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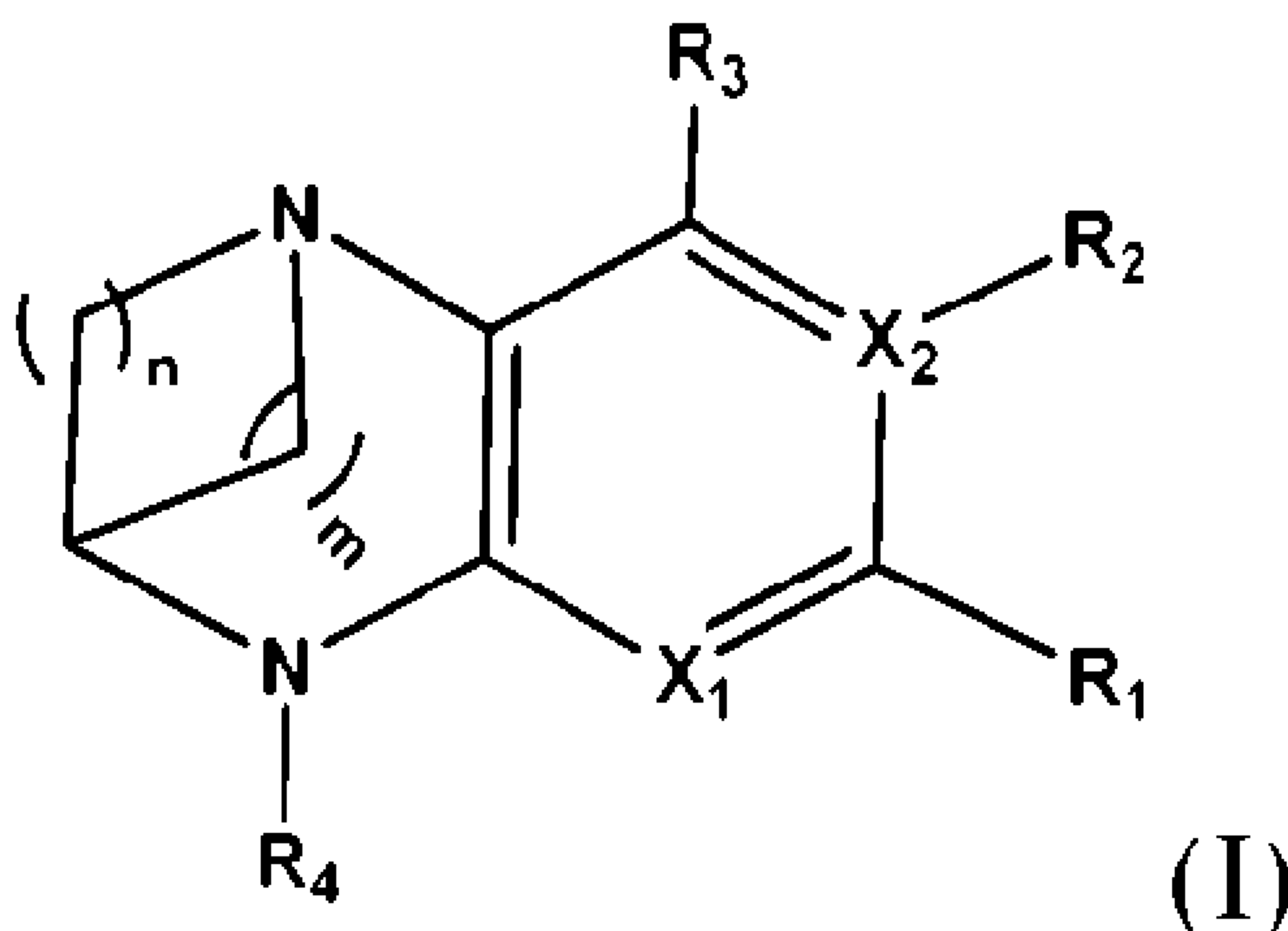
(71) Demandeur/Applicant:
GLAXOSMITHKLINE INTELLECTUAL PROPERTY
(NO.2) LIMITED, GB

(72) Inventeurs/Inventors:
ELLIS, JAMES LAMOND, US;
EVANS, KAREN ANDERSON, US;
FOX, RYAN MICHAEL, US;
MILLER, WILLIAM HENRY, US;
SEEFELD, MARK ANDREW, US

(74) Agent: GOWLING WLG (CANADA) LLP

(54) Titre : ANALOGUES D'UREE PONTES SUBSTITUES EN TANT QUE MODULATEURS DE SIRTUINE

(54) Title: SUBSTITUTED BRIDGED UREA ANALOGS AS SIRTUIN MODULATORS



(57) Abrégé/Abstract:

The present invention relates to novel substituted bridged urea analog compounds of Formula (I) or pharmaceutically acceptable salts thereof, corresponding pharmaceutical compositions, processes for making and use of such compounds, alone or in combination with other therapeutic agents, as Sirtuin Modulators useful for increasing lifespan of a cell, and in treating and/or preventing a wide variety of diseases and disorders, which include, but are not limited to, for example, diseases or disorders related to aging or stress, diabetes, obesity, neurodegenerative diseases, cardiovascular disease, blood clotting disorders, inflammation, cancer, and/or flushing as well as diseases or disorders that would benefit from increased mitochondrial activity. (I)

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(71) Applicant: GLAXOSMITHKLINE INTELLECTUAL
 PROPERTY (NO.2) LIMITED [GB/GB]; 980 Great
 West Road, Brentford Middlesex TW89GS (GB).

(72) Inventors: ELLIS, James Lamond; 1250 South Col-
 legeville Road, Collegeville, Pennsylvania 19426 (US).
 EVANS, Karen Anderson; 1250 South Collegeville Road,
 Collegeville, Pennsylvania 19426 (US). FOX, Ryan Mi-
 chael; 1250 South Collegeville Road, Collegeville,
 Pennsylvania 19426 (US). MILLER, William Henry;
 1250 South Collegeville Road, Collegeville, Pennsylvania
 19426 (US). SEEFELD, Mark Andrew; 1250 South Col-
 legeville Road, Collegeville, Pennsylvania 19426 (US).

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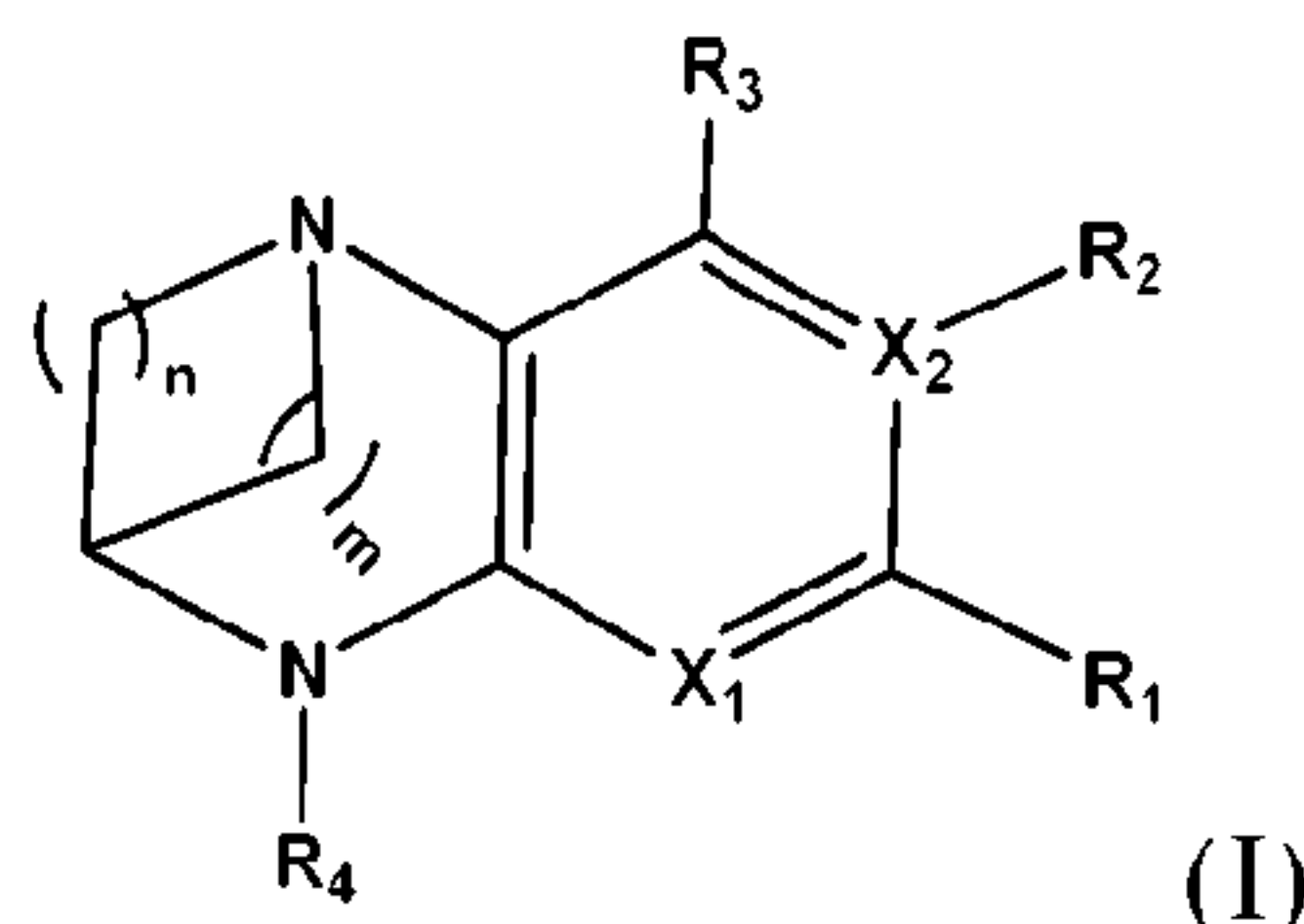
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(54) Title: SUBSTITUTED BRIDGED UREA ANALOGS AS SIRTUIN MODULATORS



(I)

(57) Abstract: The present invention relates to novel substituted bridged urea analog compounds of Formula (I) or pharmaceutically acceptable salts thereof, corresponding pharmaceutical compositions, processes for making and use of such compounds, alone or in combination with other therapeutic agents, as Sirtuin Modulators useful for increasing lifespan of a cell, and in treating and/or preventing a wide variety of diseases and disorders, which include, but are not limited to, for example, diseases or disorders related to aging or stress, diabetes, obesity, neurodegenerative diseases, cardiovascular disease, blood clotting disorders, inflammation, cancer, and/or flushing as well as diseases or disorders that would benefit from increased mitochondrial activity. (I)



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SUBSTITUTED BRIDGED UREA ANALOGS AS SIRTUIN MODULATORS**FIELD OF THE INVENTION**

In general, the present invention relates to substituted bridged urea analog
5 compounds of Formulas (I) to (VI), corresponding analogs or derivatives thereof, or
pharmaceutically acceptable salts thereof, corresponding pharmaceutical compositions,
processes for making and methods or uses of such compounds, alone or in combination
with other therapeutic agents, as Sirtuin Modulators useful for increasing lifespan of a
cell, and in treating and/or preventing a wide variety of diseases and disorders, which
10 include, but are not limited to, for example, diseases or disorders related to aging or
stress, diabetes, obesity, neurodegenerative diseases, cardiovascular disease, blood
clotting disorders, inflammation, cancer, and/or flushing as well as diseases or disorders
that would benefit from increased mitochondrial activity.

BACKGROUND

15 The Silent Information Regulator (SIR) family of genes represents a highly
conserved group of genes present in the genomes of organisms ranging from
archaebacteria to eukaryotes. The encoded SIR proteins are involved in diverse processes
from regulation of gene silencing to DNA repair. A well-characterized gene in this family
is *S. cerevisiae* SIR2, which is involved in silencing HM loci that contain information
20 specifying yeast mating type, telomere position effects and cell aging. The yeast Sir2
protein belongs to a family of histone deacetylases. The proteins encoded by members of
the SIR gene family show high sequence conservation in a 250 amino acid core domain.
The Sir2 homolog, CobB, in *Salmonella typhimurium*, functions as an NAD (nicotinamide
adenine dinucleotide)-dependent ADP-ribosyl transferase.

25 The Sir2 protein is a class III deacetylase which uses NAD as a cosubstrate.
Unlike other deacetylases, many of which are involved in gene silencing, Sir2 is
insensitive to class I and II histone deacetylase inhibitors like trichostatin A (TSA).

Deacetylation of acetyl-lysine by Sir2 is tightly coupled to NAD hydrolysis,
producing nicotinamide and a novel acetyl-ADP ribose compound. The NAD-dependent
30 deacetylase activity of Sir2 is essential for its functions, which can connect its biological
role with cellular metabolism in yeast. Mammalian Sir2 homologs have NAD-dependent
histone deacetylase activity.

Biochemical studies have shown that Sir2 can readily deacetylate the amino-terminal tails of histones H3 and H4, resulting in the formation of 2'/3'-O-acetyl-ADP-ribose (OAADPR) and nicotinamide. Strains with additional copies of SIR2 display increased rDNA silencing and a 30% longer life span. It has also been shown that additional copies of the *C. elegans* SIR2 homolog, sir-2.1, and the *D. melanogaster* dSir2 gene extend life span in those organisms. This implies that the SIR2-dependent regulatory pathway for aging arose early in evolution and has been well conserved. Today, Sir2 genes are believed to have evolved to enhance an organism's health and stress resistance to increase its chance of surviving adversity.

In humans, there are seven Sir2-like genes (SIRT1-SIRT7) that share the conserved catalytic domain of Sir2. SIRT1 is a nuclear protein with the highest degree of sequence similarity to Sir2. SIRT1 regulates multiple cellular targets by deacetylation including the tumor suppressor p53, the cellular signaling factor NF- κ B, and the FOXO transcription factor.

SIRT3 is a homolog of SIRT1 that is conserved in prokaryotes and eukaryotes. The SIRT3 protein is targeted to the mitochondrial cristae by a unique domain located at the N-terminus. SIRT3 has NAD⁺-dependent protein deacetylase activity and is ubiquitously expressed, particularly in metabolically active tissues. Upon transfer to the mitochondria, SIRT3 is believed to be cleaved into a smaller, active form by a mitochondrial matrix processing peptidase (MPP).

Caloric restriction has been known for over 70 years to improve the health and extend the lifespan of mammals. Yeast life span, like that of metazoans, is also extended by interventions that resemble caloric restriction, such as low glucose. The discovery that both yeast and flies lacking the SIR2 gene do not live longer when calorically restricted provides evidence that SIR2 genes mediate the beneficial health effects of a restricted calorie diet. Moreover, mutations that reduce the activity of the yeast glucose-responsive cAMP (adenosine 3',5'-monophosphate)-dependent (PKA) pathway extend life span in wild type cells but not in mutant sir2 strains, demonstrating that SIR2 is likely to be a key downstream component of the caloric restriction pathway.

In addition to therapeutic potential, structural and biophysical studies of SIRT1 activity and activation by small molecule sirtuin modulators would be useful to advance understanding of the biological function of sirtuins, to further the understanding of the

mechanism of action of sirtuin activation and to aid in the development of assays that identify novel sirtuin modulators.

The present invention is directed to overcoming these and other problems encountered in the art.

5

SUMMARY OF THE INVENTION

In general, the present invention relates to substituted bridged urea analog compounds of Formulas (I) to (VI), corresponding analogs or derivatives thereof, or pharmaceutically acceptable salts thereof, corresponding pharmaceutical compositions, processes for making and use of such compounds, alone or in combination with other therapeutic agents, as Sirtuin Modulators useful for increasing lifespan of a cell, and in treating and/or preventing a wide variety of diseases and disorders, which include, but are not limited to, for example, diseases or disorders related to aging or stress, diabetes, obesity, neurodegenerative diseases, cardiovascular disease, blood clotting disorders, inflammation, cancer, and/or flushing as well as diseases or disorders that would benefit from increased mitochondrial activity.

15

In particular, the present invention relates to novel compounds of Formulas (I) to (VI), corresponding analogs (i.e., with hydrogen substitution at the R^2 position) and corresponding pharmaceutical compositions comprising compounds of Formulas (I) to (VI) respectively.

20

The present invention also relates to processes for making compounds of Formulas (I) to (VI), and corresponding analogs (i.e., with hydrogen substitution at the R^2 position), respectively.

The present invention also relates to methods for using or uses of Sirtuin Modulator compounds as defined herein in treating and/or preventing a wide variety of diseases and disorders, which include, but are not limited to, for example, diseases or disorders related to aging or stress, diabetes, obesity, neurodegenerative diseases, cardiovascular disease, blood clotting disorders, inflammation, cancer, and/or flushing as well as diseases or disorders that would benefit from increased mitochondrial activity, further which may be selected from or include, but are not limited to psoriasis, atopic dermatitis, acne, rosacea, inflammatory bowel disease, osteoporosis, sepsis, arthritis, COPD, systemic lupus erythematosus and ophthalmic inflammation.

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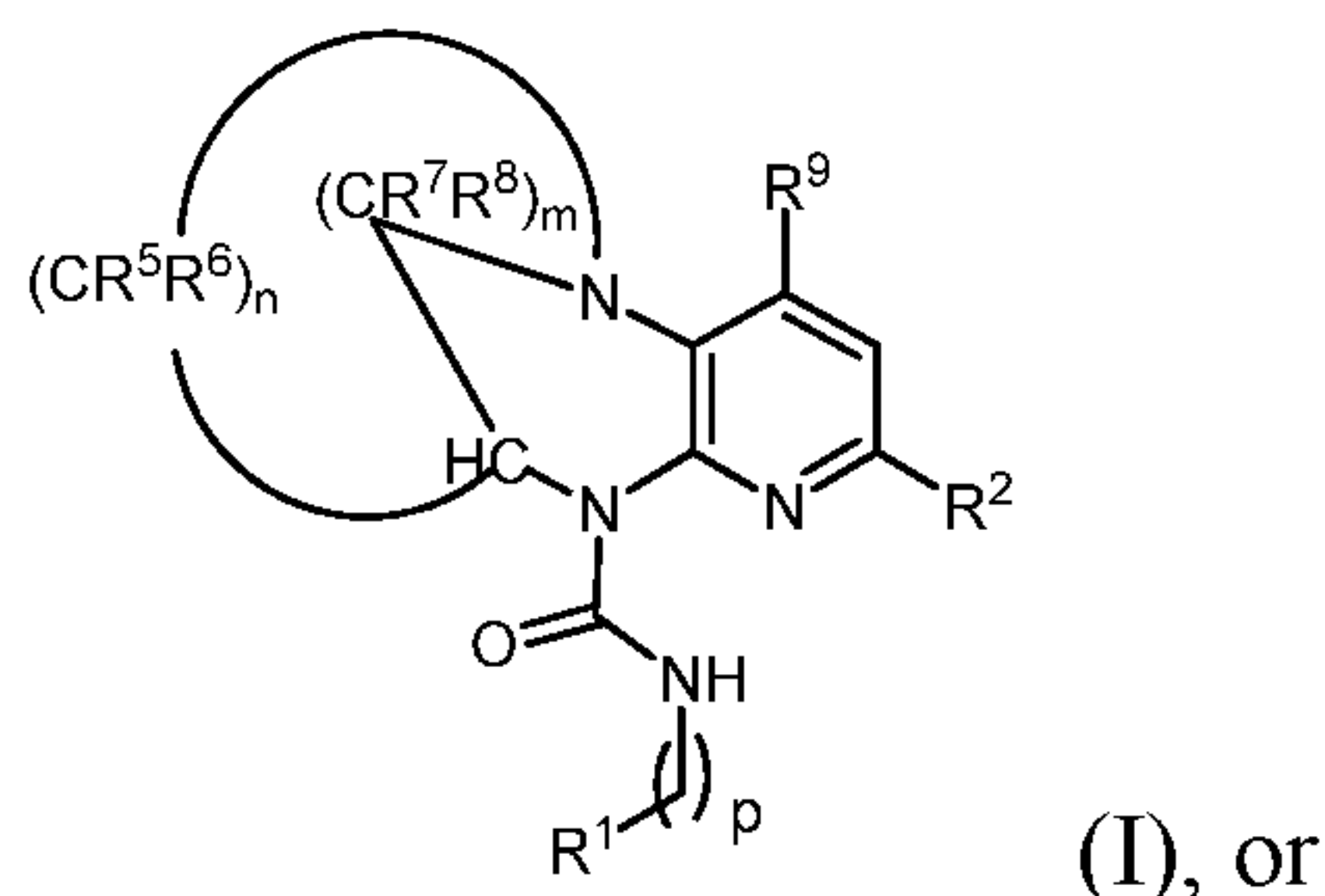
DETAILED DESCRIPTION OF THE INVENTION

In general, the present invention relates to substituted bridged urea analog compounds of Formulas (I) to (VI), corresponding analogs or derivatives thereof, or pharmaceutically acceptable salts thereof, corresponding pharmaceutical compositions, processes for making and use of such compounds, alone or in combination with other therapeutic agents, as Sirtuin Modulators useful for increasing lifespan of a cell, and in treating and/or preventing a wide variety of diseases and disorders, which include, but are not limited to, for example, diseases or disorders related to aging or stress, diabetes, obesity, neurodegenerative diseases, cardiovascular disease, blood clotting disorders, inflammation, cancer, and/or flushing as well as diseases or disorders that would benefit from increased mitochondrial activity.

COMPOUNDS

In particular, the present invention relates to novel compounds of Formulas (I) to (VI), corresponding analogs (i.e., with hydrogen substitution at the R² position) and corresponding pharmaceutical compositions comprising compounds of Formulas (I) to (VI), respectively.

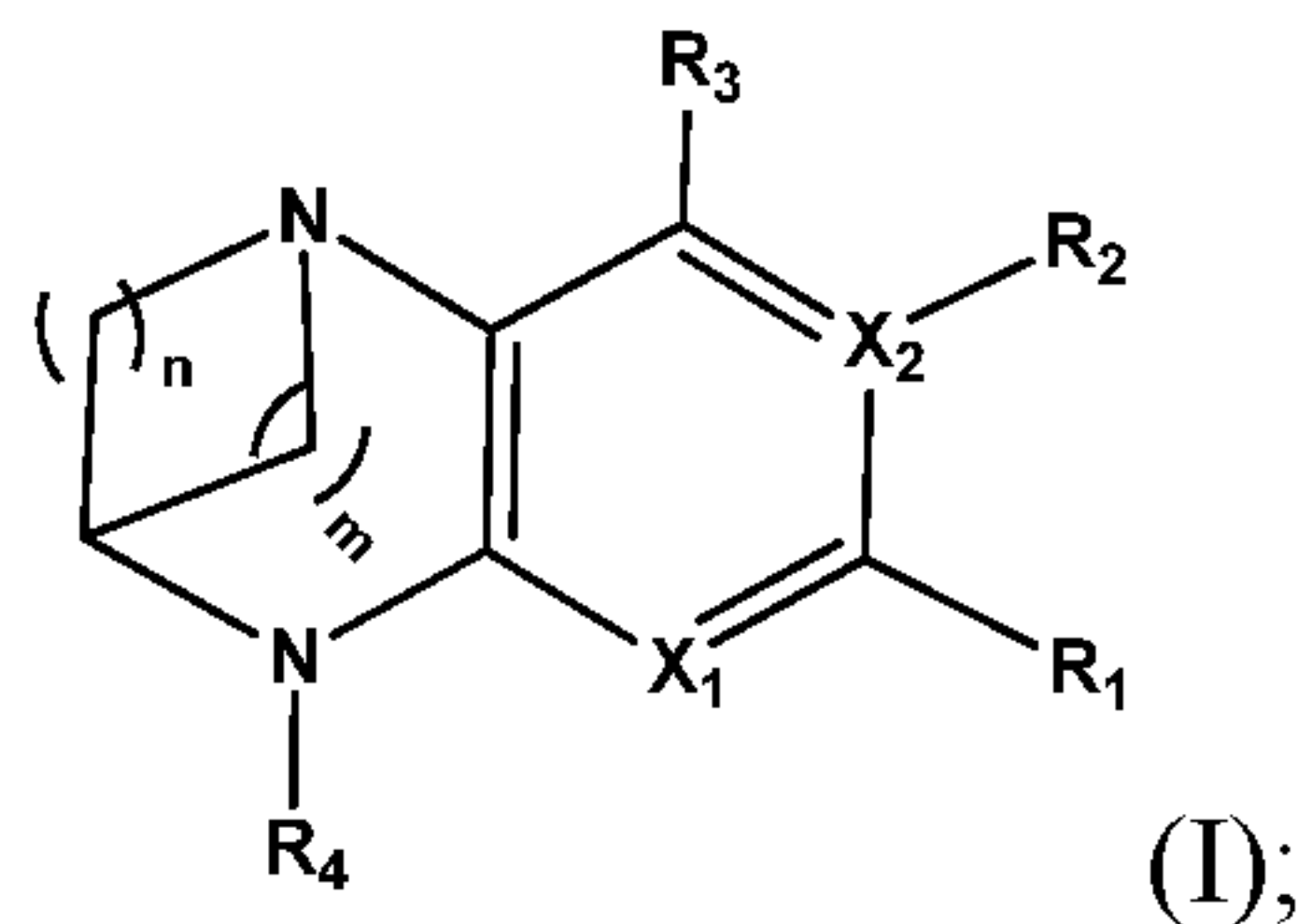
International Patent Application No. WO09/061879, International Filing Date: 13 May 2014 discloses novel sirtuin-modulating substituted bridged urea and related analogs compounds of Formula (I):



a pharmaceutically acceptable salt thereof, corresponding pharmaceutical compositions, combinations with other therapeutic agents, methods for making and methods or uses for increasing the lifespan of a cell, and treating and/or preventing a wide variety of diseases and disorders including, for example, diseases or disorders related to aging or stress, diabetes, obesity, neurodegenerative diseases, cardiovascular disease, blood clotting disorders, inflammation, cancer, and/or flushing as well as diseases or disorders that would benefit from increased mitochondrial activity.

In one aspect, the present invention provides novel sirtuin-modulating compounds of Structural Formulas (I) to (VI), respectively corresponding analogs (i.e., with hydrogen substitution at the R² position) as are described in detail below.

