the at least one antigen.

22. The single-dose vaccine composition of claim 21, wherein the compound is *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, or a pharmaceutically acceptable salt thereof.

23. A method of treating or preventing a disease in a patient in need thereof comprising administering to the patient a pharmaceutical composition comprising: (i) at least one antigen; (ii) a compound of any one of claims 1-10, or a pharmaceutically acceptable salt

thereof; (iii) one or more emulsifiers; (iv) a terpene; and (v) a pharmaceutically acceptable carrier.

24. A method of treating or preventing a disease in a patient in need thereof comprising administering to the patient a pharmaceutical composition comprising: (i) at least one antigen; (ii) a compound of any one of claims 1-10, or a pharmaceutically acceptable salt thereof; (iii) sorbitan trioleate (SPAN-85); (iv) polysorbate-20 (PS-20) or polysorbate-80 (PS-80); (v) squalene; and (vi) a pharmaceutically acceptable carrier.

25. The method of claim 24, wherein the compound is *N*-(5-(4-(4-((5-amino-7-(butylamino)-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)methyl)-3-methoxyphenyl)piperazin-1-yl)-5-oxopentyl)stearamide, or a pharmaceutically acceptable salt thereof.

26. The method of any one of claims 23-25, wherein the patient is a human.

27. The method of any one of claims 23-25, wherein the patient is a non-human animal.