In one aspect, the present invention relates to a compound of Formula (I):



where:

X_1 or X_2 independently is selected from -N or -C;

R¹ is hydrogen, halogen, -CN, carbocyclyl, heterocyclyl, -N-substituted heterocyclyl, aryl, heteroaryl, -C(O)R_a or -C(O)-NR_bR_c;

R² is halogen, -straight or branched C₁-C₆ alkyl, -straight or branched-C₁-C₆ haloalkyl, or -C(O)-NR_bR_c;

R³ is hydrogen, halogen, -hydroxy, -straight or branched C₁-C₆ alkyl, or -straight or branched-C₁-C₆ haloalkyl;

R^4 is hydrogen or $-C(O)NR_bR_c$;

where:

when X_2 is -N, R_2 is non-existent; or

when X_2 is -C, R_2 is as defined above;

each R^1 , R^2 , R^3 or R^4 as defined above optionally is further substituted with one or more substituents selected from hydrogen, halogen, -OH, $-(CH_2)_xOH$, $-C\equiv N$, $-NR_dR_e$, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl, -straight or branched C_1 - C_6 alkoxy, -straight or branched C_1 - C_6 haloalkoxy, -O-straight or branched- C_1 - C_6 haloalkyl, $-C_1$ - C_6 cycloalkyl, $-(CH_2)_x$ -cycloalkyl, heterocyclyl, aryl, -heteroaryl, $-(CH_2)_x$ -heteroaryl, $-O-(CH_2)_xCH(OH)CH_2(OH)$, or $-C(O)OR_f$;

each R_a, R_b, R_c, R_d, R_e, or R_f as defined above independently is selected from hydrogen, -straight or branched C₁-C₆ alkyl, -straight or branched-C₁-C₆ haloalkyl, -C₁-C₆-cycloalkyl, -(CH₂)_xC₁-C₆-cycloalkyl, heterocyclyl, -N-heterocyclyl, aryl, heteroaryl, or -(CH₂)_xheteroaryl, -(CHR_g)_xheteroaryl;

where:

R_g is -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl;

each R_a , R_b , R_c , R_d , R_e , or R_f as defined above optionally is further substituted with one or more substituents selected from hydrogen, halogen, -OH, -C $\equiv$ N, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl, -straight or branched C_1 - C_6 alkoxy, -O-straight or branched- C_1 - C_6 haloalkyl, - C_1 - C_6 cycloalkyl, carbocyclyl, -(CH₂)_x-carbocyclyl, -heterocyclyl, -O-heterocyclyl aryl, -heteroaryl, -(CH₂)_x-heteroaryl, -O-(CH₂)_xCH(OH)CH₂(OH), -(CH₂)_x-OH, or -C(O)-OH;

10 m is an integer from 1 to 3;

n is an integer selected from 1 to 3;

x is 0 or an integer from 1 to 6; or

a pharmaceutically salt thereof.

In another aspect, the present invention relates to a compound of the present invention as defined above (i.e., compounds of Structural Formulas (I) to (VI), respectively corresponding analogs (i.e., with hydrogen substitution at the R^2 position) and throughout the instant application, where it is provided that:

when $n = 1$, $m \neq 1$; and

when $n = 3$, $m \neq 3$.

20 In another aspect, the present invention relates to a compound of the present invention, where R^2 is C(O)-NR_bR_c; wherein R_b and R_c are as defined above and throughout the present application.

In another aspect, the present invention relates to a compound of Formulas (I) to (VI), where:

25 m is 1;

n is 2 or 3; and

R^4 is hydrogen.

In another aspect, the present invention relates to a compound of Formulas (I) to (VI), where:

30 m is 1;

n is 2 or 3; and

R^4 is -C(O)NR_bR_c, wherein each R_b and R_c is as defined above.

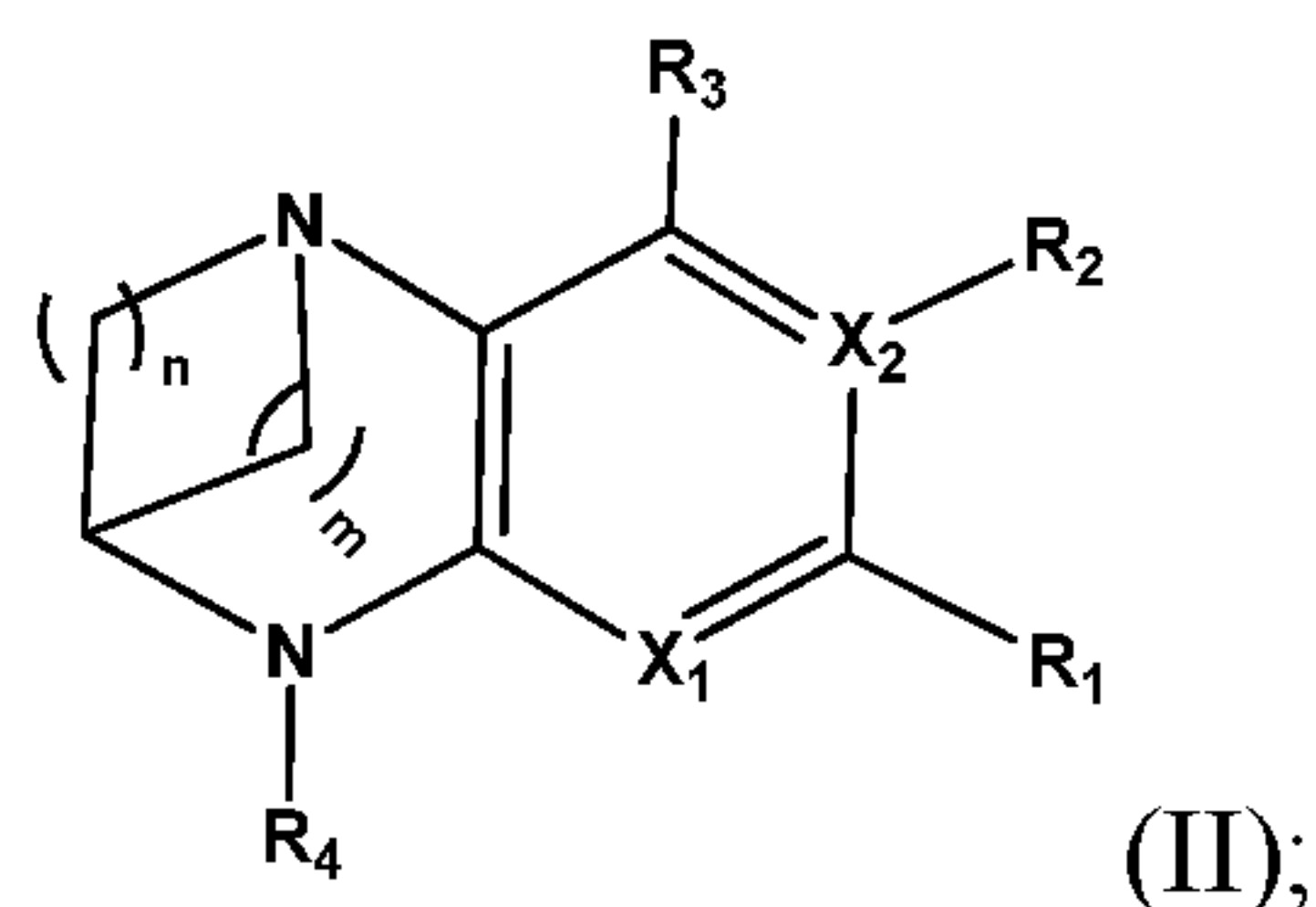
In another aspect, the present invention relates to a compound of Formulas (I) to (VI), where:

m is 1;

n is 3; and

5 R^4 is R^4 is hydrogen or $-C(O)NR_bR_c$, wherein R_b and R_c is as defined above in claim 1.

In one aspect, the present invention relates to a compound of Formula (II):



where:

10 X_1 or X_2 independently is selected from -N or -C;

R^1 is hydrogen, halogen, -CN, carbocyclyl, heterocyclyl, -N-substituted heterocyclyl, aryl or heteroaryl.

R^2 is halogen, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl, or $-C(O)NR_bR_c$;

15 R^3 is hydrogen, halogen, -hydroxy, -straight or branched C_1 - C_6 alkyl, or -straight or branched- C_1 - C_6 haloalkyl;

R^4 is hydrogen or $-C(O)NR_bR_c$;

where:

when X_2 is -N, R_2 is non-existent; or

20 when X_2 is -C, R_2 is as defined above;

each R^1 , R^2 , R^3 or R^4 as defined above optionally is further substituted with one or more substituents selected from hydrogen, halogen, -OH, $-(CH_2)_xOH$, $-C\equiv N$, $-NR_dR_e$, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl, -straight or branched C_1 - C_6 alkoxy, -straight or branched C_1 - C_6 haloalkoxy, -O-straight or branched- C_1 - C_6 haloalkyl, $-C_1$ - C_6 cycloalkyl, $-(CH_2)_x$ -cycloalkyl, heterocyclyl, aryl, -heteroaryl, $-(CH_2)_x$ -heteroaryl, $-O-(CH_2)_xCH(OH)CH_2(OH)$, or $-C(O)OR_f$;

each R_a , R_b , R_c , R_d , R_e , or R_f as defined above independently is selected from hydrogen, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6

haloalkyl, -C₁-C₆-cycloalkyl, -(CH₂)_xC₁-C₆-cycloalkyl, heterocyclyl, -N-heterocyclyl, aryl, heteroaryl, or -(CH₂)_xheteroaryl, -(CHR_g)_xheteroaryl;

where:

R_g is -straight or branched C₁-C₆ alkyl, -straight or branched-C₁-C₆ haloalkyl;

each R_a, R_b, R_c, R_d, R_e, or R_f as defined above optionally is further substituted with one or more substituents selected from hydrogen, halogen, -OH, -C≡N, -straight or branched C₁-C₆ alkyl, -straight or branched-C₁-C₆ haloalkyl, -straight or branched C₁-C₆ alkoxy, -O-straight or branched-C₁-C₆ haloalkyl, -C₁-C₆ cycloalkyl, carbocyclyl, -(CH₂)_x-carbocyclyl, -heterocyclyl, -O-heterocyclyl aryl, -heteroaryl, -(CH₂)_x-heteroaryl, -O-(CH₂)_xCH(OH)CH₂(OH), -(CH₂)_x-OH, or -C(O)-OH;

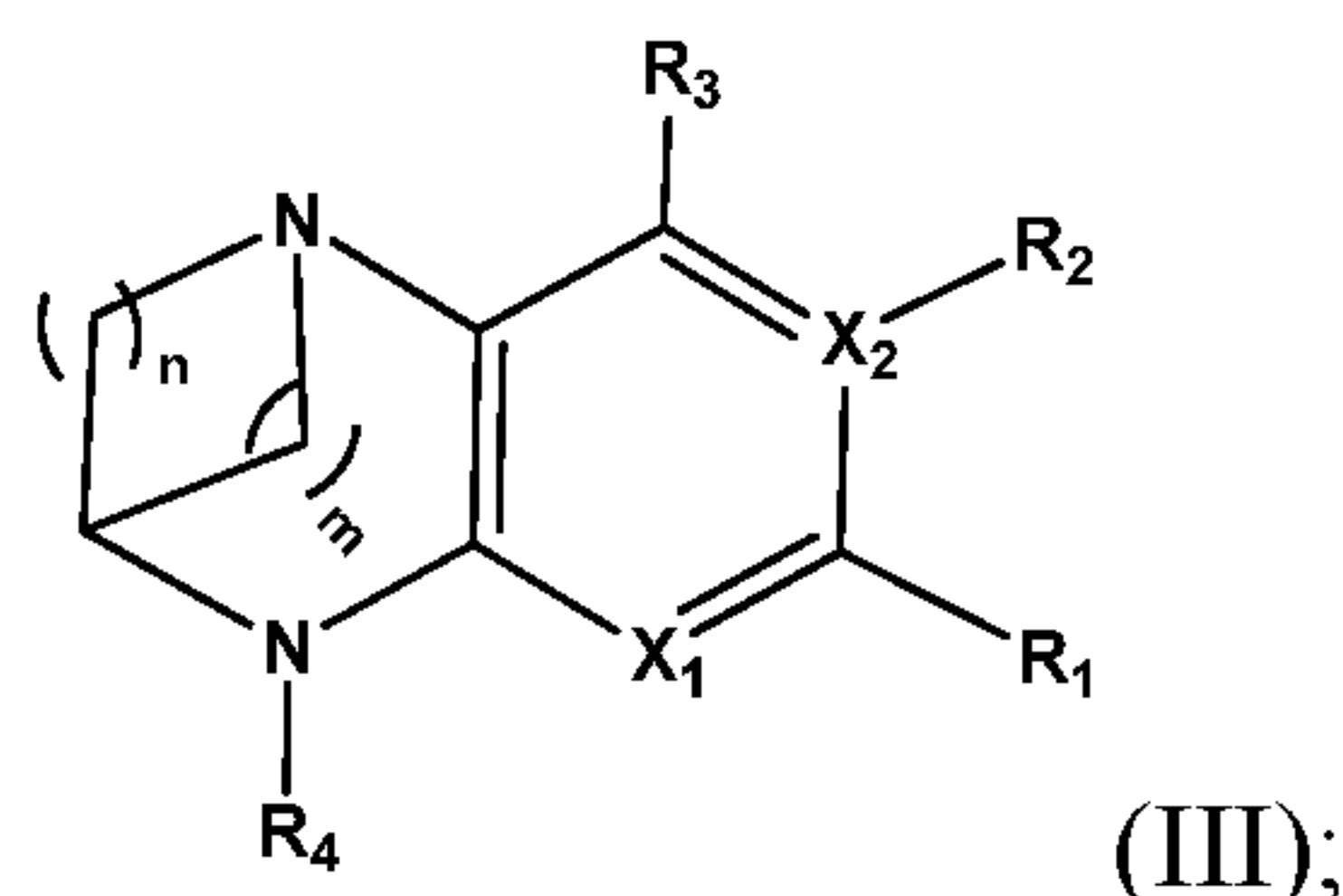
m is an integer from 1 to 3;

n is an integer selected from 1 to 3;

x is 0 or an integer from 1 to 6; or

a pharmaceutically salt thereof.

In one aspect, the present invention relates to a compound of Formula (III):



where:

X₁ or X₂ independently is selected from -N or -C;

R¹ is -C(O)R_a or -C(O)-NR_bR_c;

R² is halogen, -straight or branched C₁-C₆ alkyl, -straight or branched-C₁-C₆ haloalkyl, or -C(O)-NR_bR_c;

R³ is hydrogen, halogen, -hydroxy, -straight or branched C₁-C₆ alkyl, or -straight or branched-C₁-C₆ haloalkyl;

R⁴ is hydrogen or -C(O)NR_bR_c;

where:

when X₂ is -N, R₂ is non-existent; or

when X₂ is -C, R₂ is as defined above;

each R^1 , R^2 , R^3 or R^4 as defined above optionally is further substituted with one or more substituents selected from hydrogen, halogen, -OH, $-(CH_2)_xOH$, $-C\equiv N$, $-NR_dR_e$, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl, -straight or branched C_1 - C_6 alkoxy, -straight or branched C_1 - C_6 haloalkoxy, -O-straight or branched- C_1 - C_6 haloalkyl, $-C_1$ - C_6 cycloalkyl, $-(CH_2)_x$ -cycloalkyl, heterocyclyl, aryl, -heteroaryl, $-(CH_2)_x$ -heteroaryl, $-O-(CH_2)_xCH(OH)CH_2(OH)$, or $-C(O)OR_f$;

each R_a , R_b , R_c , R_d , R_e , or R_f as defined above independently is selected from hydrogen, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl, $-C_1$ - C_6 -cycloalkyl, $-(CH_2)_xC_1$ - C_6 -cycloalkyl, heterocyclyl, -N-heterocyclyl, aryl, heteroaryl, or $-(CH_2)_x$ -heteroaryl, $-(CHR_g)_x$ -heteroaryl;

where:

R_g is -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl;

each R_a , R_b , R_c , R_d , R_e , or R_f as defined above optionally is further substituted with one or more substituents selected from hydrogen, halogen, -OH, $-C\equiv N$, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl, -straight or branched C_1 - C_6 alkoxy, -O-straight or branched- C_1 - C_6 haloalkyl, $-C_1$ - C_6 cycloalkyl, carbocyclyl, $-(CH_2)_x$ -carbocyclyl, -heterocyclyl, -O-heterocyclyl aryl, -heteroaryl, $-(CH_2)_x$ -heteroaryl, -O- $(CH_2)_xCH(OH)CH_2(OH)$, $-(CH_2)_x$ -OH, or $-C(O)-OH$;

m is an integer from 1 to 3;

n is an integer selected from 1 to 3;

x is 0 or an integer from 1 to 6; or

a pharmaceutically salt thereof.

In another aspect, the present invention relates to a compound of the present invention as defined above (i.e., compounds of Structural Formulas (I) to (VI), respectively corresponding analogs (i.e., with hydrogen substitution at the R^2 position) and throughout the instant application, where it is provided that:

when $n = 1$, $m \neq 1$; and

when $n = 3$, $m \neq 3$.

In another aspect, the present invention relates to a compound of the present invention as described herein, where R^2 is $C(O)-NR_bR_c$; wherein R_b and R_c are as defined above and throughout the present application.

In another aspect, the present invention relates to a compound of Formulas (I) to (VI) or any compound as described herein, where:

m is 1;

n is 2 or 3; and

R^4 is hydrogen.

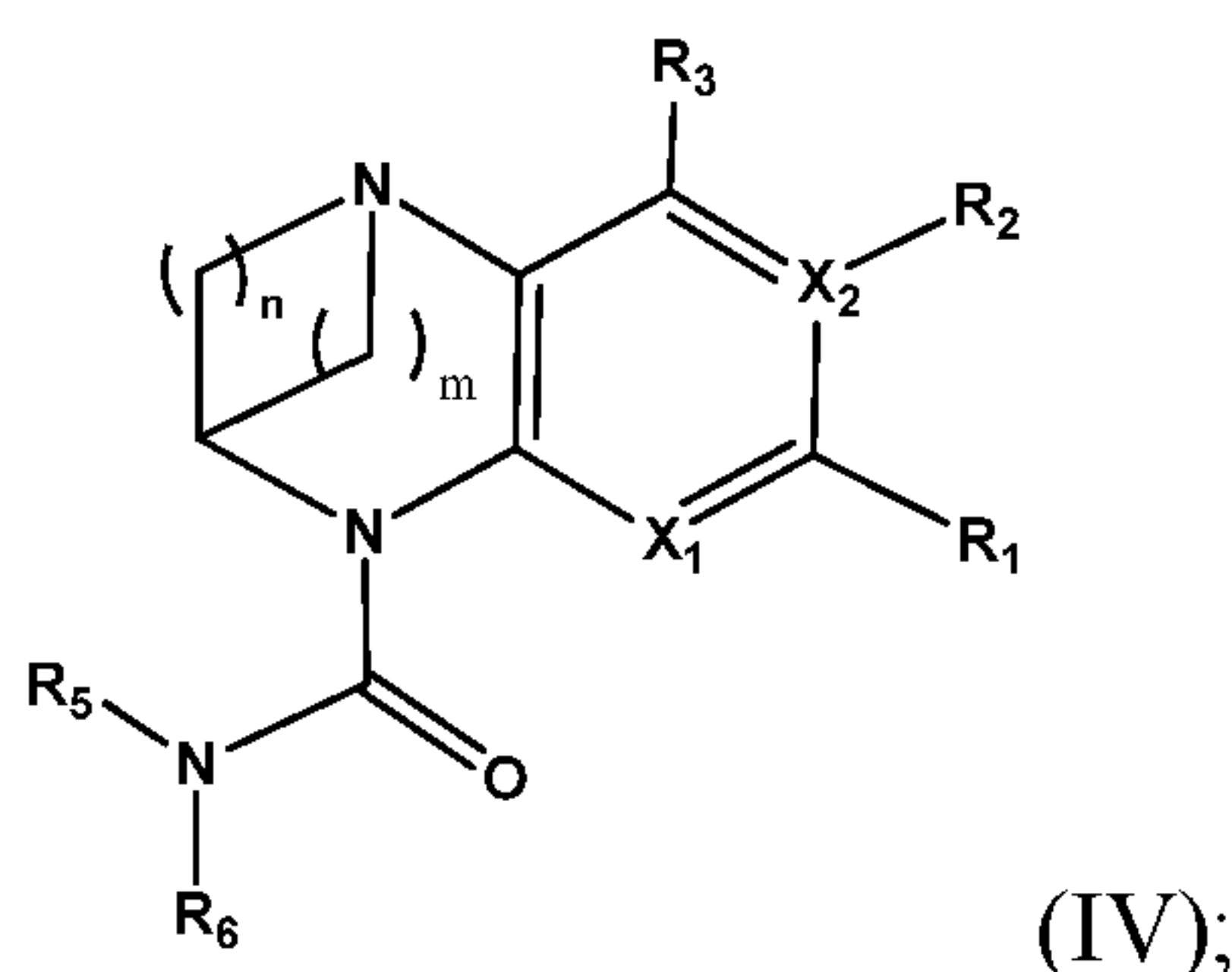
In another aspect, the present invention relates to a compound of Formulas (I) to (VI), where:

m is 1;

n is 2 or 3; and

R^4 is $-C(O)NR_bR_c$, wherein each R_b and R_c is as defined above.

In another aspect, the present invention relates to a compound of Formula (IV):



where:

X_1 or X_2 independently is selected from -N or -C;

where:

when X_2 is -N, R_2 is non-existent; or

when X_2 is -C, R_2 is as defined above;

R^1 is hydrogen, halogen, -CN, carbocyclyl, heterocyclyl, -N-substituted heterocyclyl, aryl, heteroaryl, $-C(O)R_a$ or $-C(O)-NR_bR_c$;

R^2 is halogen, -straight or branched C_1-C_6 alkyl, -straight or branched- C_1-C_6 haloalkyl, or $-C(O)-NR_bR_c$;

R^3 is hydrogen, halogen, -hydroxy, -straight or branched C_1-C_6 alkyl, or -straight or branched- C_1-C_6 haloalkyl;

each R^5 and R^6 independently is selected from hydrogen, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl, - C_1 - C_6 cycloalkyl, $-(CH_2)_xC_1$ - C_6 cycloalkyl, heterocyclyl, -N-heterocyclyl, aryl, heteroaryl, or $-(CH_2)_x$ heteroaryl, $-(CHR_g)_x$ heteroaryl; wherein:

5 each R^1 , R^2 , R^3 , R^5 and R^6 as defined above optionally is further substituted with one or more substituents selected from hydrogen, halogen, -OH, $-(CH_2)_xOH$, $-C\equiv N$, $-NR_dR_e$, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl, -straight or branched C_1 - C_6 alkoxy, -straight or branched C_1 - C_6 haloalkoxy, -O-straight or branched- C_1 - C_6 haloalkyl, - C_1 - C_6 cycloalkyl, $-(CH_2)_x$ -
10 cycloalkyl, heterocyclyl, aryl, -heteroaryl, $-(CH_2)_x$ -heteroaryl, -O- $(CH_2)_xCH(OH)CH_2(OH)$, or $-C(O)OR_f$;

each R_a , R_b , R_c , R_d , R_e , R_f or R_g as defined above independently is selected from hydrogen, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl, - C_1 - C_6 -cycloalkyl, $-(CH_2)_xC_1$ - C_6 -cycloalkyl, heterocyclyl, -N-
15 heterocyclyl, aryl, heteroaryl, or $-(CH_2)_x$ heteroaryl;

where:

each R_a , R_b , R_c , R_d , R_e , R_f or R_g as defined above optionally is further substituted with one or more substituents selected from hydrogen, halogen, -OH, $-(CH_2)_xOH$, $-C\equiv N$, NR_hR_i , -straight or branched C_1 - C_6 alkyl, -straight
20 or branched- C_1 - C_6 haloalkyl, -straight or branched C_1 - C_6 alkoxy, -straight or branched- C_1 - C_6 haloalkoxy, - C_1 - C_6 cycloalkyl, $-(CH_2)_x$ -cycloalkyl, heterocyclyl, -heterocyclyl, -O-heterocyclyl, aryl, -heteroaryl, $-(CH_2)_x$ -heteroaryl, -O- $(CH_2)_xCH(OH)CH_2(OH)$, $-(CH_2)_x-OH$, or $-C(O)OR_j$;

where:

25 each R_h , R_i and R_j independently is selected from hydrogen, -straight or branched C_1 - C_6 alkyl or -straight or branched- C_1 - C_6 haloalkyl;

m is an integer from 1 to 3;

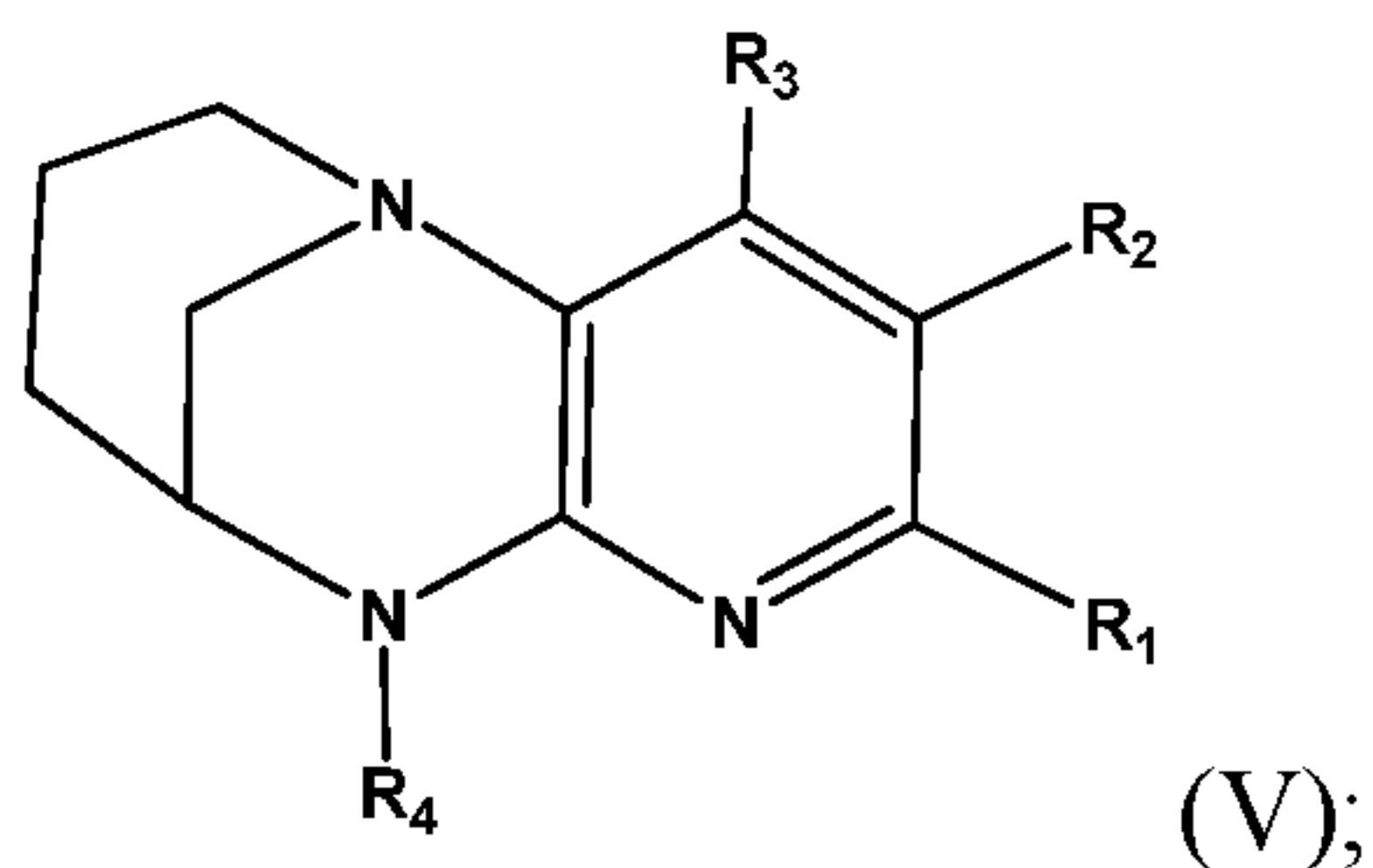
n is an integer selected from 2 to 3;

x is 0 or an integer from 1 to 6; or

30 a pharmaceutically salt thereof.

In another aspect, the present invention relates to a compound of the present invention, where n is 2 or 3 and m is 1.

In another aspect, the present invention relates to a compound of Formula (V):



where:

R^1 is hydrogen, halogen, -CN, carbocyclyl, heterocyclyl, -N-substituted heterocyclyl, aryl, heteroaryl, -C(O) R_a or -C(O)-NR $_b$ R $_c$;

R^2 halogen, -straight or branched C₁-C₆ alkyl, -straight or branched-C₁-C₆ haloalkyl, or -C(O)-NR $_b$ R $_c$;

R^3 is hydrogen, halogen, -hydroxy, -straight or branched C₁-C₆ alkyl, or -straight or branched-C₁-C₆ haloalkyl;

R^4 is hydrogen or -C(O)-NR $_b$ R $_c$;

where:

each R^1 , R^2 , R^3 or R^4 as defined above optionally is further substituted with one or more substituents selected from hydrogen, halogen, -OH, -(CH₂)_xOH, -C≡N, -NR $_d$ R $_e$, -straight or branched C₁-C₆ alkyl, -straight or branched-C₁-C₆ haloalkyl, -straight or branched C₁-C₆ alkoxy, -straight or branched C₁-C₆ haloalkoxy, -O-straight or branched-C₁-C₆ haloalkyl, -C₁-C₆ cycloalkyl, -(CH₂)_x-cycloalkyl, heterocyclyl, aryl, -heteroaryl, -(CH₂)_x-heteroaryl, -O-(CH₂)_xCH(OH)CH₂(OH), or -C(O)OR $_f$;

each R_a , R_b , R_c , R_d , R_e , or R_f as defined above independently is selected from hydrogen, -straight or branched C₁-C₆ alkyl, -straight or branched-C₁-C₆ haloalkyl, -C₁-C₆-cycloalkyl, -(CH₂)_xC₁-C₆-cycloalkyl, heterocyclyl, -N-heterocyclyl, aryl, heteroaryl, or -(CH₂)_xheteroaryl;

where:

each R_a , R_b , R_c , R_d , R_e , or R_f as defined above optionally is further substituted with one or more substituents selected from hydrogen, halogen, -OH, -(CH₂)_xOH, -C≡N, -NR $_g$ R $_h$, -straight or branched C₁-C₆ alkyl, -straight or branched-C₁-C₆ haloalkyl, -straight or branched C₁-C₆ alkoxy, -straight or branched-C₁-C₆ haloalkoxy, -C₁-C₆ cycloalkyl, -(CH₂)_x-cycloalkyl, heterocyclyl, -heterocyclyl, -O-heterocyclyl, aryl, -heteroaryl, -(CH₂)_x-heteroaryl, -O-(CH₂)_xCH(OH)CH₂(OH), -(CH₂)_x-OH, or -C(O)OR $_i$;

wherein:

each R_g , R_h and R_i as defined above independently is selected from hydrogen, -straight or branched C_1 - C_6 alkyl or -straight or branched- C_1 - C_6 haloalkyl;

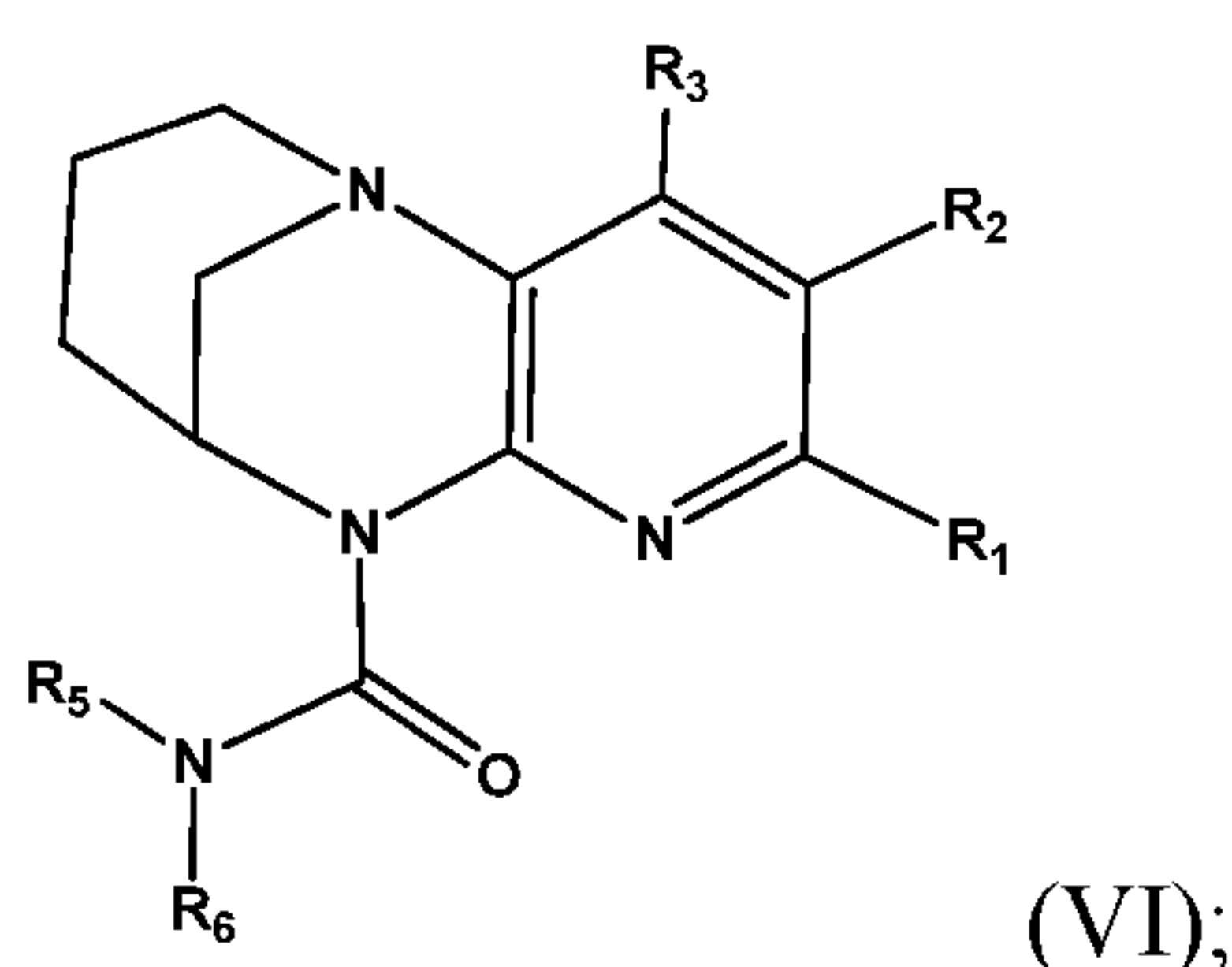
5 m is an integer from 1 to 3;

n is an integer selected from 2 to 3;

x is 0 or an integer from 1 to 6; or

a pharmaceutically salt thereof;

10 In another aspect, the present invention relates to a compound of Formula (IV):



where:

R^1 is hydrogen, halogen, -CN, carbocyclyl, heterocyclyl, -N-substituted heterocyclyl, aryl, heteroaryl, -C(O) R_a or -C(O)-NR $_b$ R $_c$;

15 R^2 halogen, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl, or -C(O)-NR $_b$ R $_c$;

R^3 is hydrogen, halogen, -hydroxy, -straight or branched C_1 - C_6 alkyl, or -straight or branched- C_1 - C_6 haloalkyl;

each R^5 and R^6 independently is selected from hydrogen, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl, - C_1 - C_6 cycloalkyl, -(CH $_2$) $_x$ C_1 - C_6 cycloalkyl, heterocyclyl, -N-heterocyclyl, aryl, heteroaryl, or -(CH $_2$) $_x$ heteroaryl, -(CHR $_g$) $_x$ heteroaryl;

where:

each R^1 , R^2 , R^3 , R^5 and R^6 as defined above optionally is further substituted with one or more substituents selected from hydrogen, halogen, -OH, -(CH $_2$) $_x$ OH, -C $\equiv$ N, -NR $_d$ R $_e$, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl, -straight or branched C_1 - C_6 alkoxy, -straight or branched C_1 - C_6 haloalkoxy, -O-straight or branched- C_1 - C_6 haloalkyl, - C_1 - C_6 cycloalkyl, -(CH $_2$) $_x$ -cycloalkyl, heterocyclyl, aryl, -heteroaryl, -(CH $_2$) $_x$ -heteroaryl, -O-(CH $_2$) $_x$ CH(OH)CH $_2$ (OH), or -C(O)OR $_f$;

each R_a , R_b , R_c , R_d , R_e , R_f or R_g as defined above independently is selected from hydrogen, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl, - C_1 - C_6 -cycloalkyl, $-(CH_2)_x$ - C_1 - C_6 -cycloalkyl, heterocyclyl, -N-heterocyclyl, aryl, heteroaryl, or $-(CH_2)_x$ -heteroaryl;

5 where:

each R_a , R_b , R_c , R_d , R_e , R_f or R_g as defined above optionally is further substituted with one or more substituents selected from hydrogen, halogen, -OH, $-(CH_2)_x$ -OH, $-C\equiv N$, NR_hR_i , -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl, -straight or branched C_1 - C_6 alkoxy, -straight or branched- C_1 - C_6 haloalkoxy, - C_1 - C_6 cycloalkyl, $-(CH_2)_x$ -cycloalkyl, heterocyclyl, -heterocyclyl, -O-heterocyclyl, aryl, -heteroaryl, $-(CH_2)_x$ -heteroaryl, -O- $-(CH_2)_x$ -CH(OH)CH₂(OH), $-(CH_2)_x$ -OH, or $-C(O)OR_j$;

where:

each R_h , R_i and R_j independently is selected from hydrogen, -straight or branched C_1 - C_6 alkyl or -straight or branched- C_1 - C_6 haloalkyl;

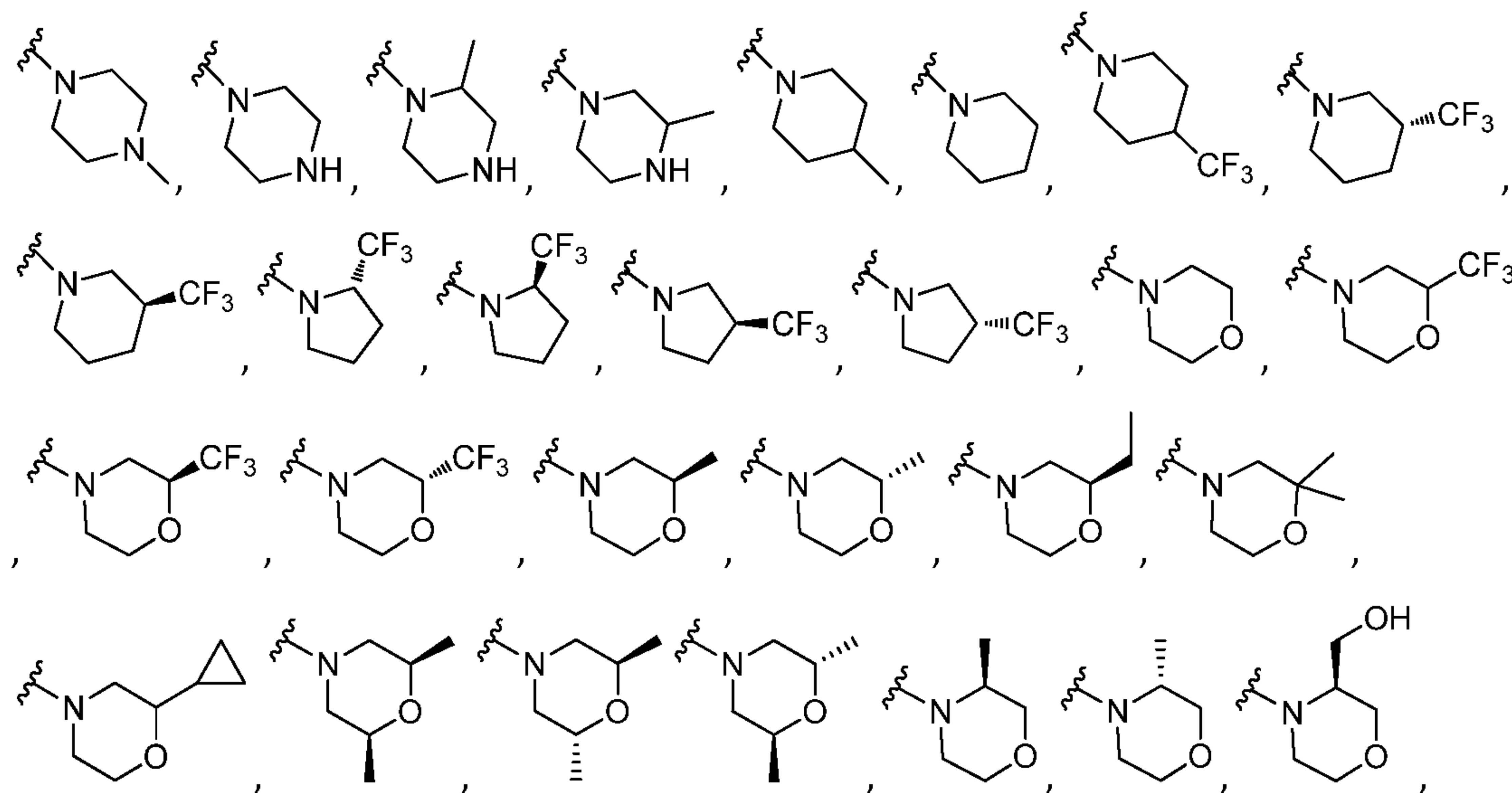
m is an integer from 1 to 3;

n is an integer selected from 2 to 3;

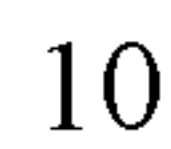
x is 0 or an integer from 1 to 6; or

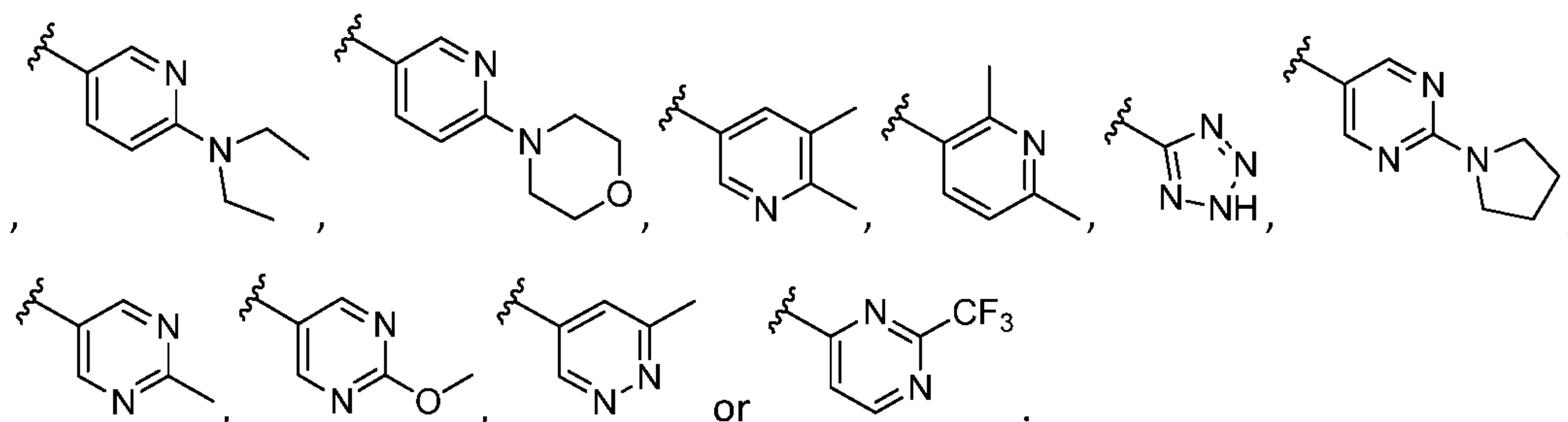
a pharmaceutically salt thereof;

20 In another aspect, the present invention relates to compounds of Formulas (I) to (IV), respectively, wherein R^1 is selected from:

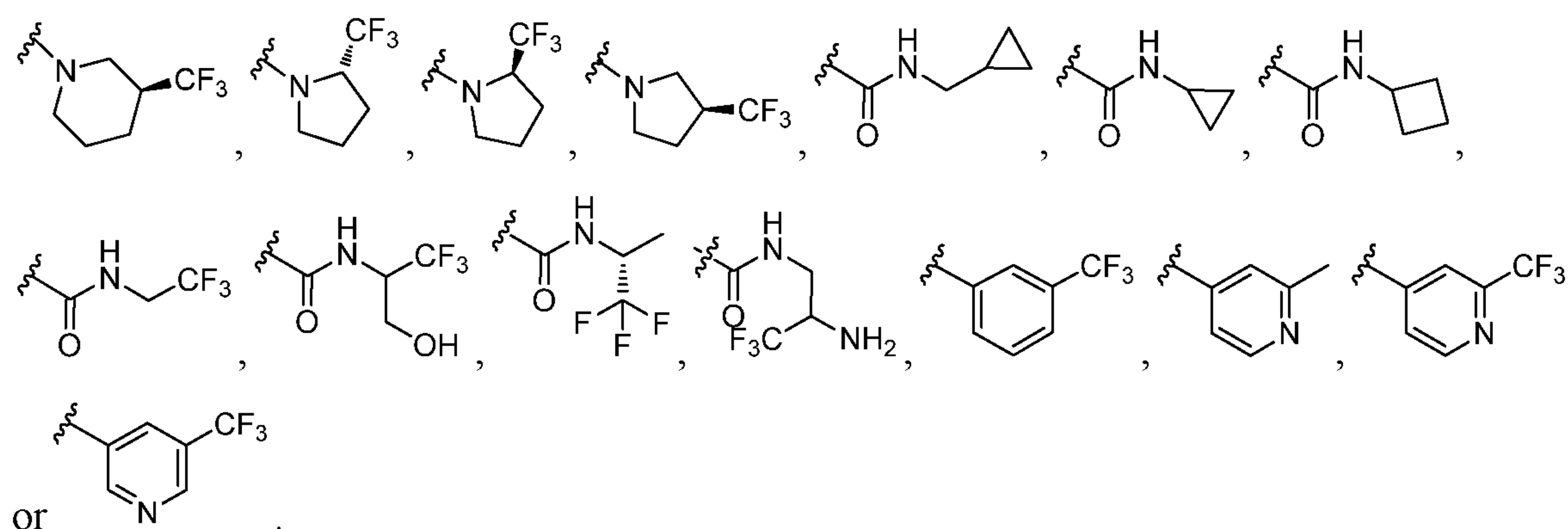


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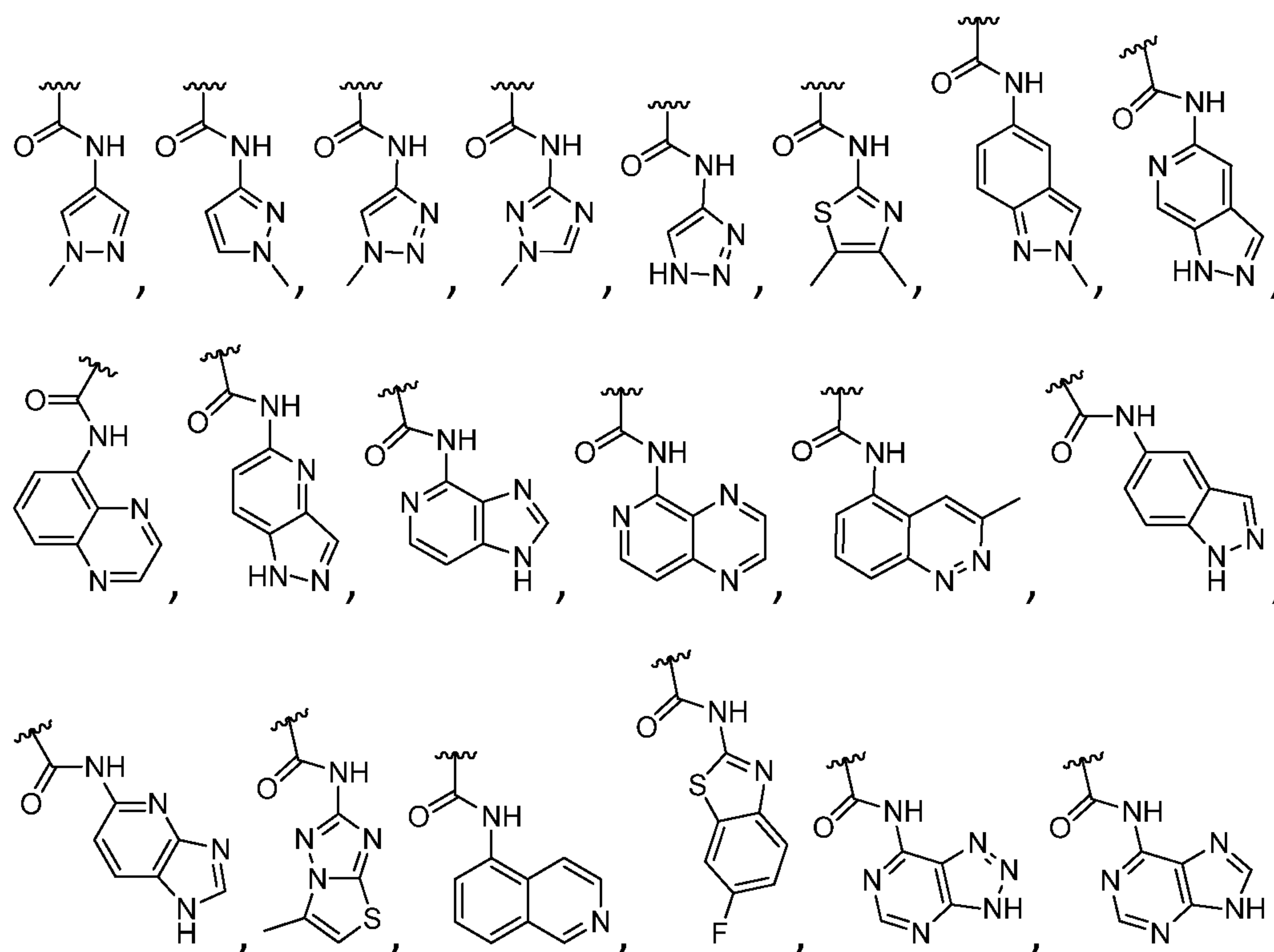




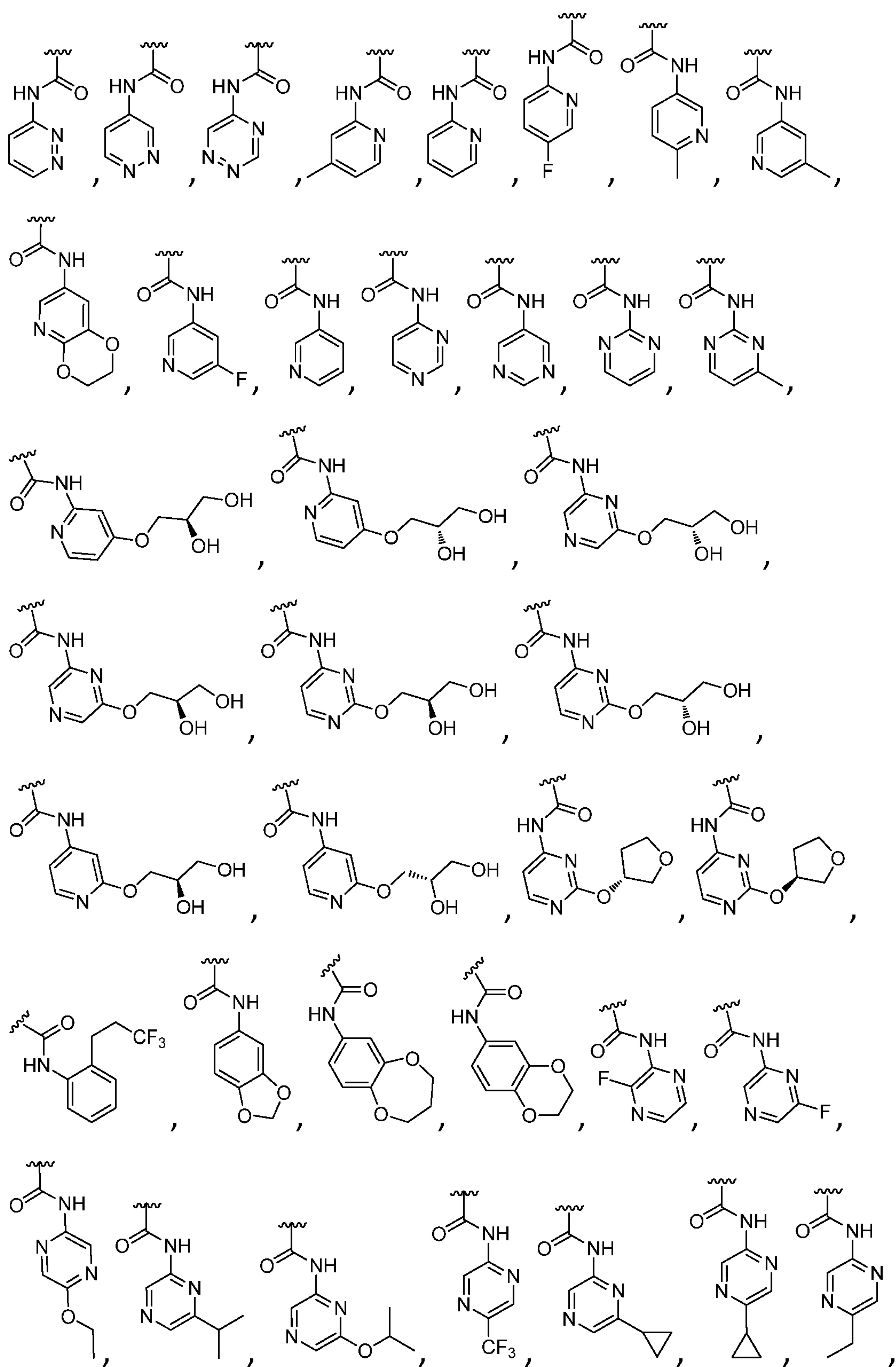
In another aspect, the present invention relates to compound(s) of Formulas (I) to
5 (IV), respectively, R1 is



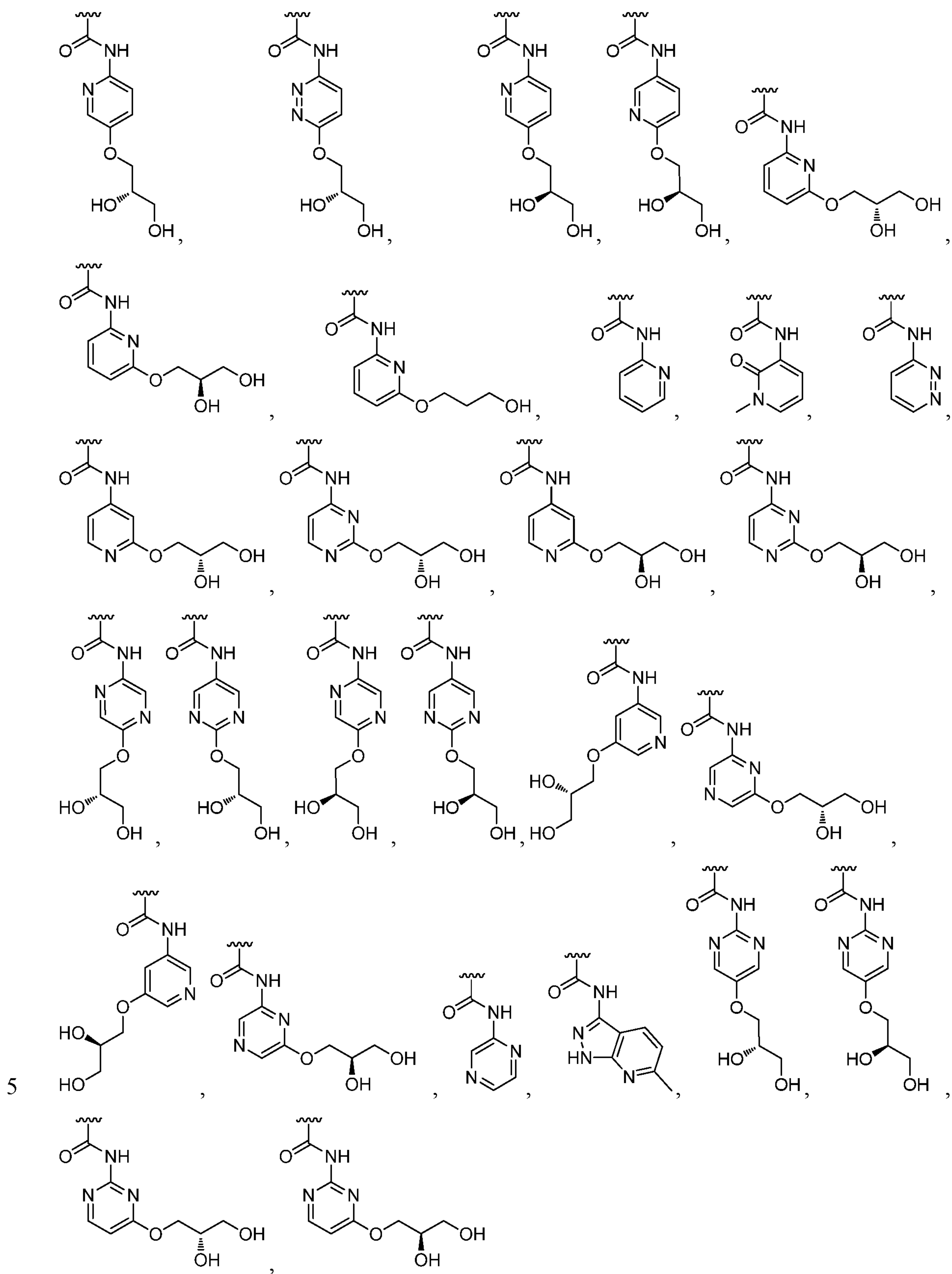
In another aspect, the present invention relates to compound(s) of Formulas (I) to (IV), respectively, where R⁴ is selected from:



5



[illegible]

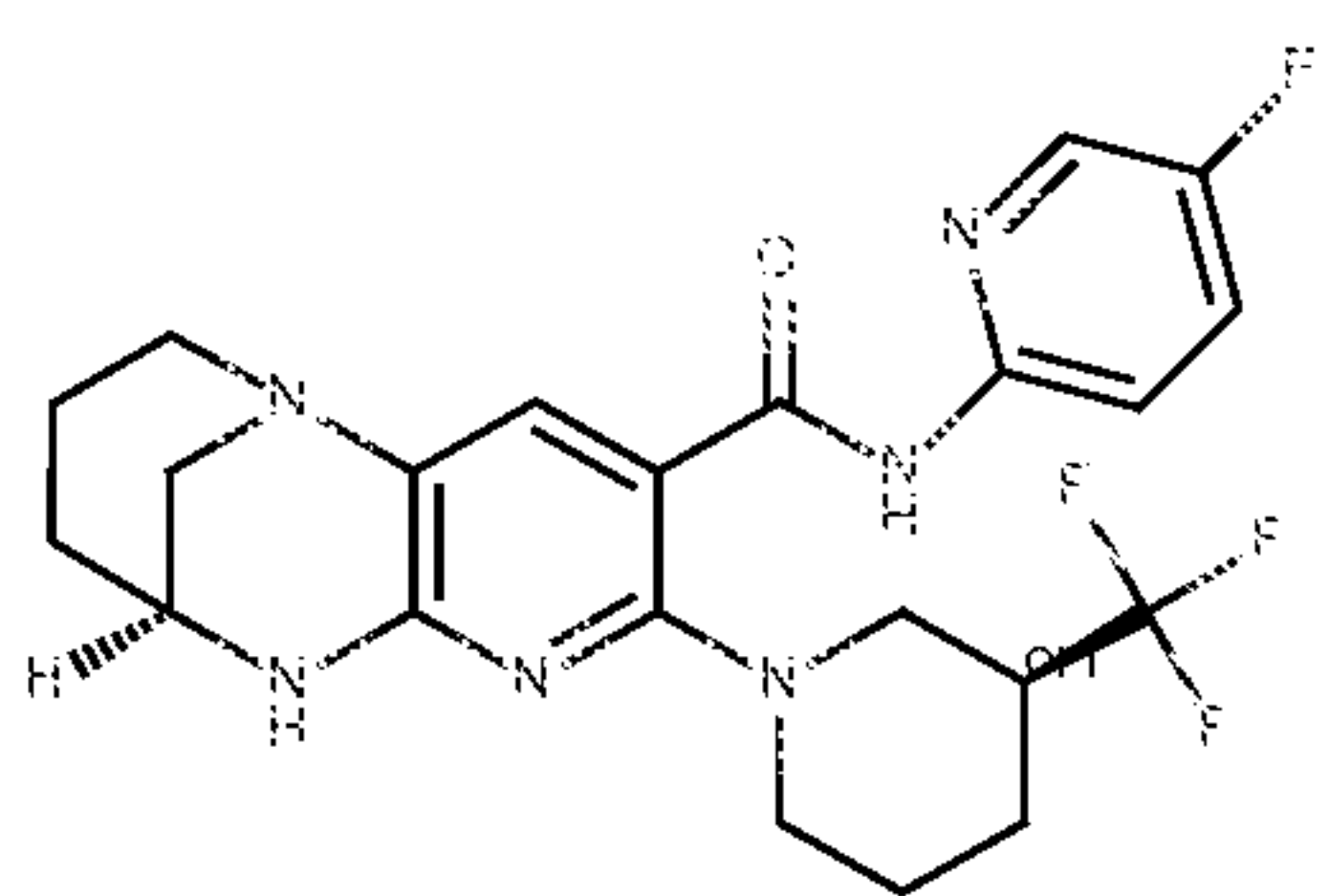
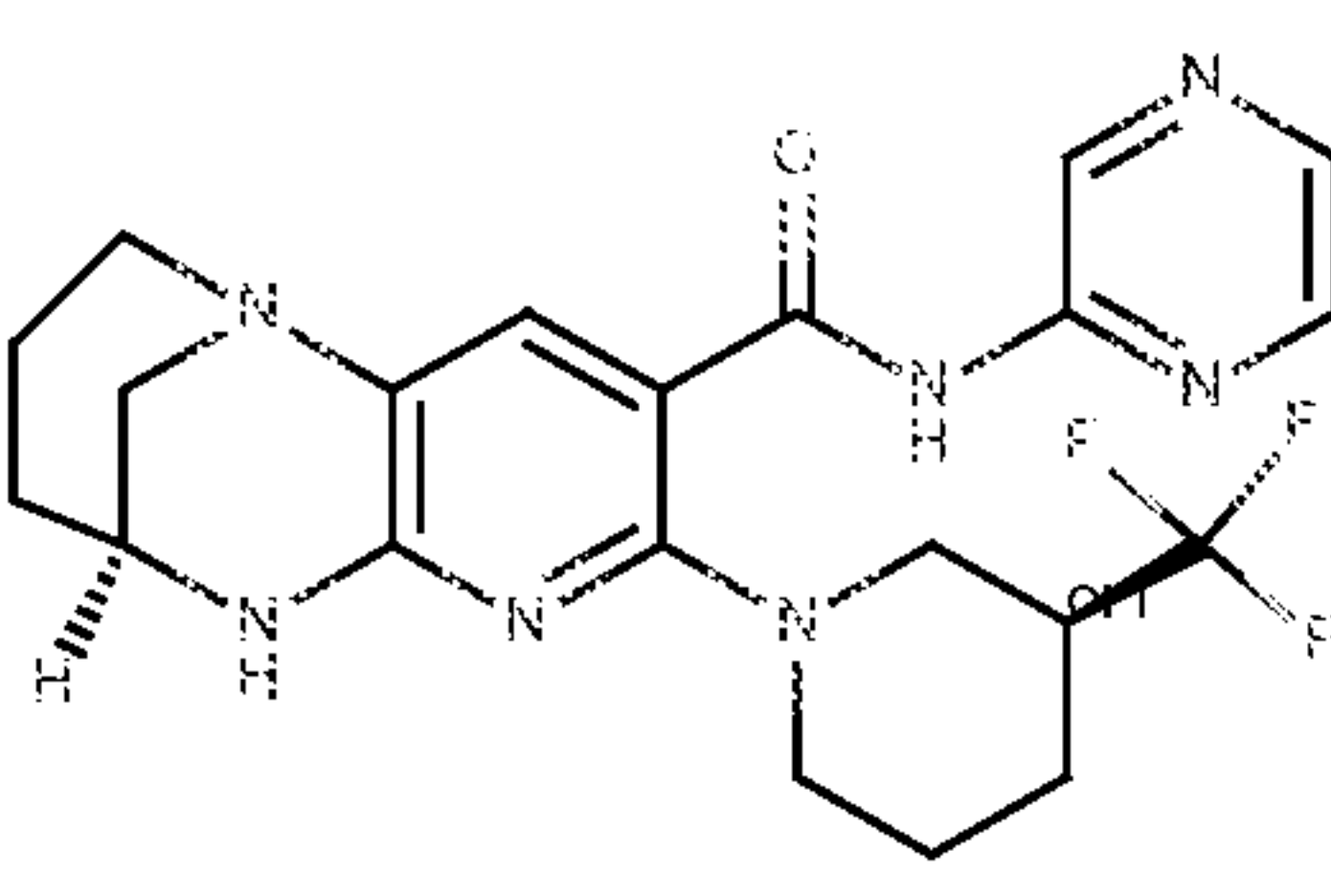
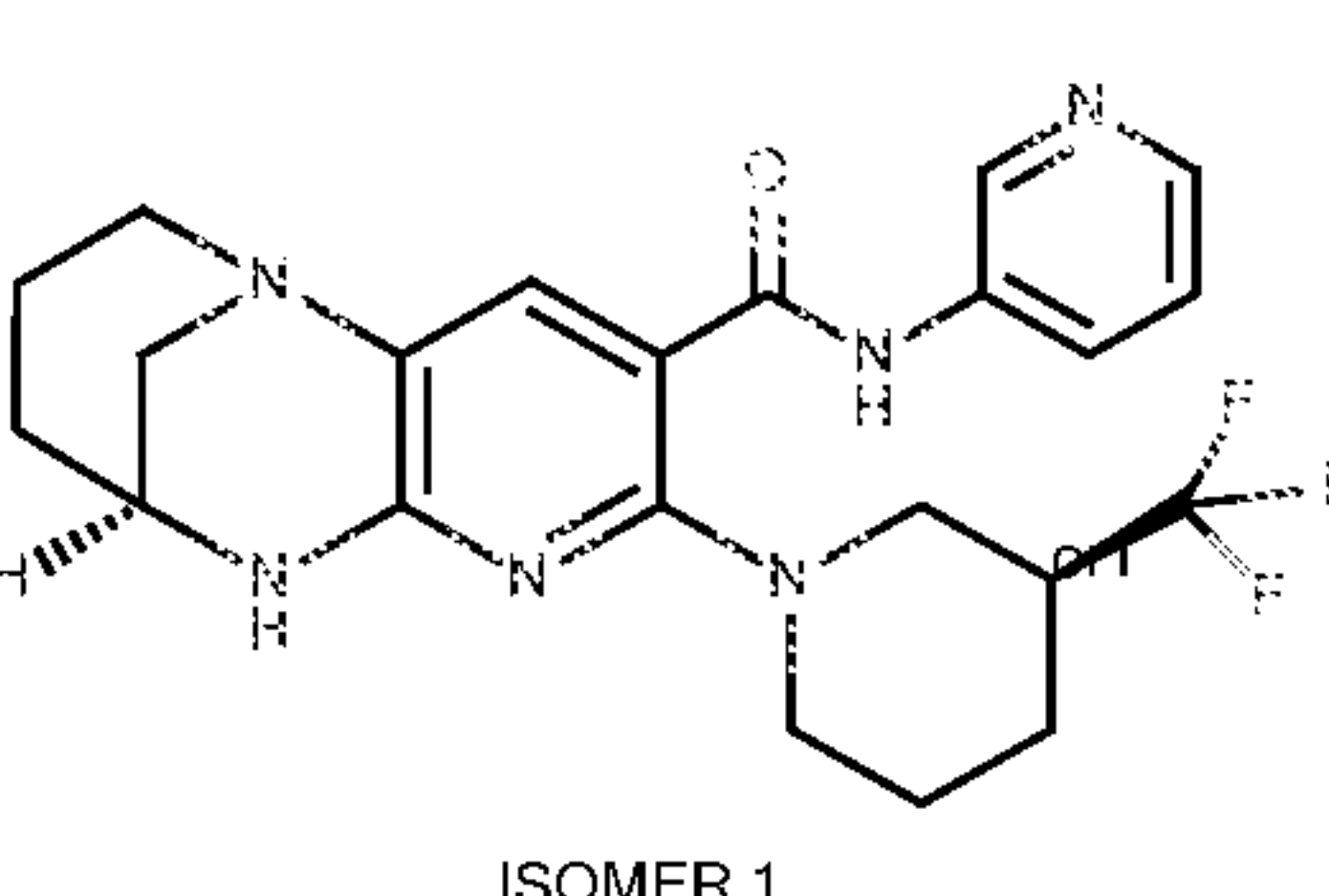
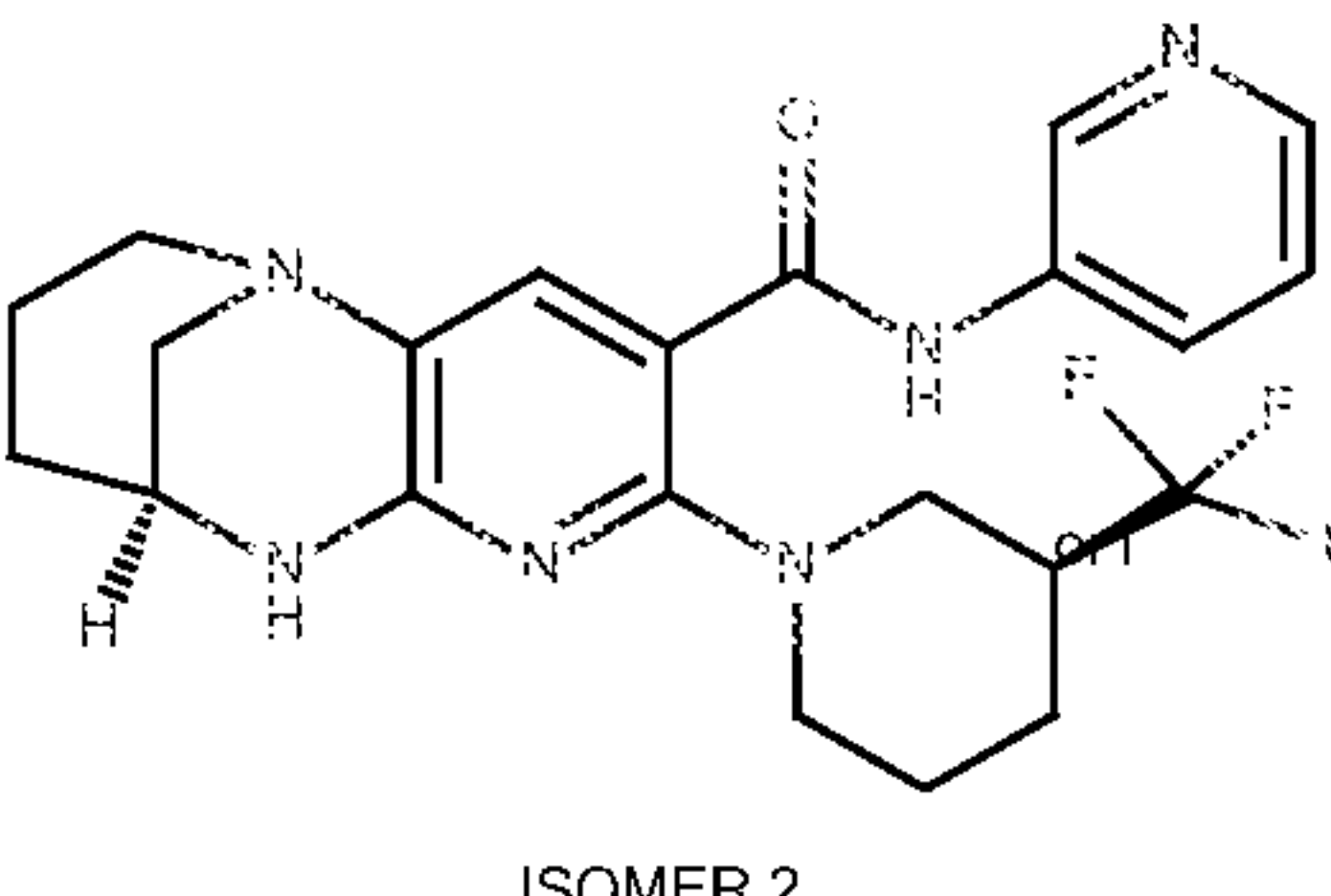


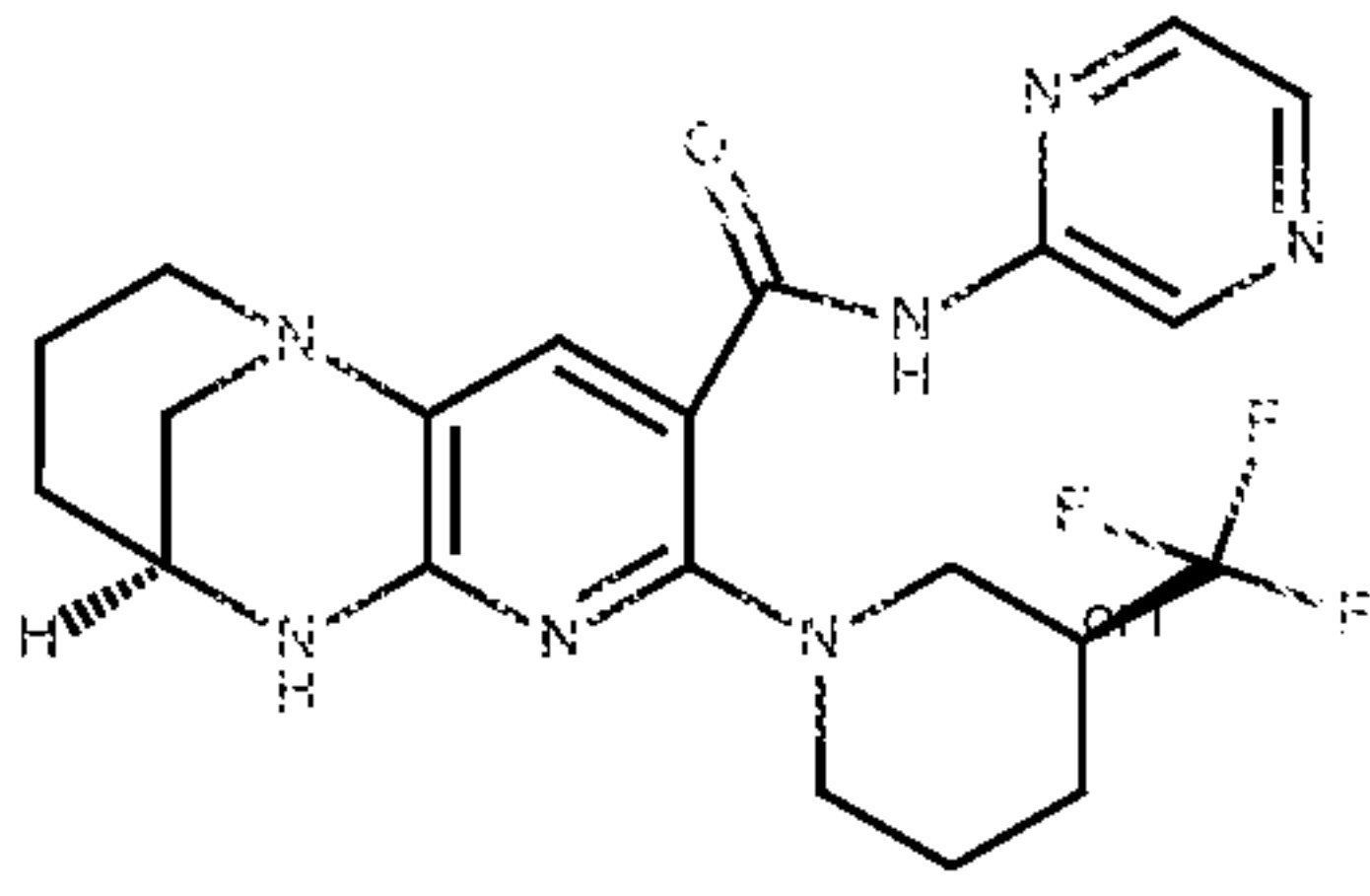
In another aspect, the present invention relates to a compound which is as defined in Table 1 of the instant specification starting at page 303:

In another aspect, the present invention relates to a compound which is:

5

Chart 3

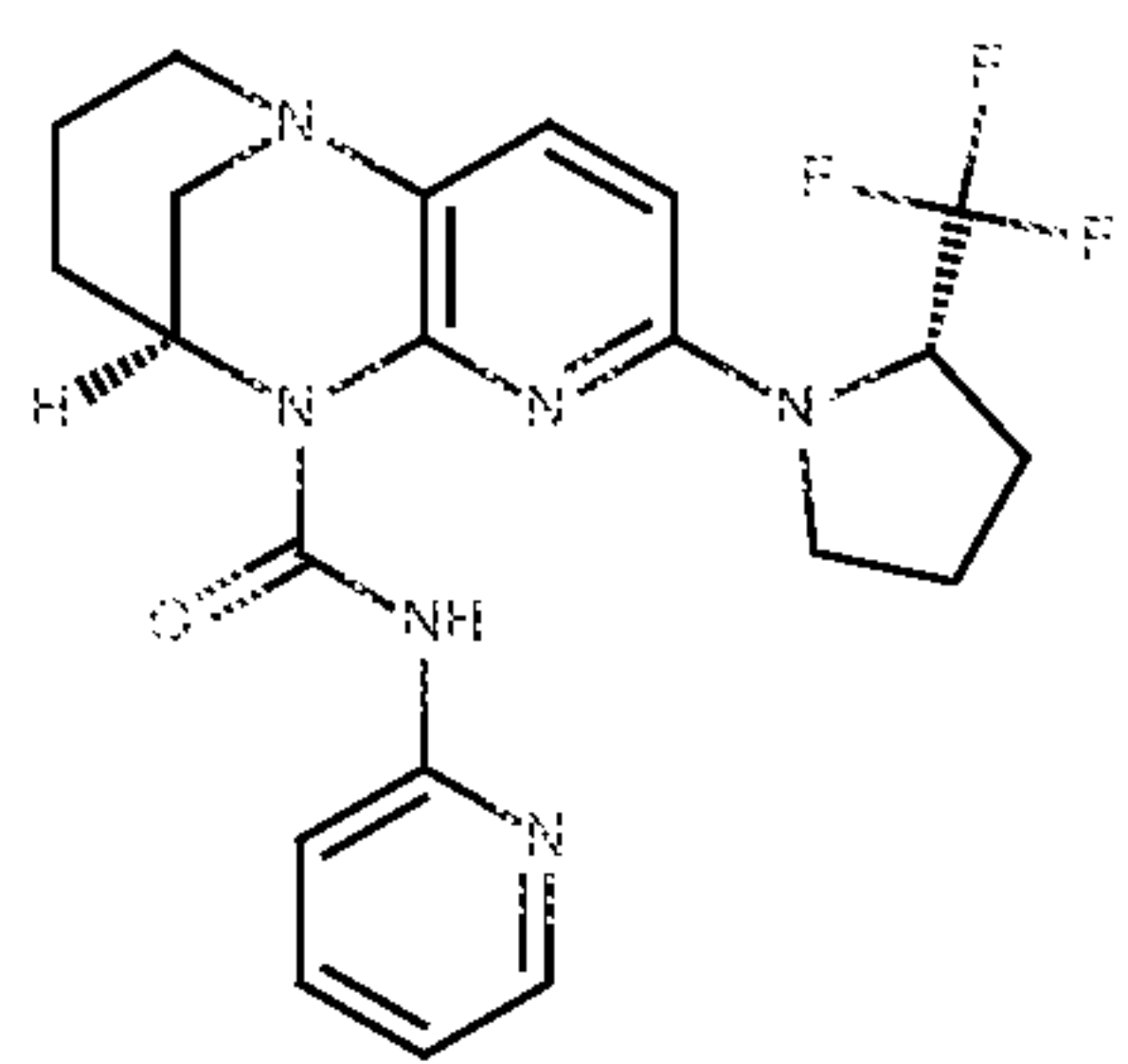
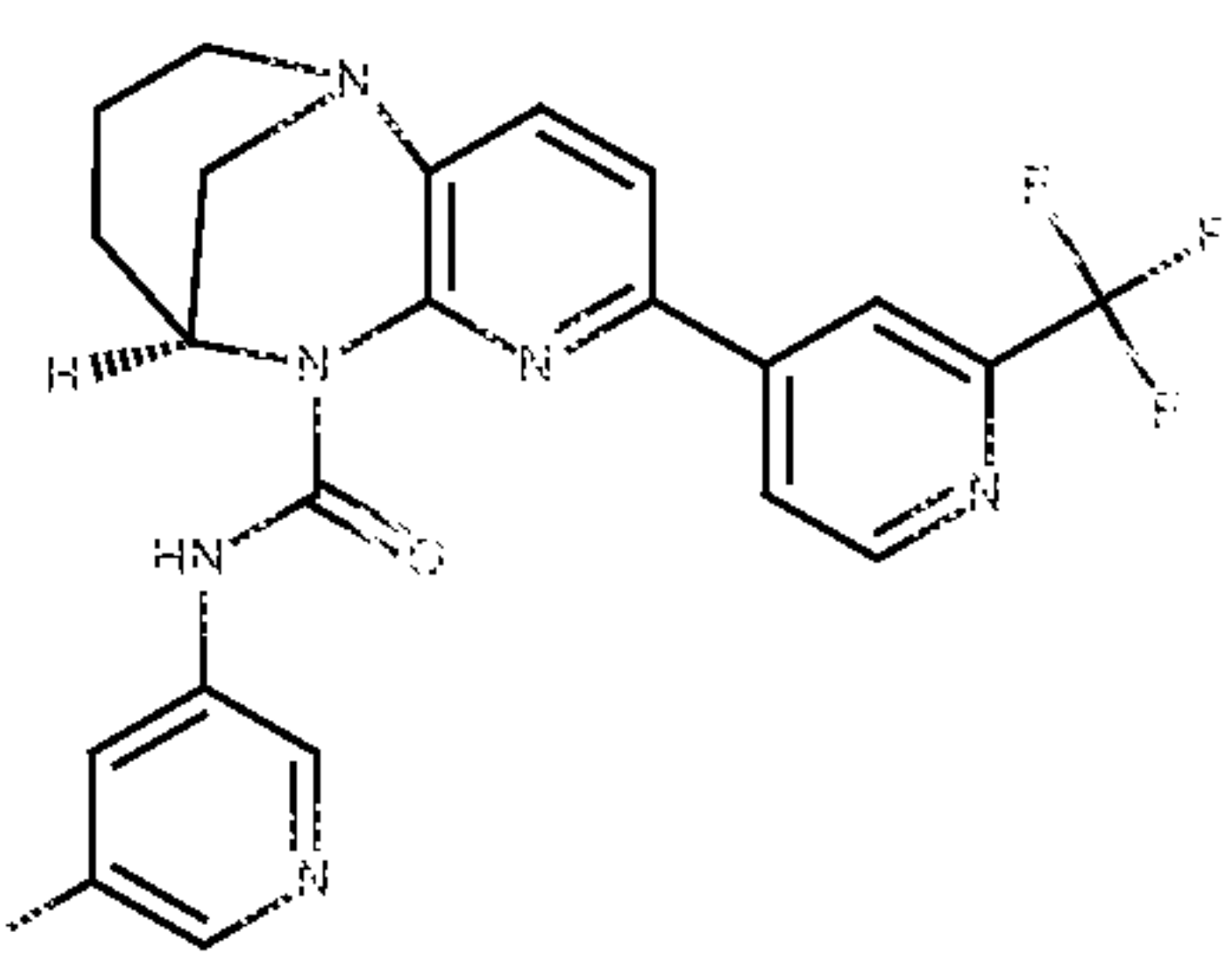
Structure	Chemical Name: Generated by ChemAxon
 ISOMER 1	(9S)-N-(5-fluoropyridin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-4-carboxamide
 ISOMER 1	(9S)-N-(pyrazin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-4-carboxamide
 ISOMER 1	(9S)-N-(pyridin-3-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-4-carboxamide
 ISOMER 2	(9S)-N-(pyridin-3-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-4-carboxamide

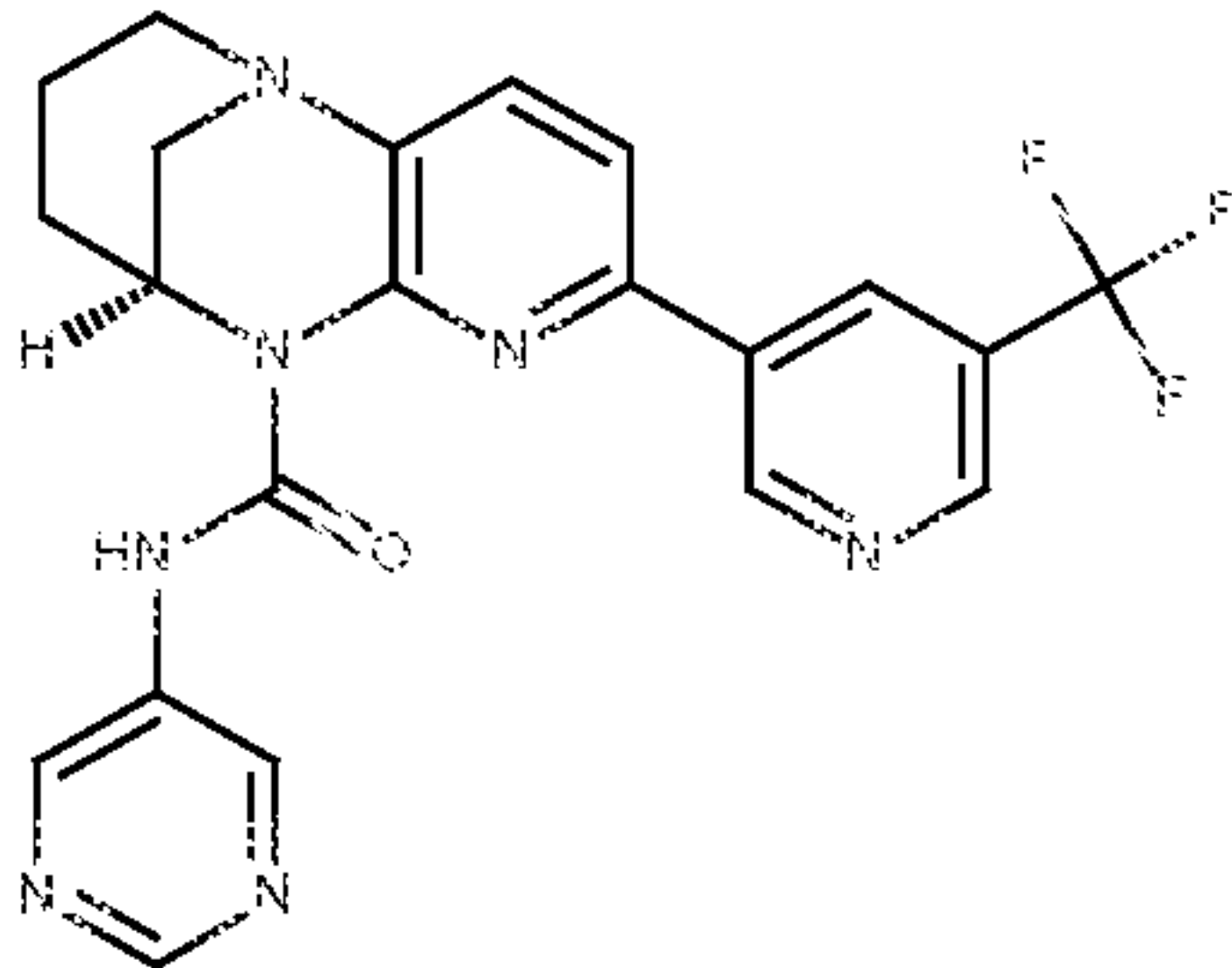
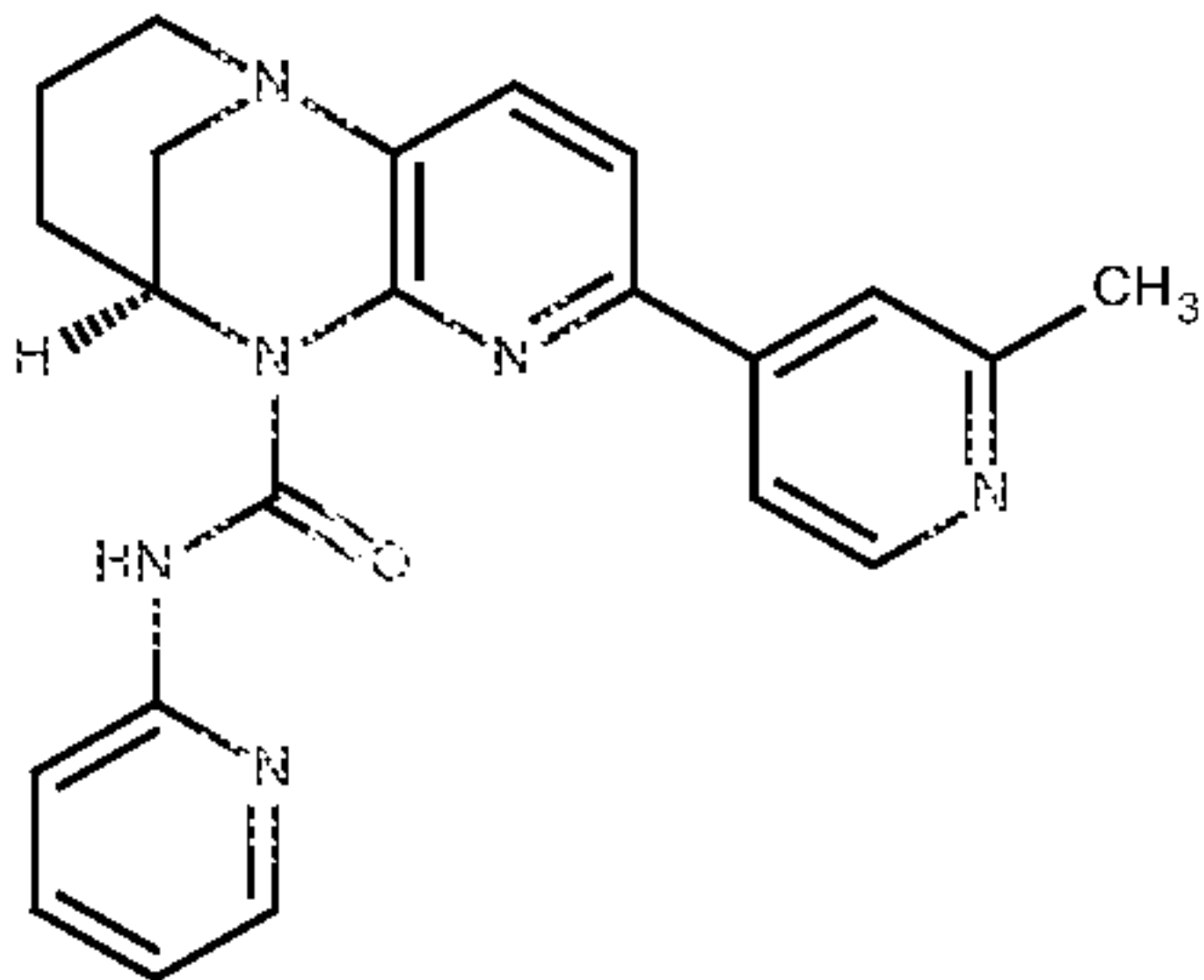
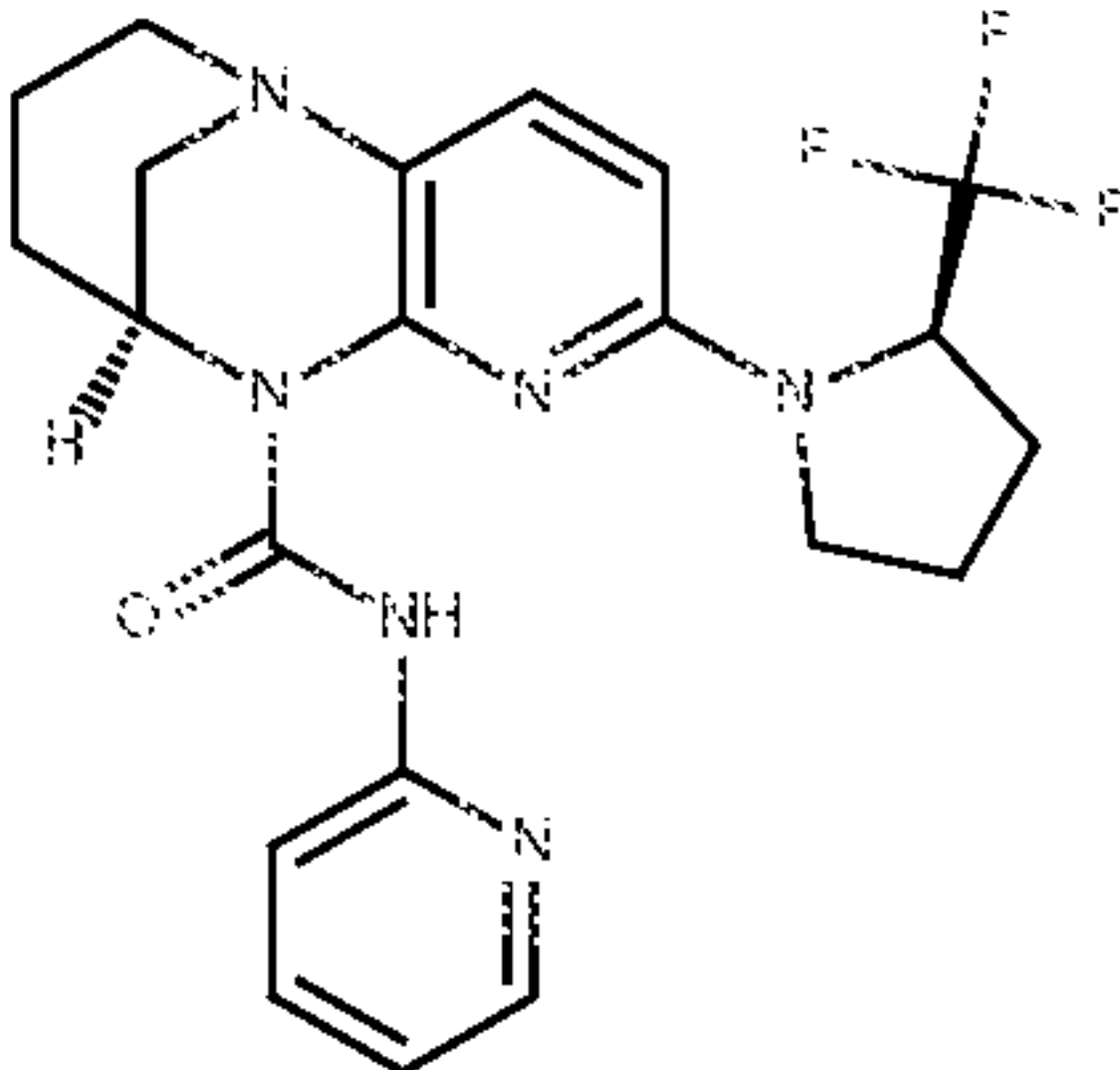
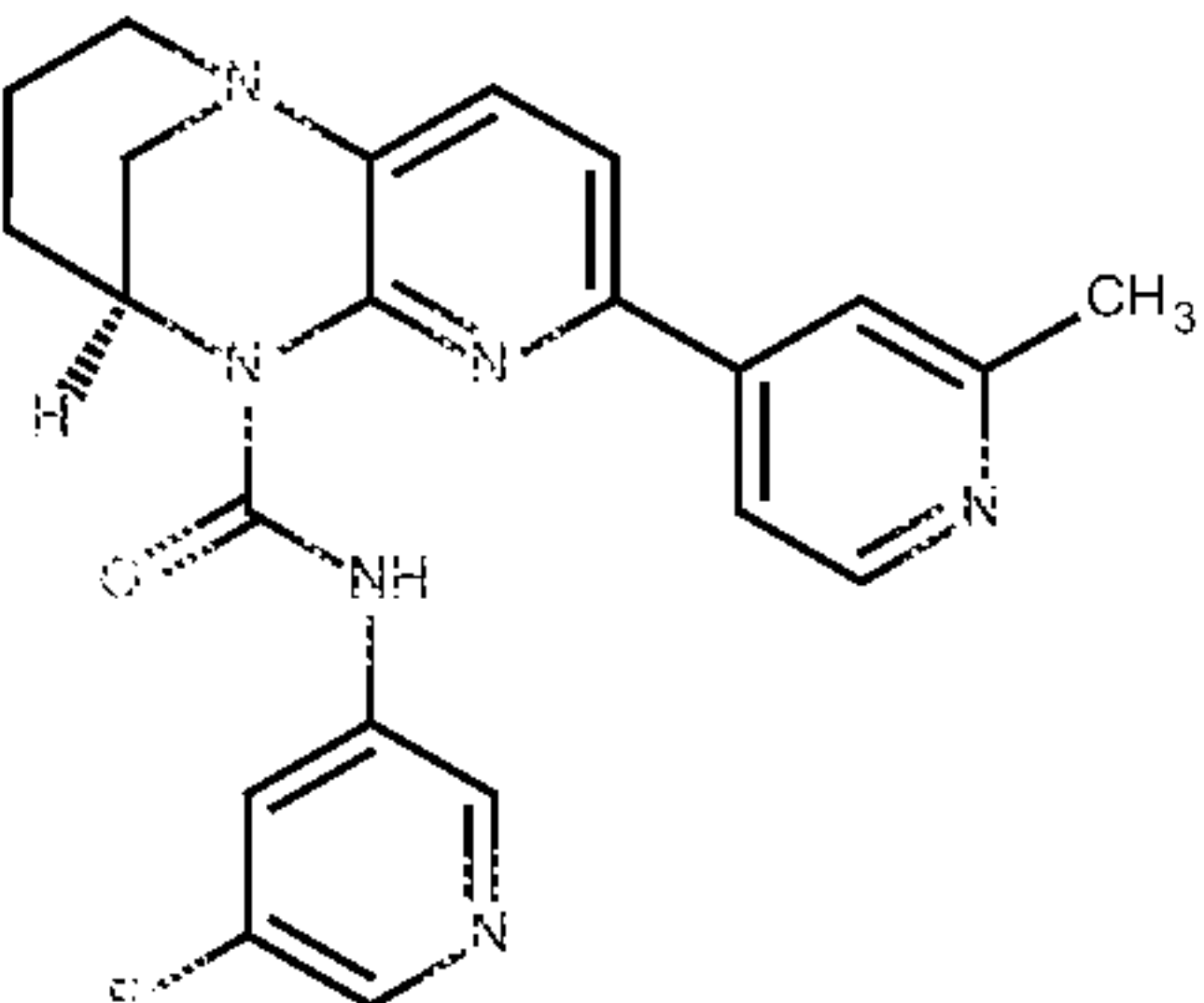
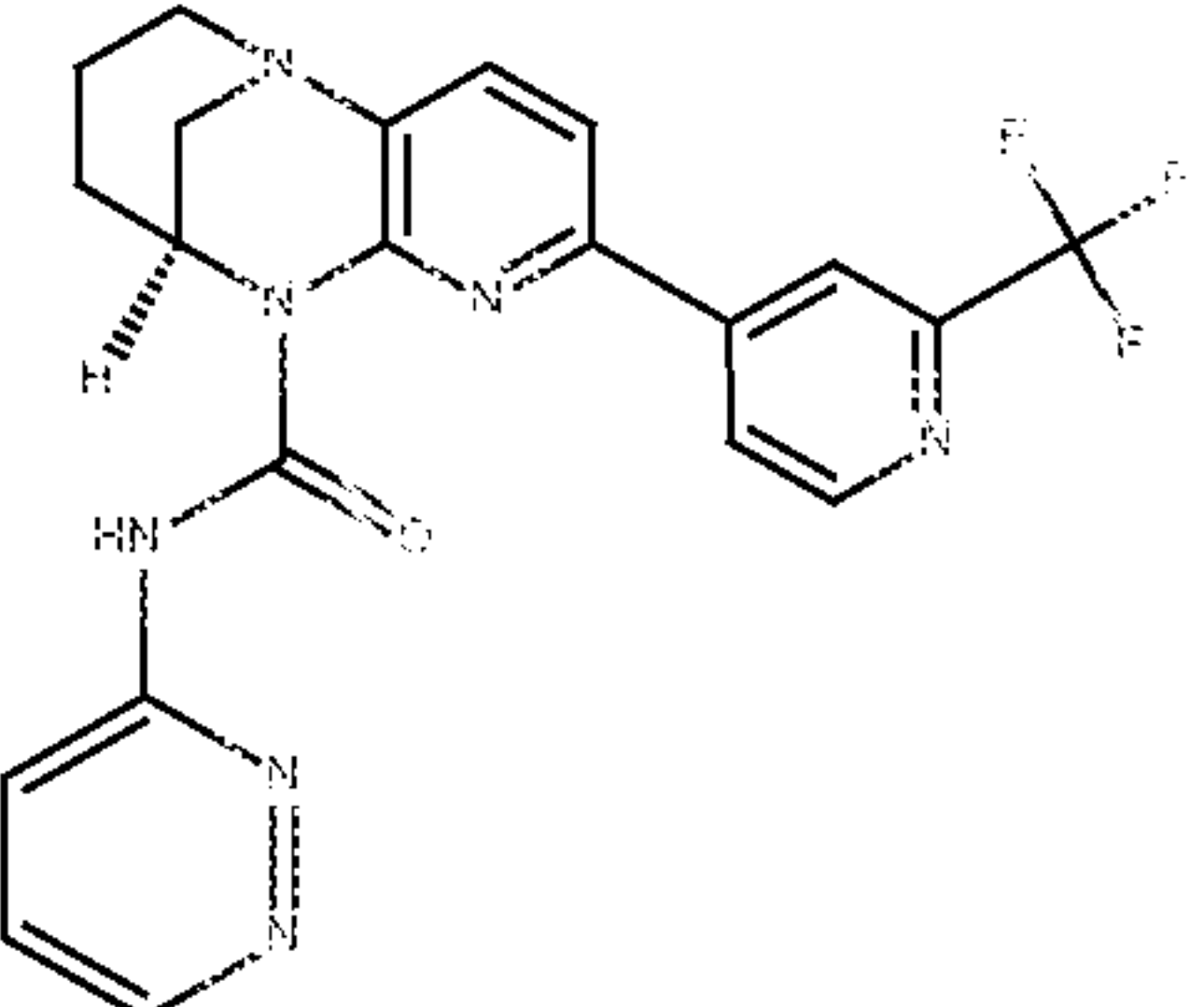
Structure	Chemical Name: Generated by ChemAxon
 ISOMER 2	(9S)-N-(pyrazin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-4-carboxamide

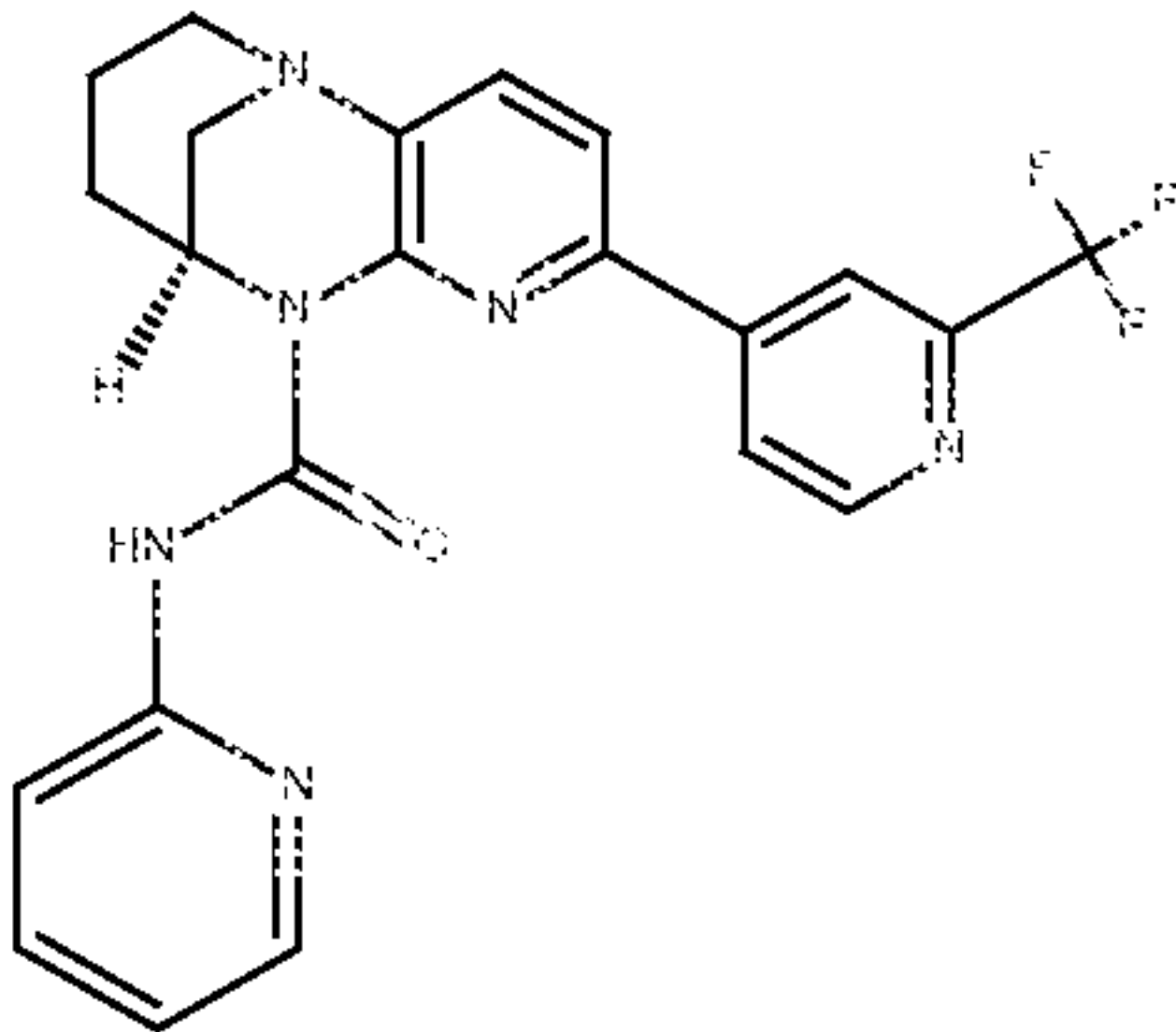
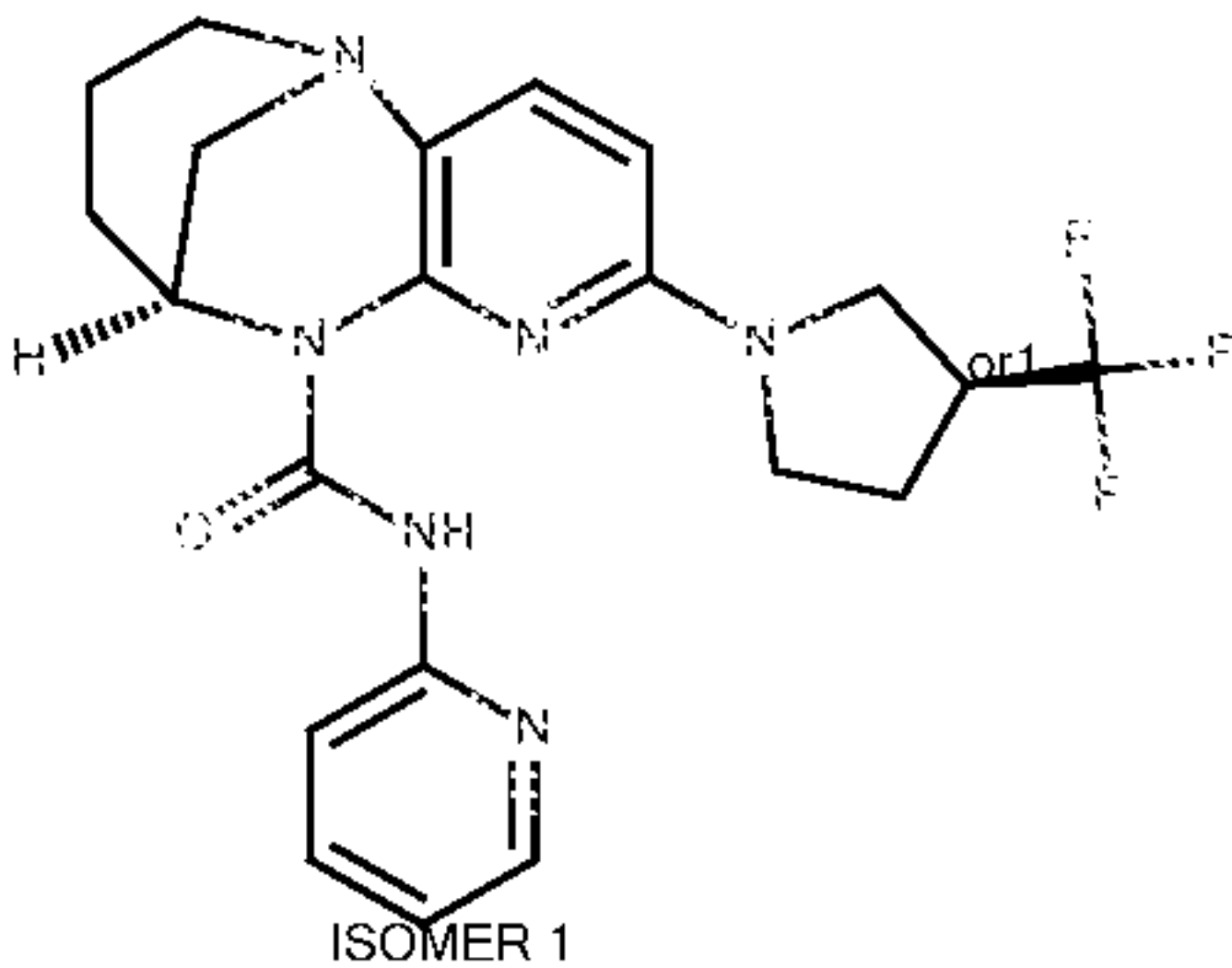
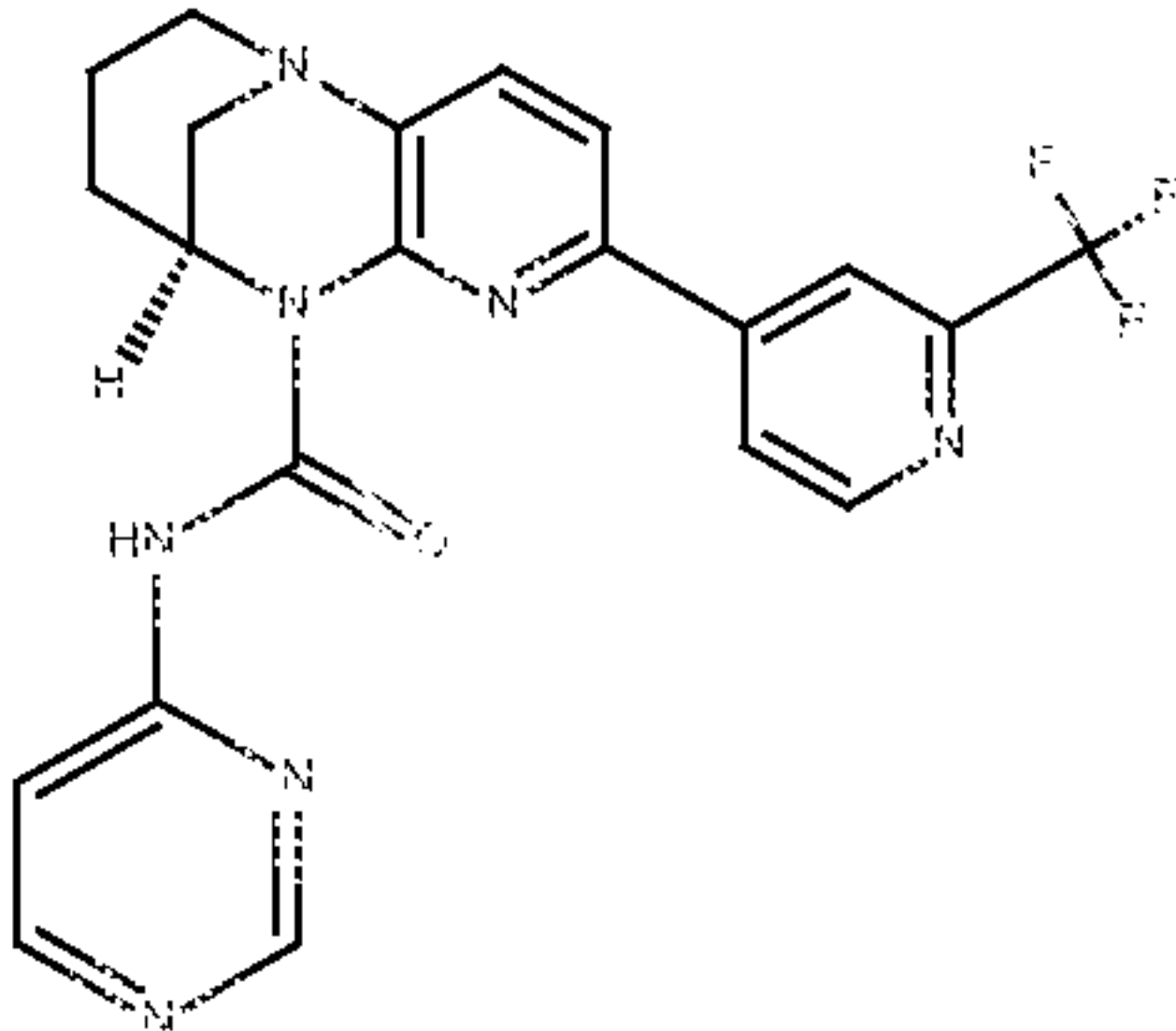
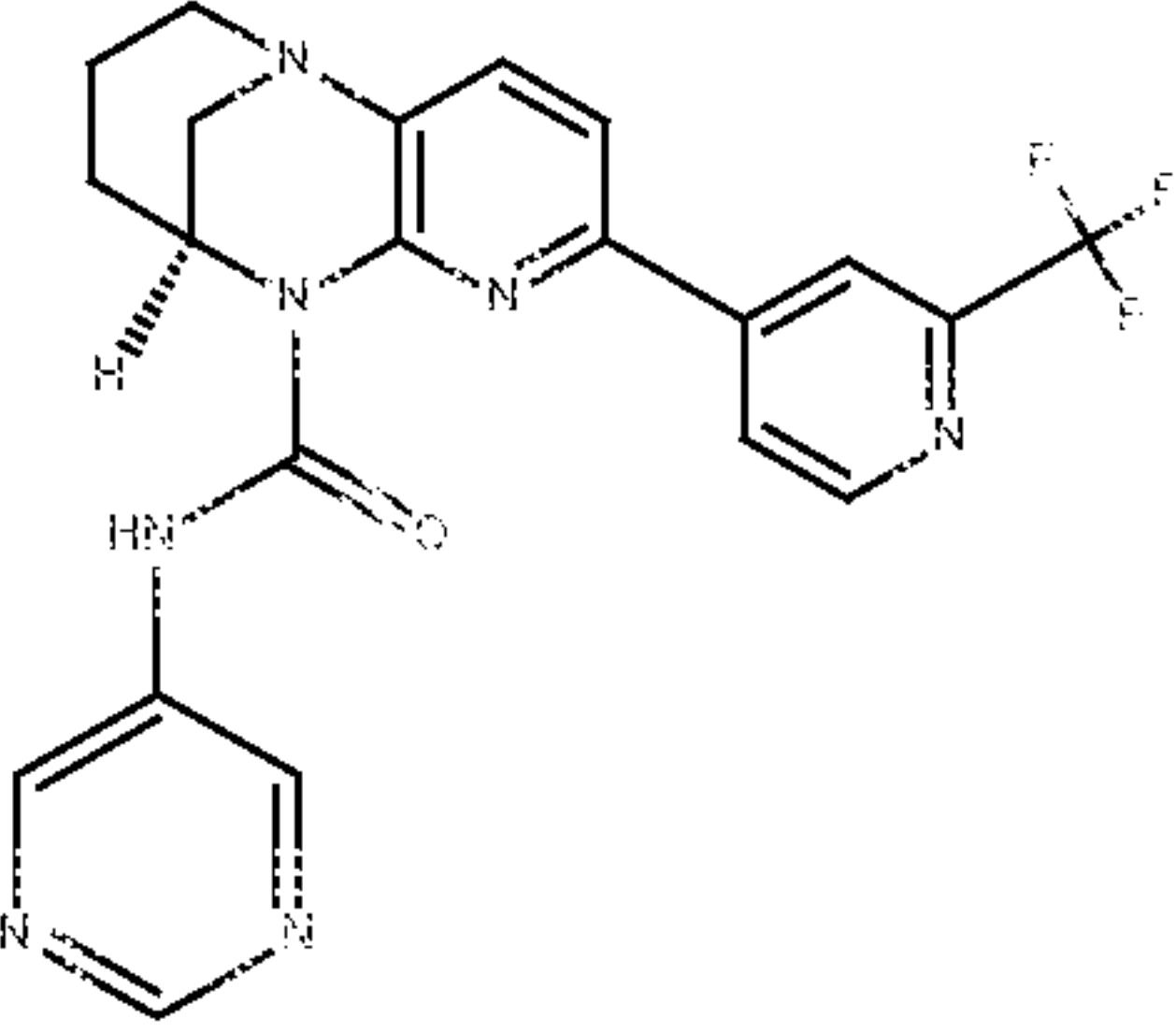
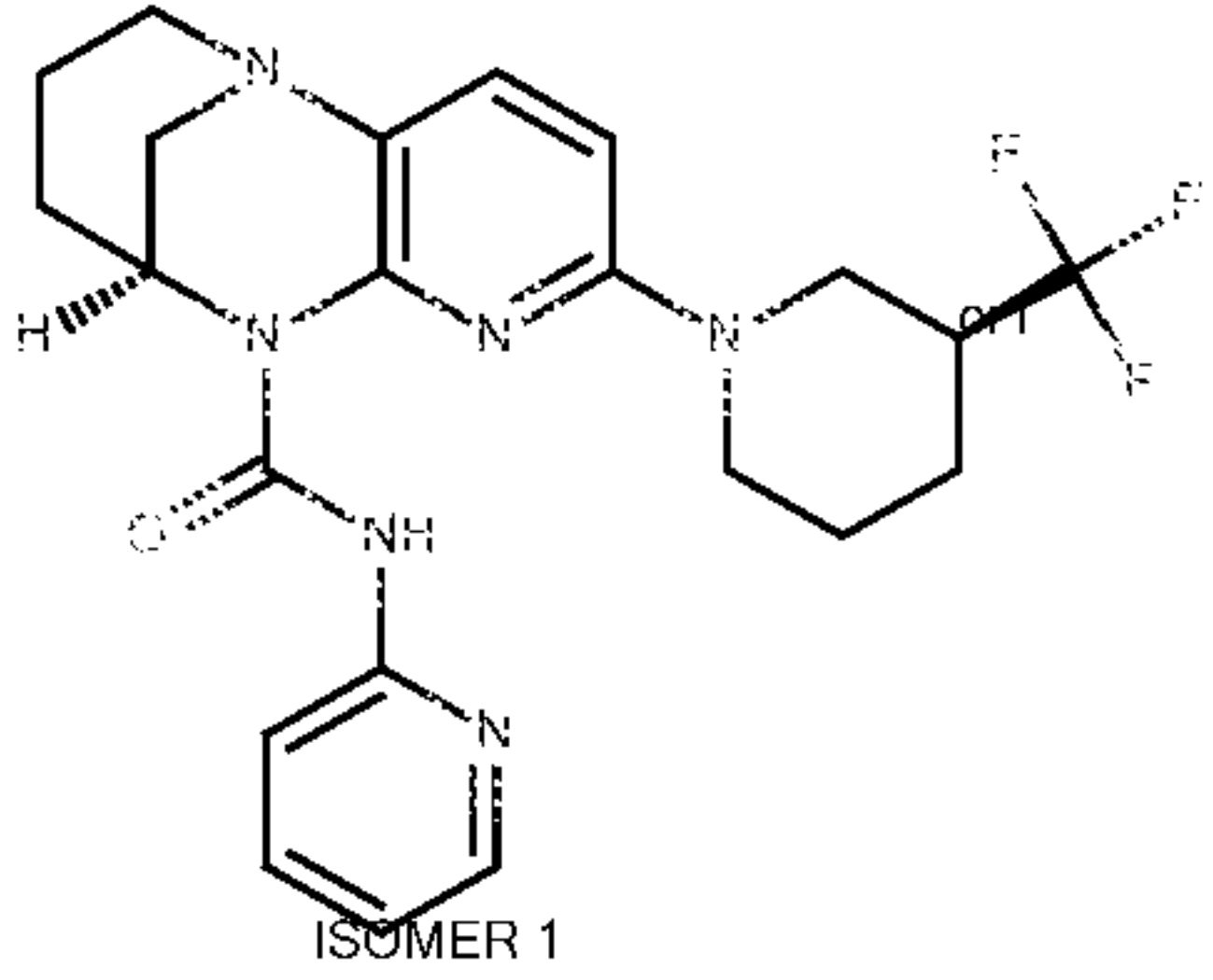
In another aspect, the present invention relates to a compound which is a corresponding analog or derivative of the present invention s (i.e., with hydrogen substitution at the R² position):

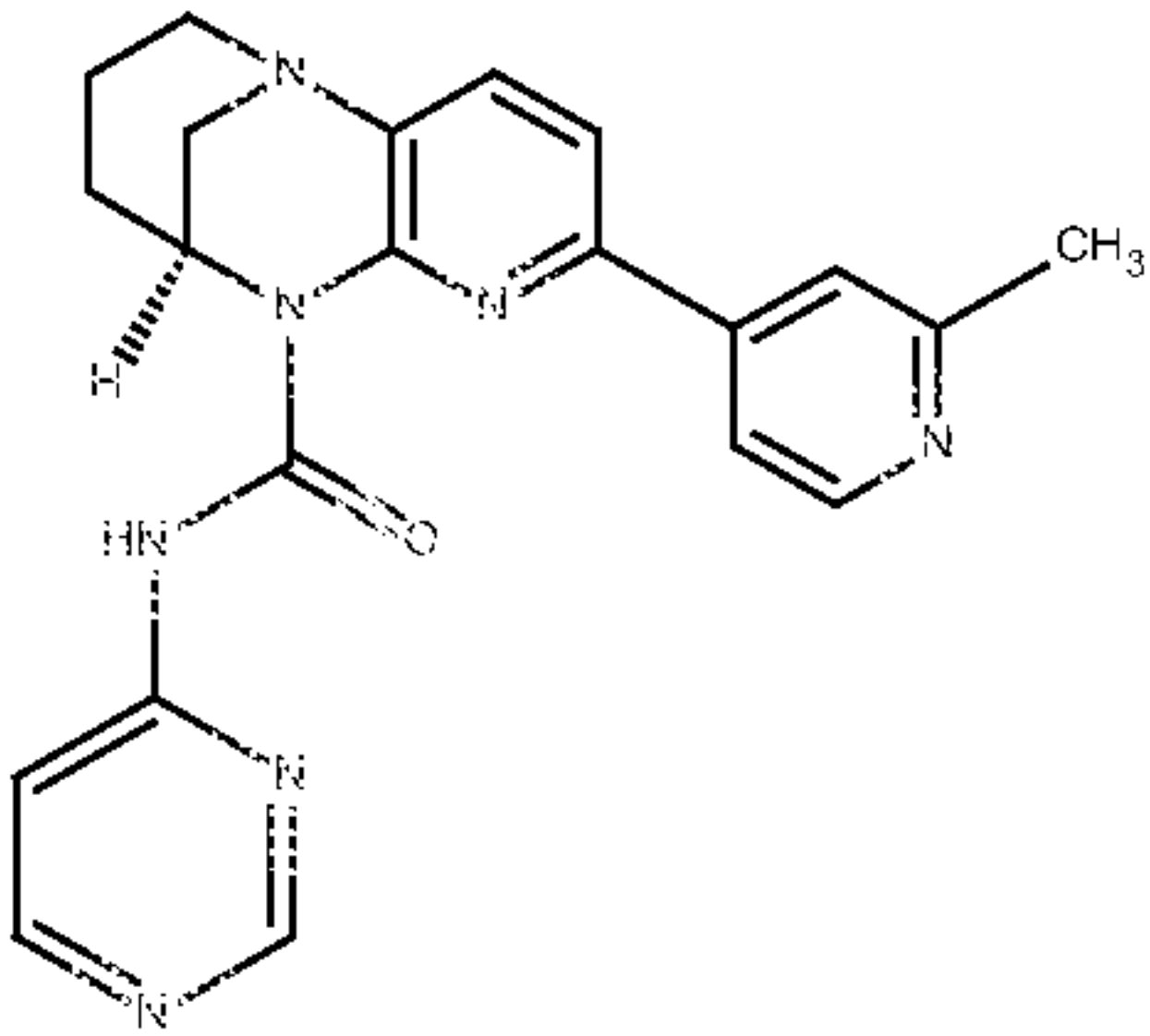
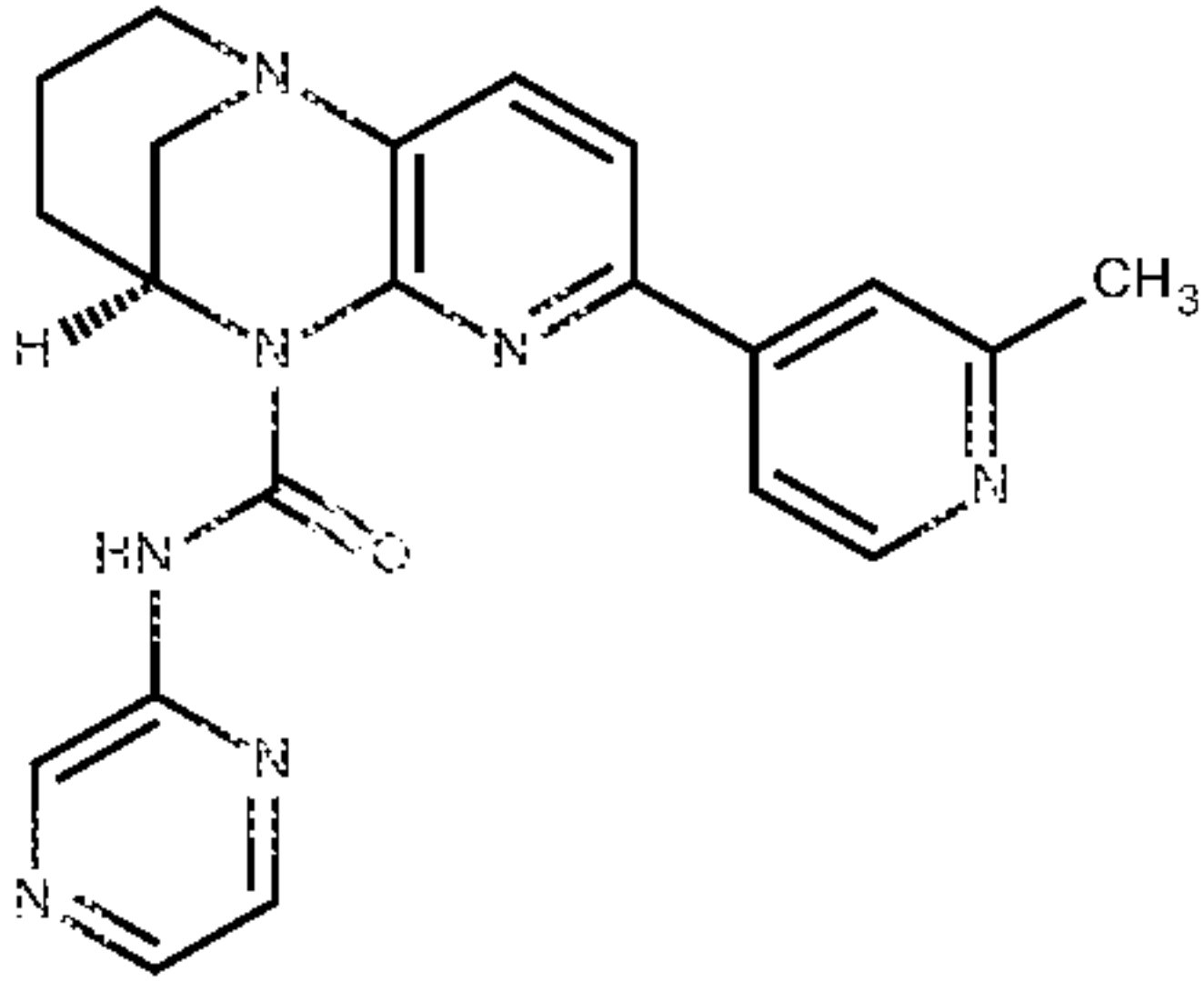
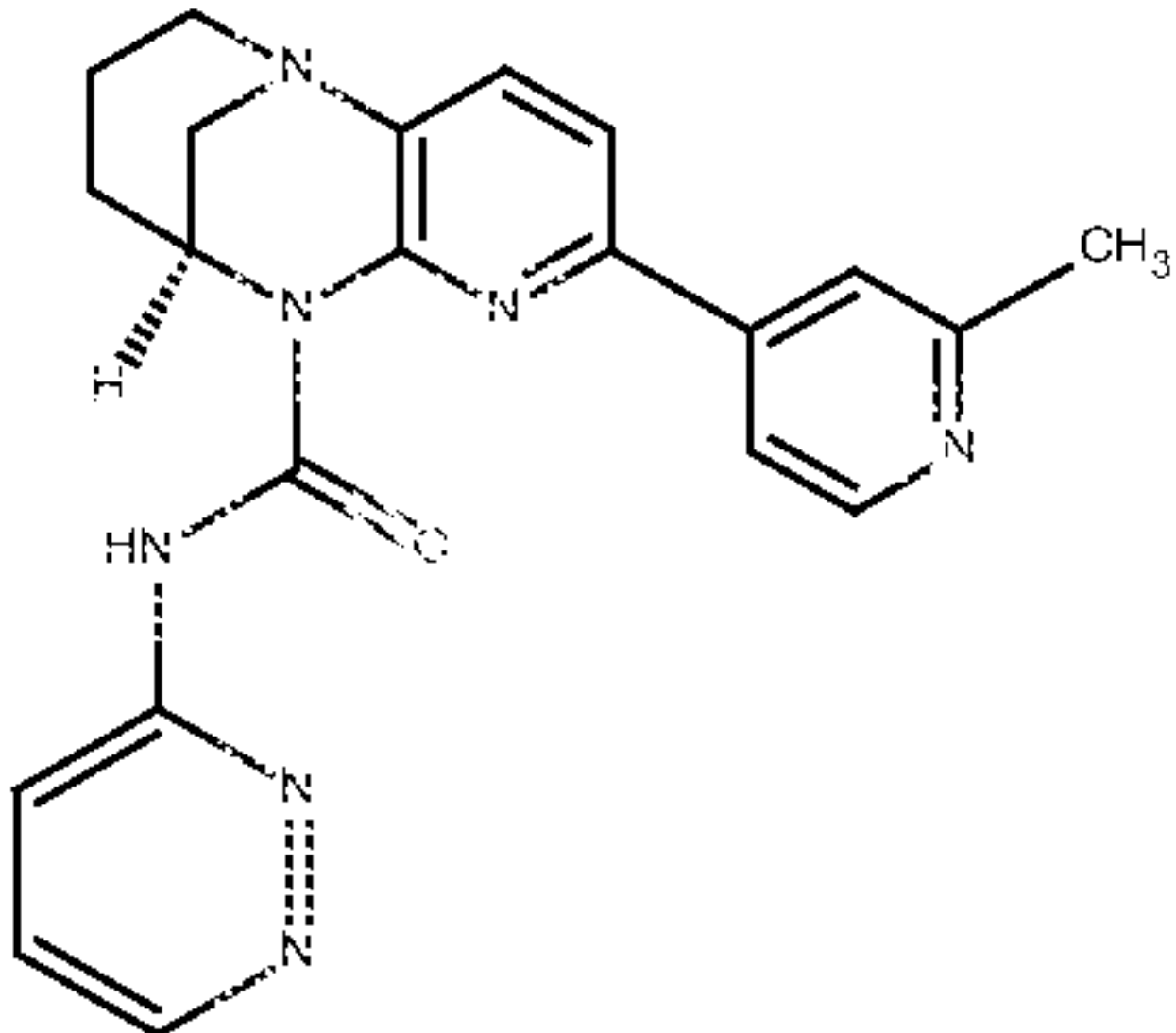
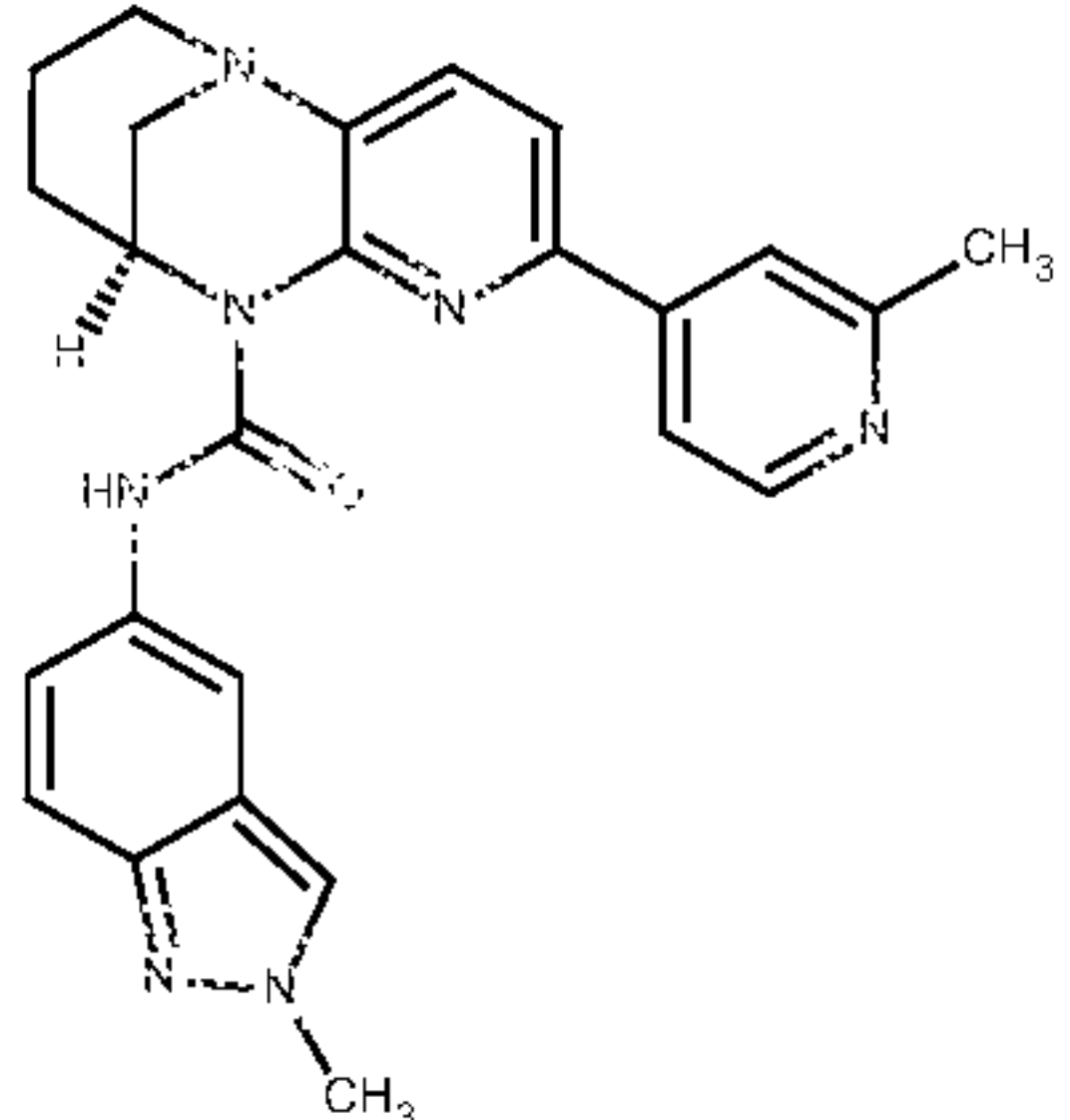
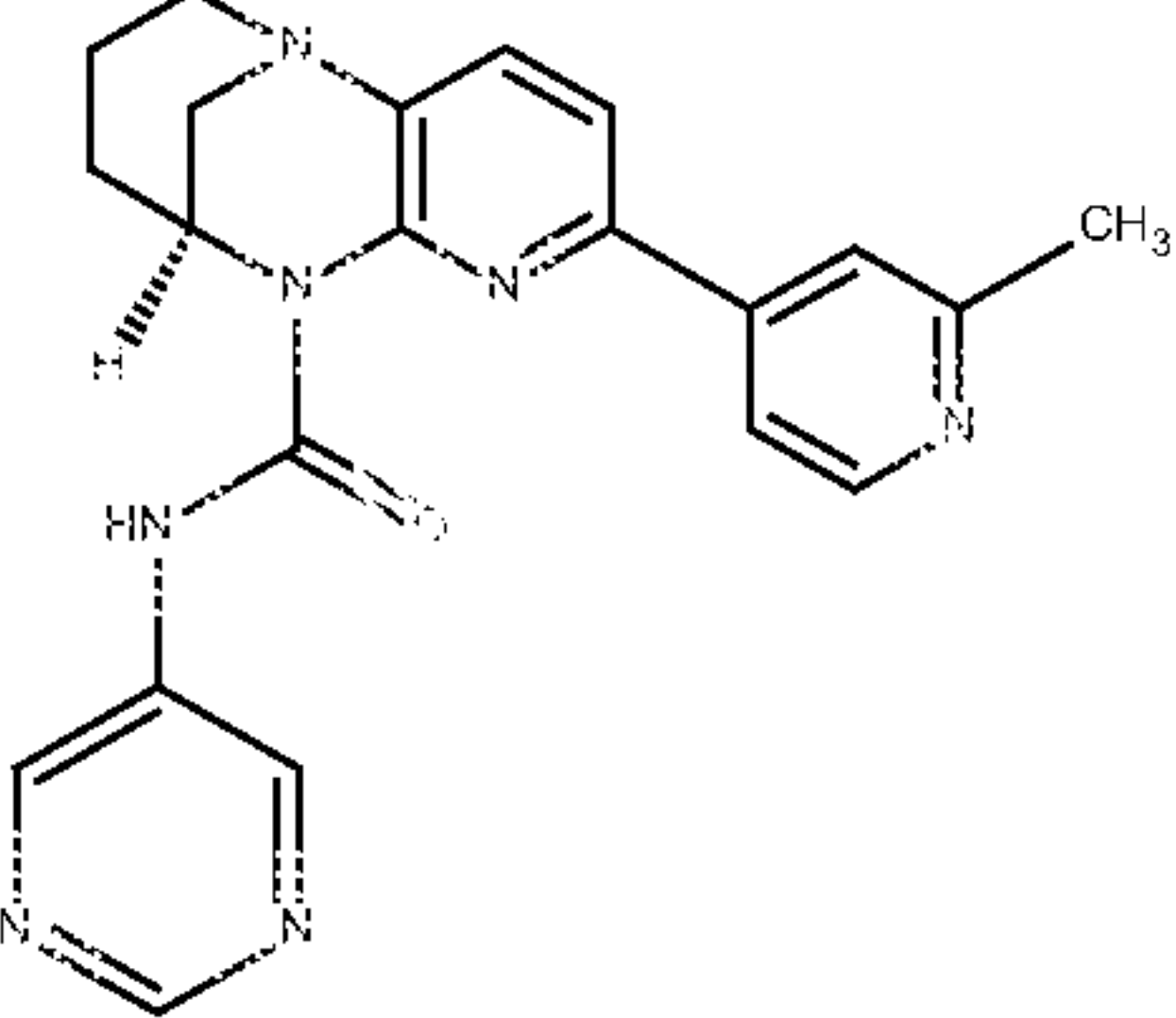
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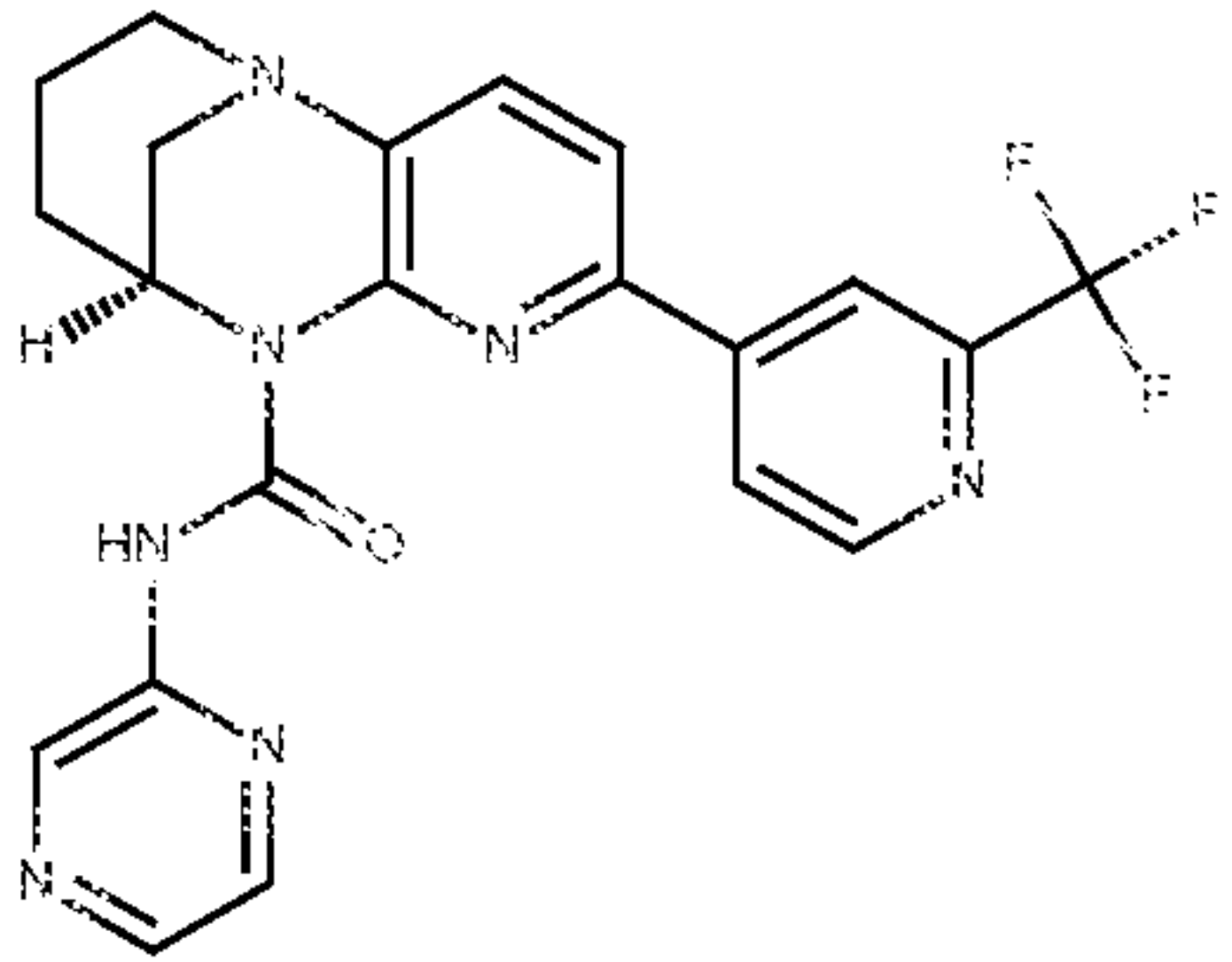
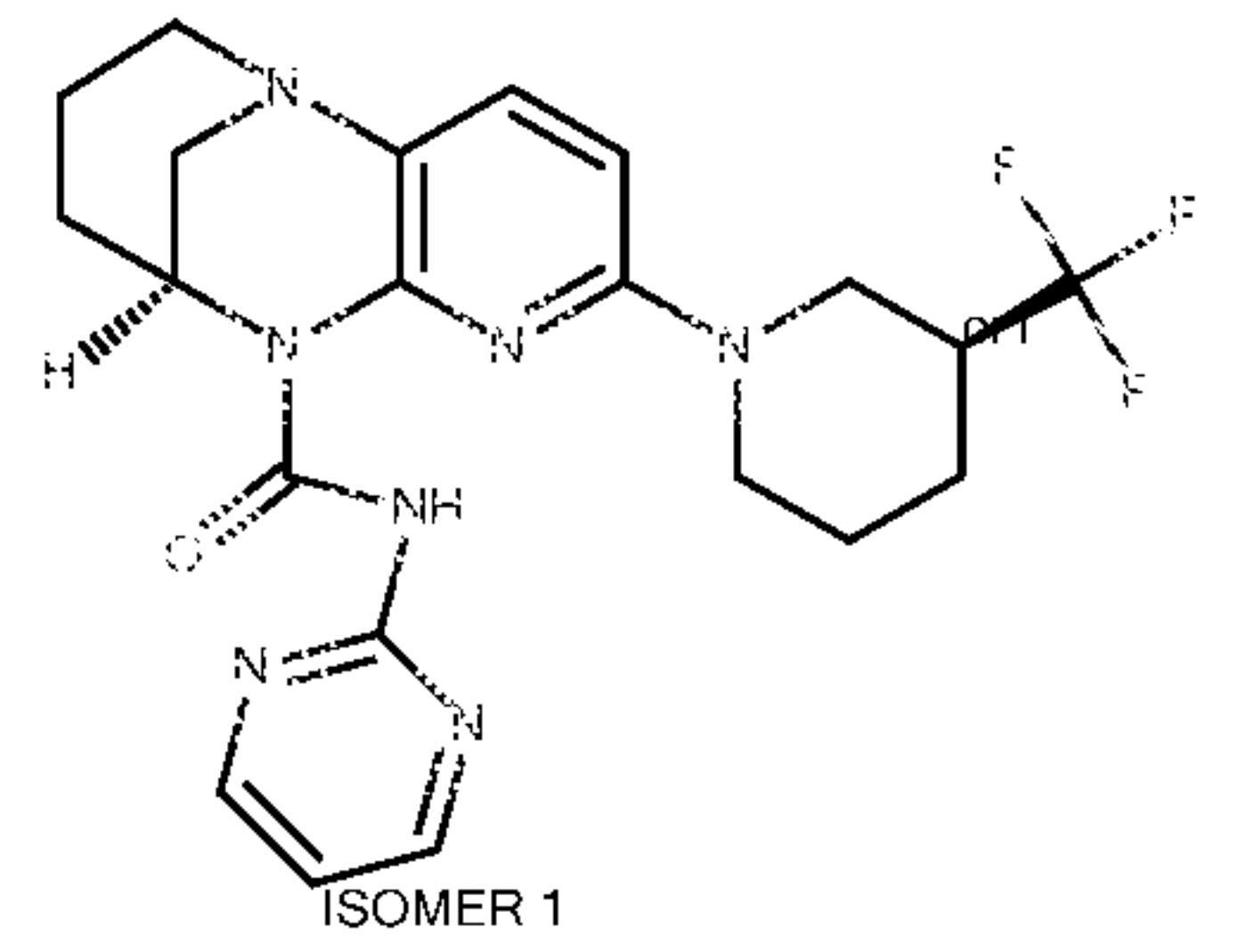
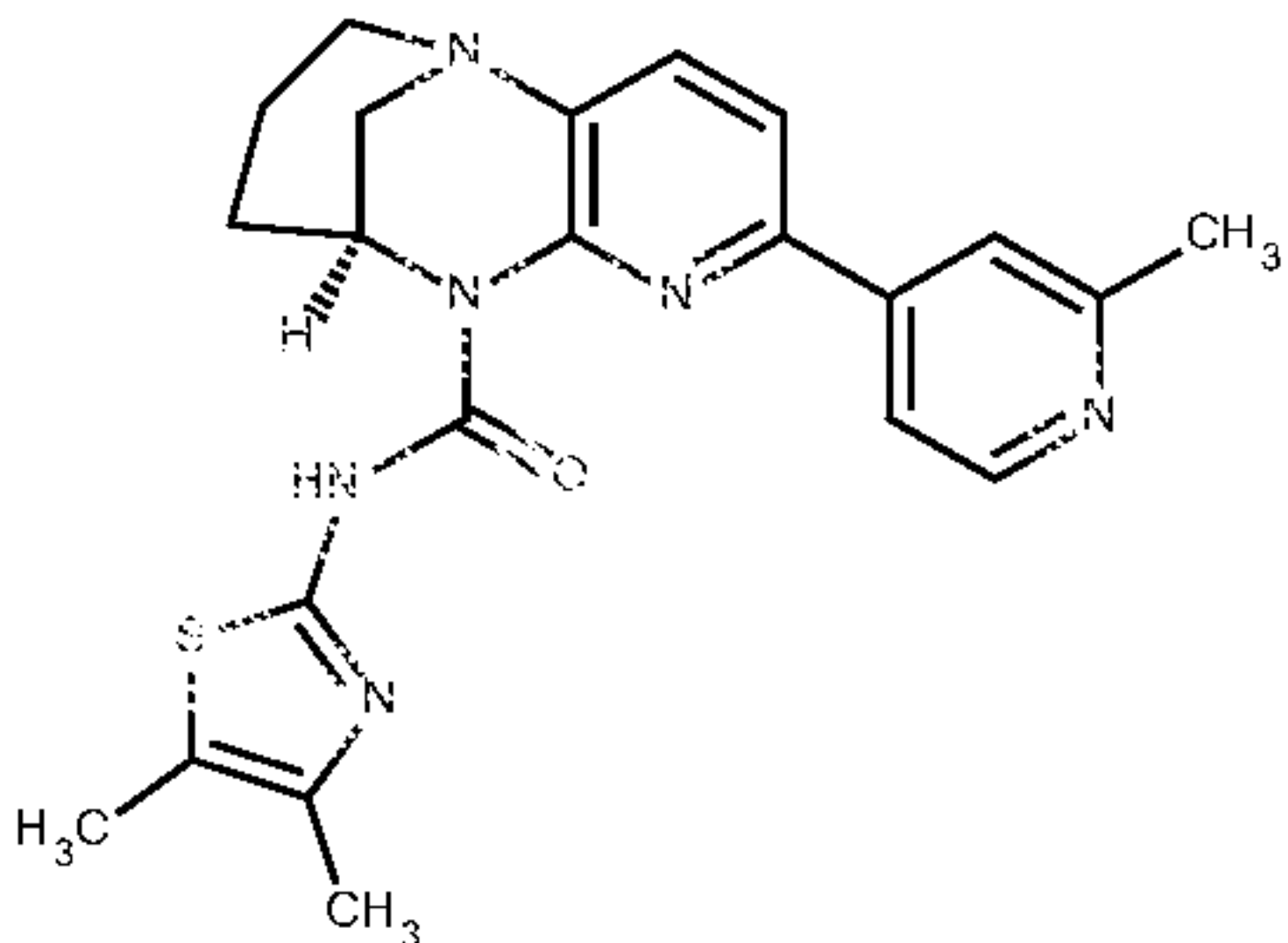
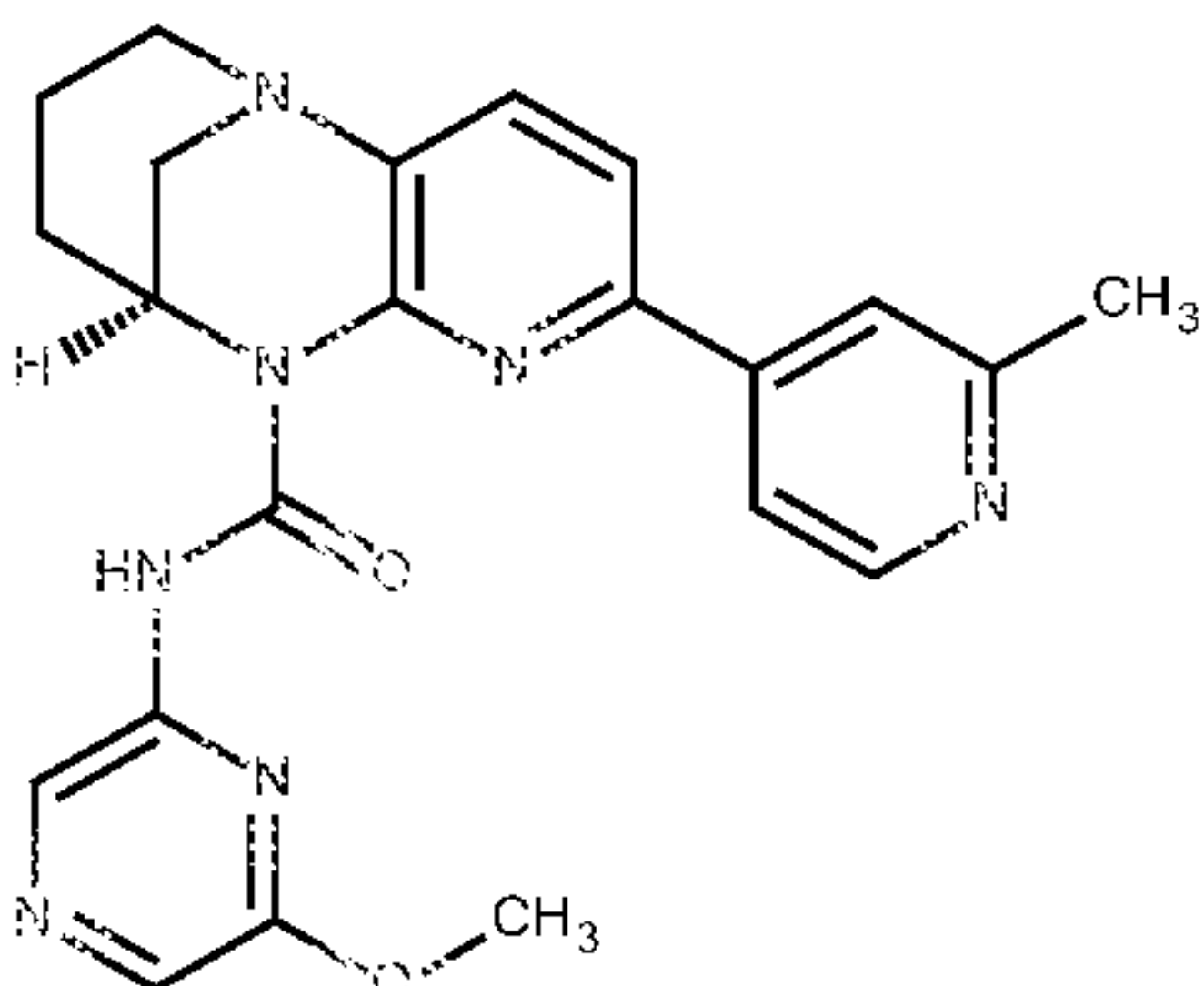
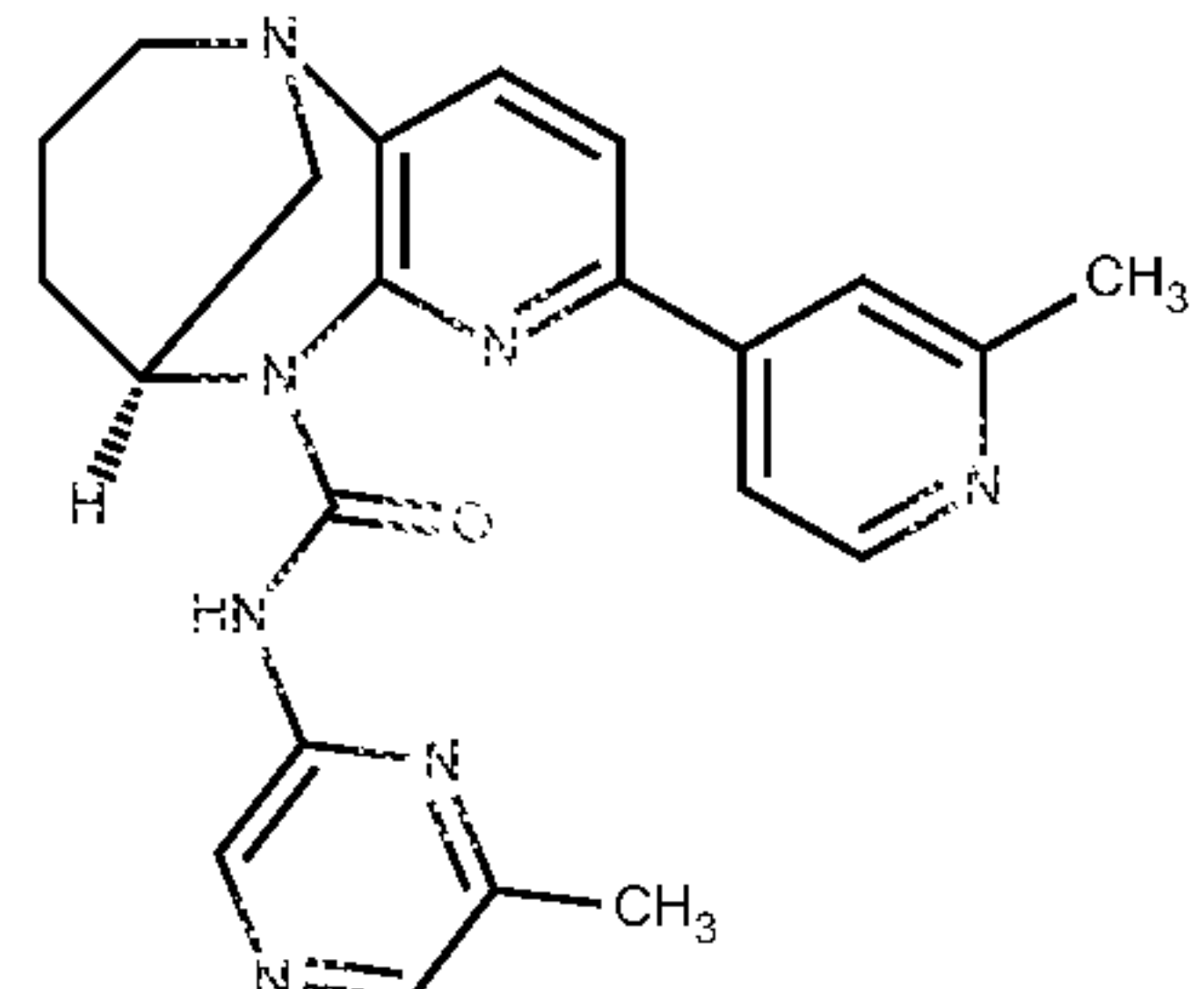
Chart 4 – Three Carbon Unit and Pyridine – No Meta Substitution

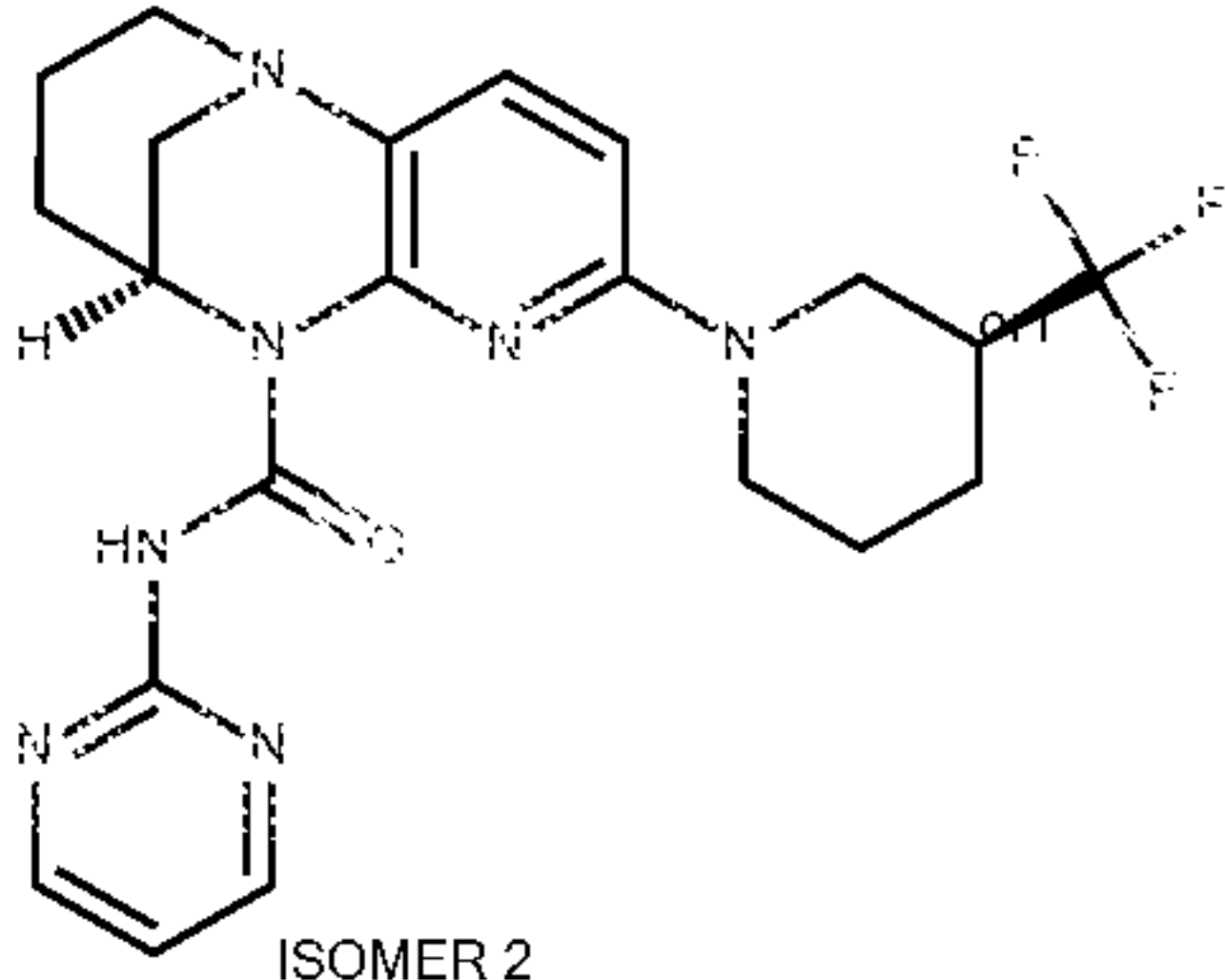
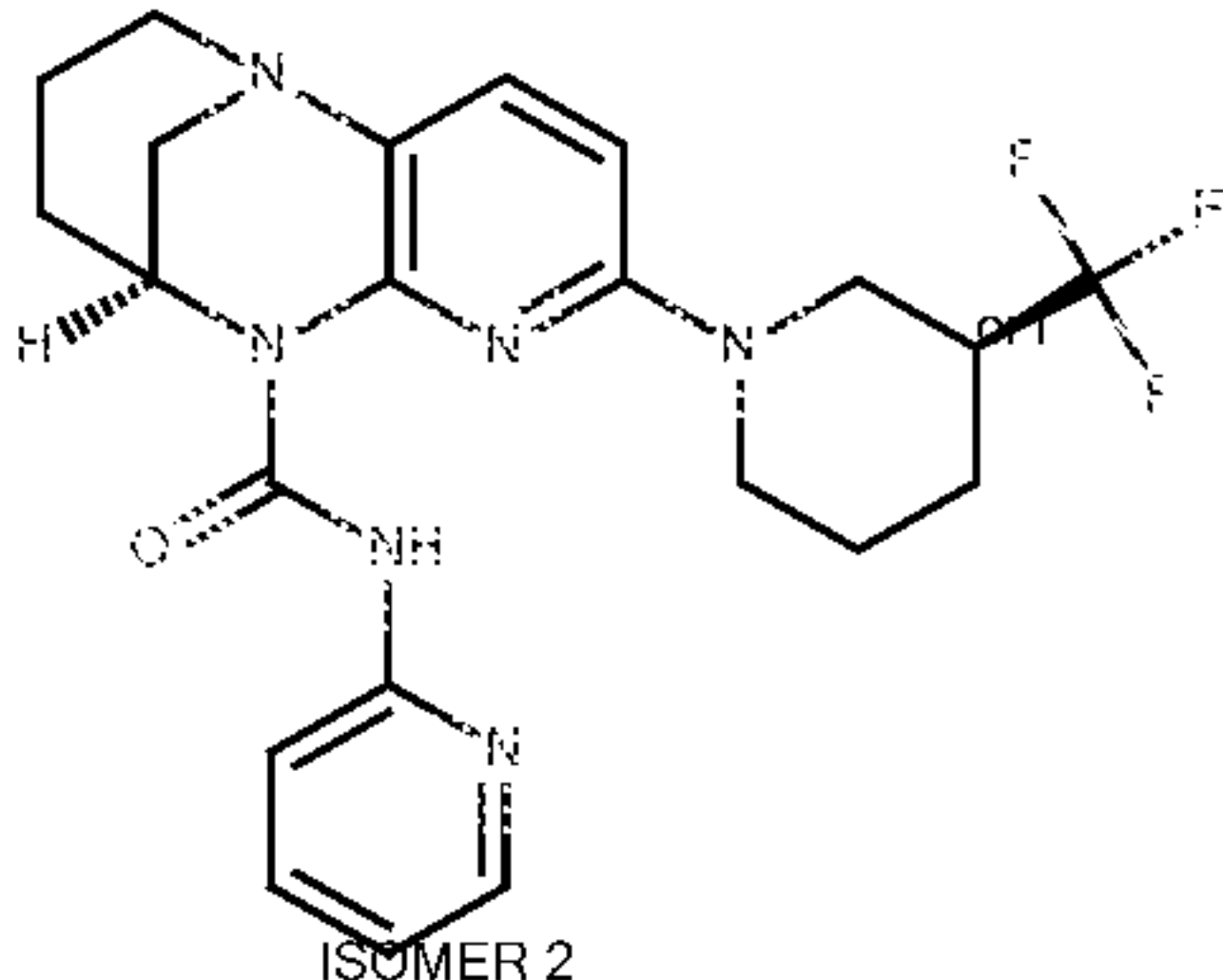
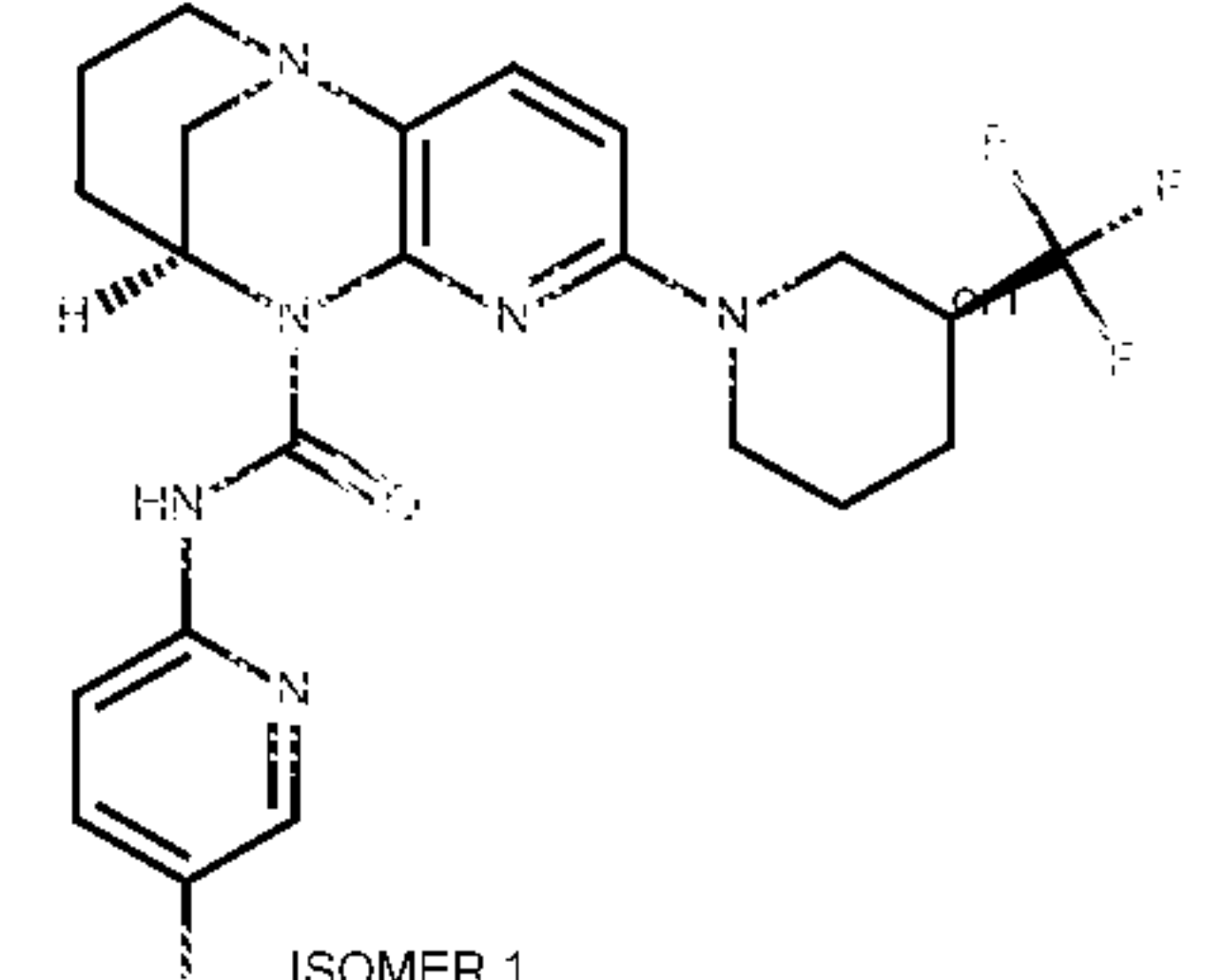
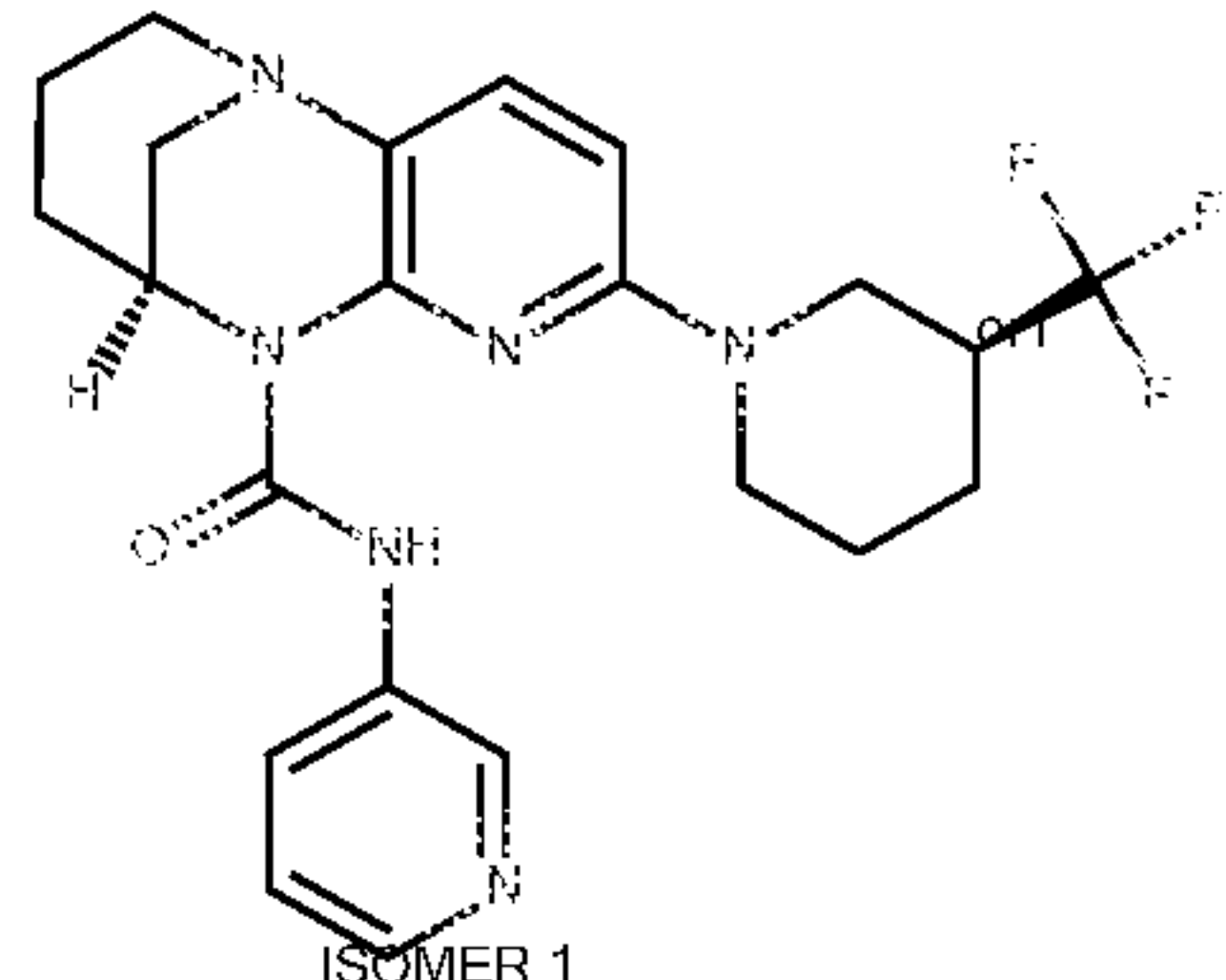
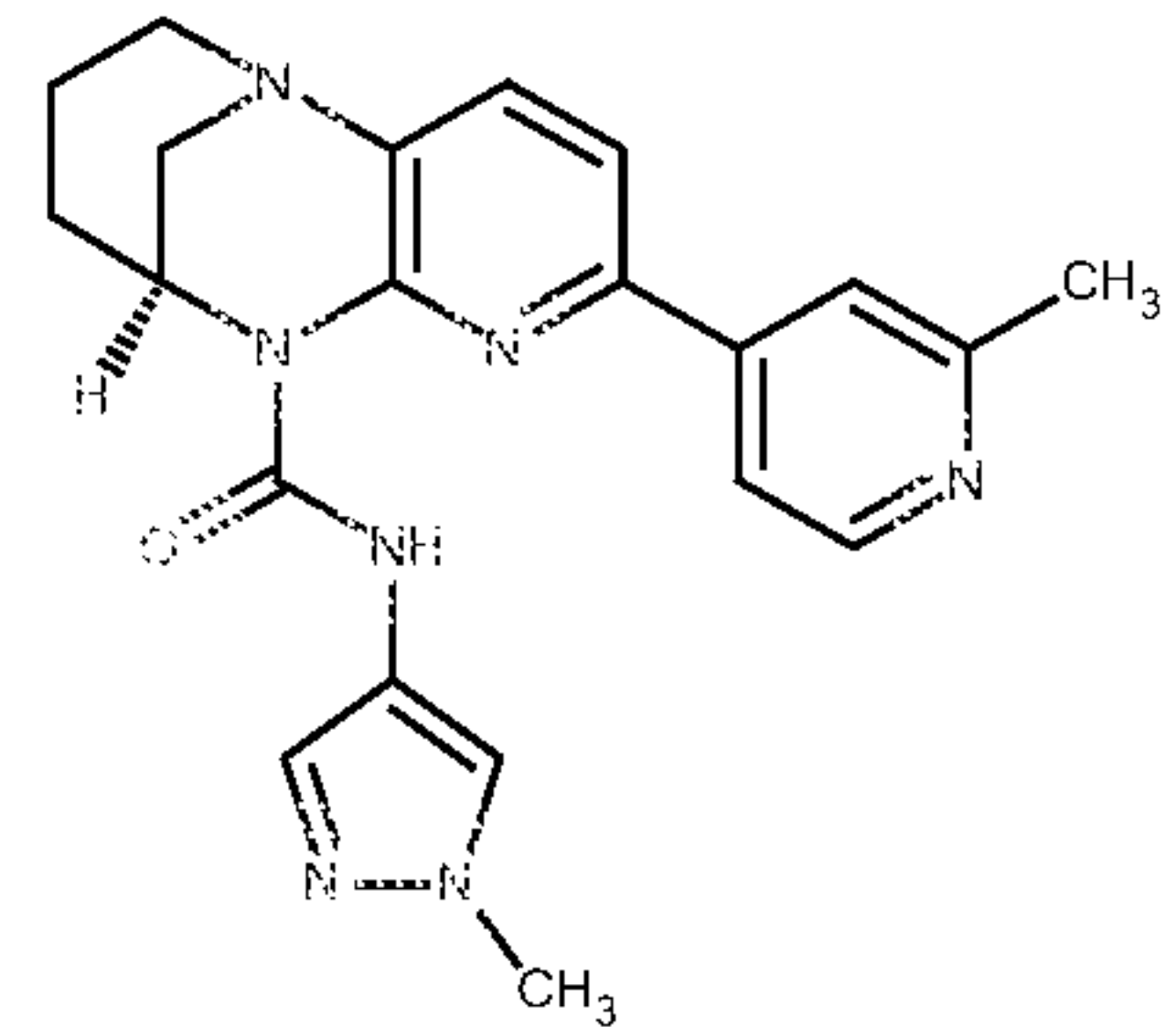
Structure	Chemical Name: Generated by ChemAxon
	(9S)-N-(pyridin-2-yl)-5-[(2S)-2-(trifluoromethyl)pyrrolidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
	(9S)-N-(5-fluoropyridin-3-yl)-5-[2-(trifluoromethyl)pyridin-4-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide

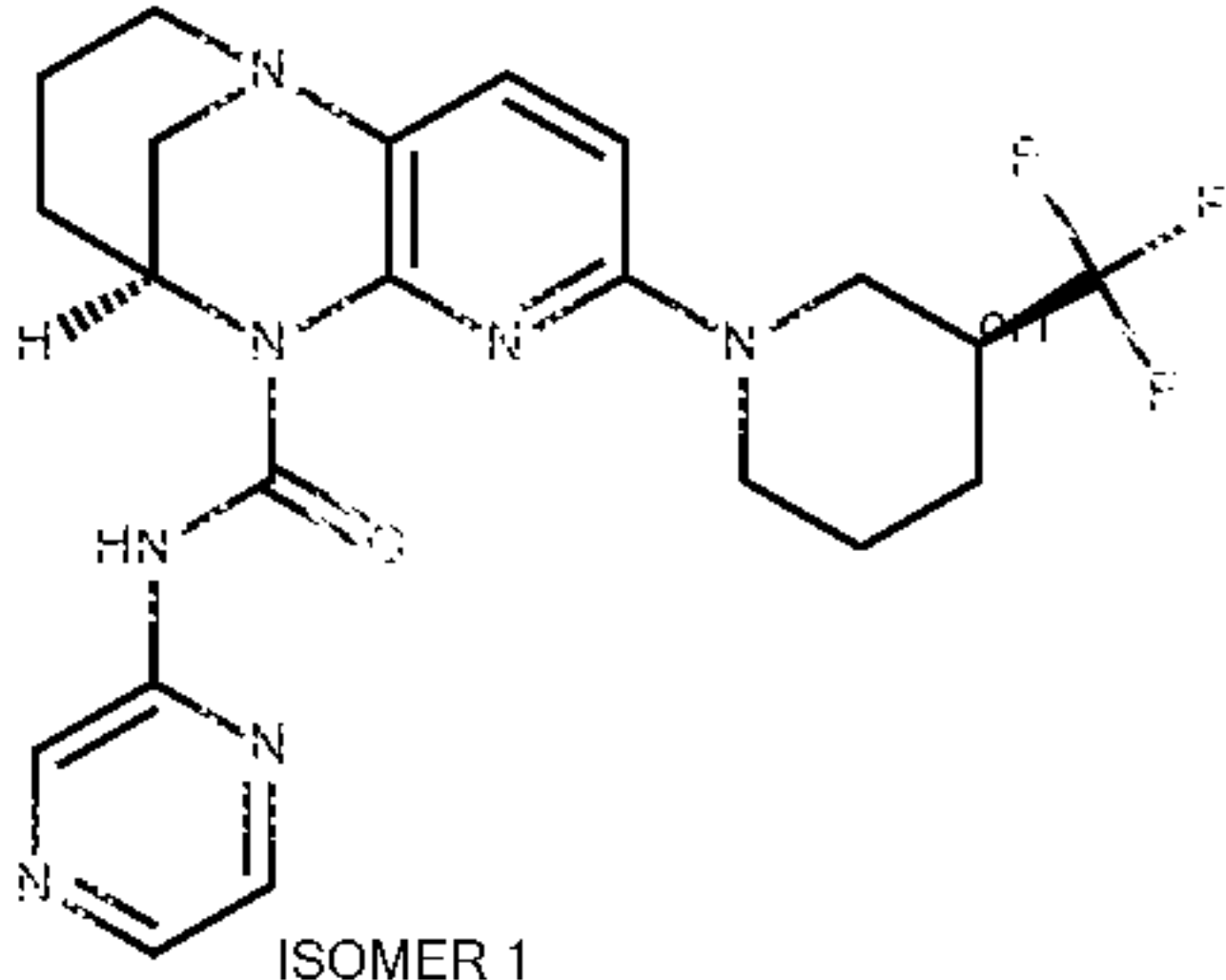
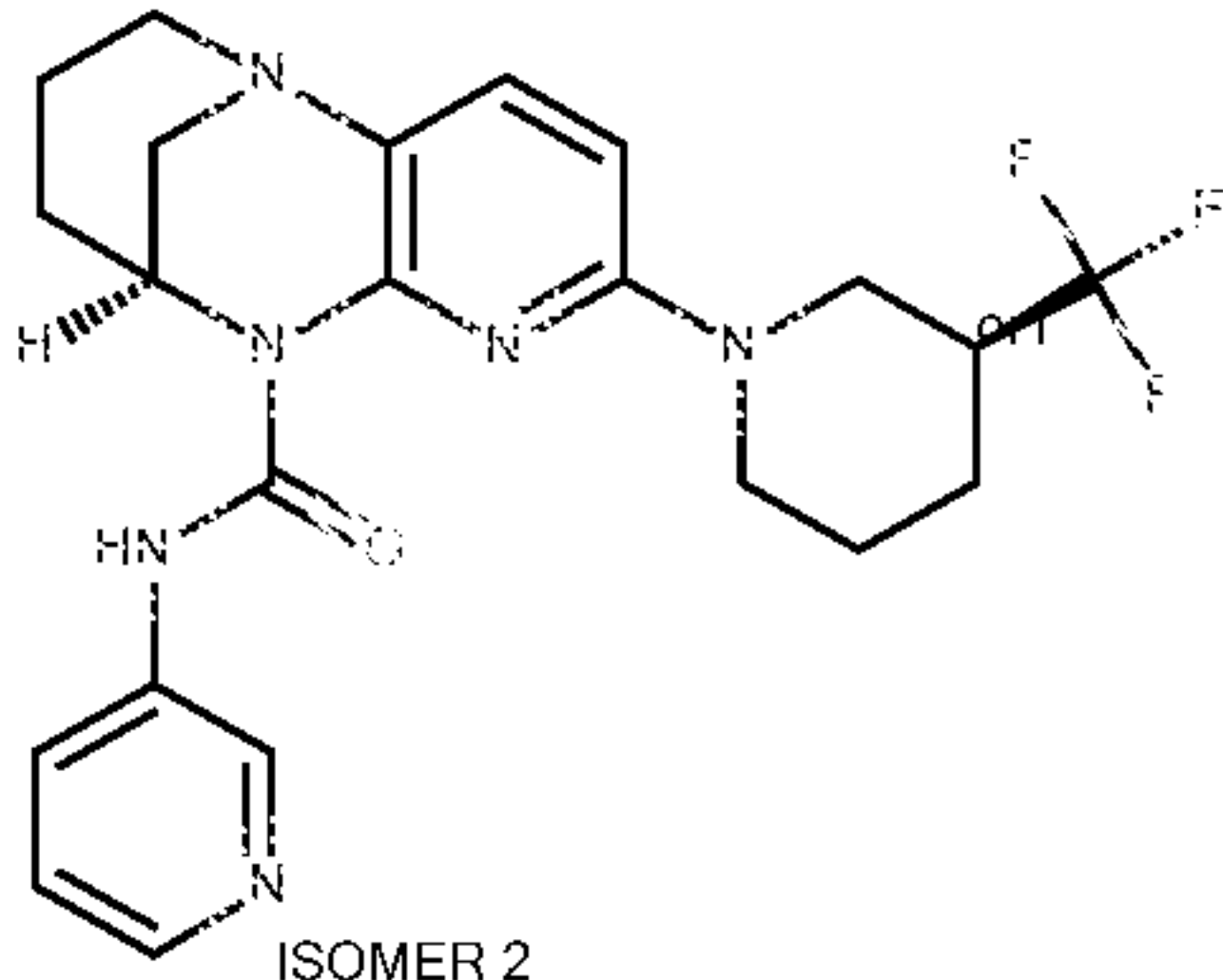
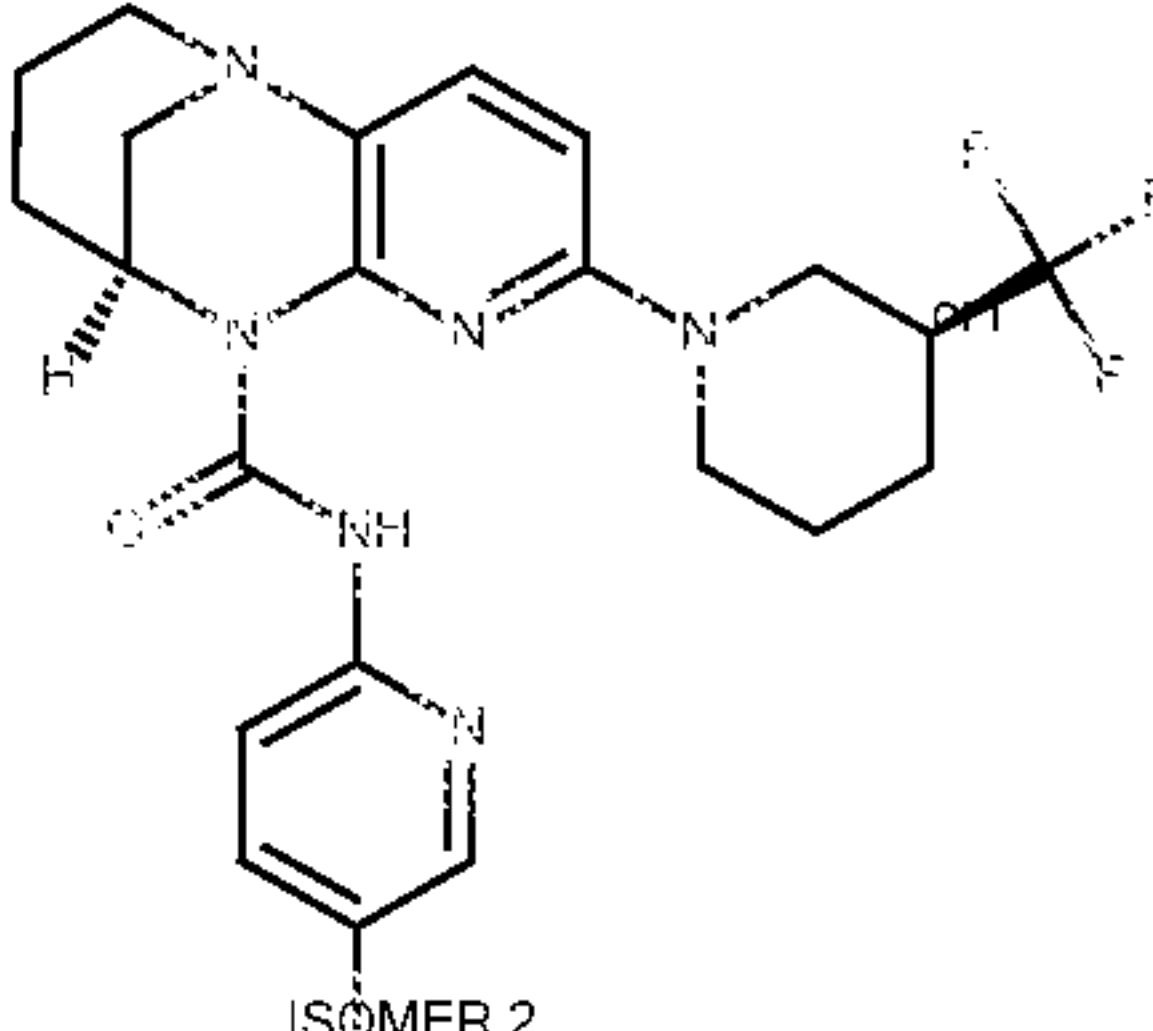
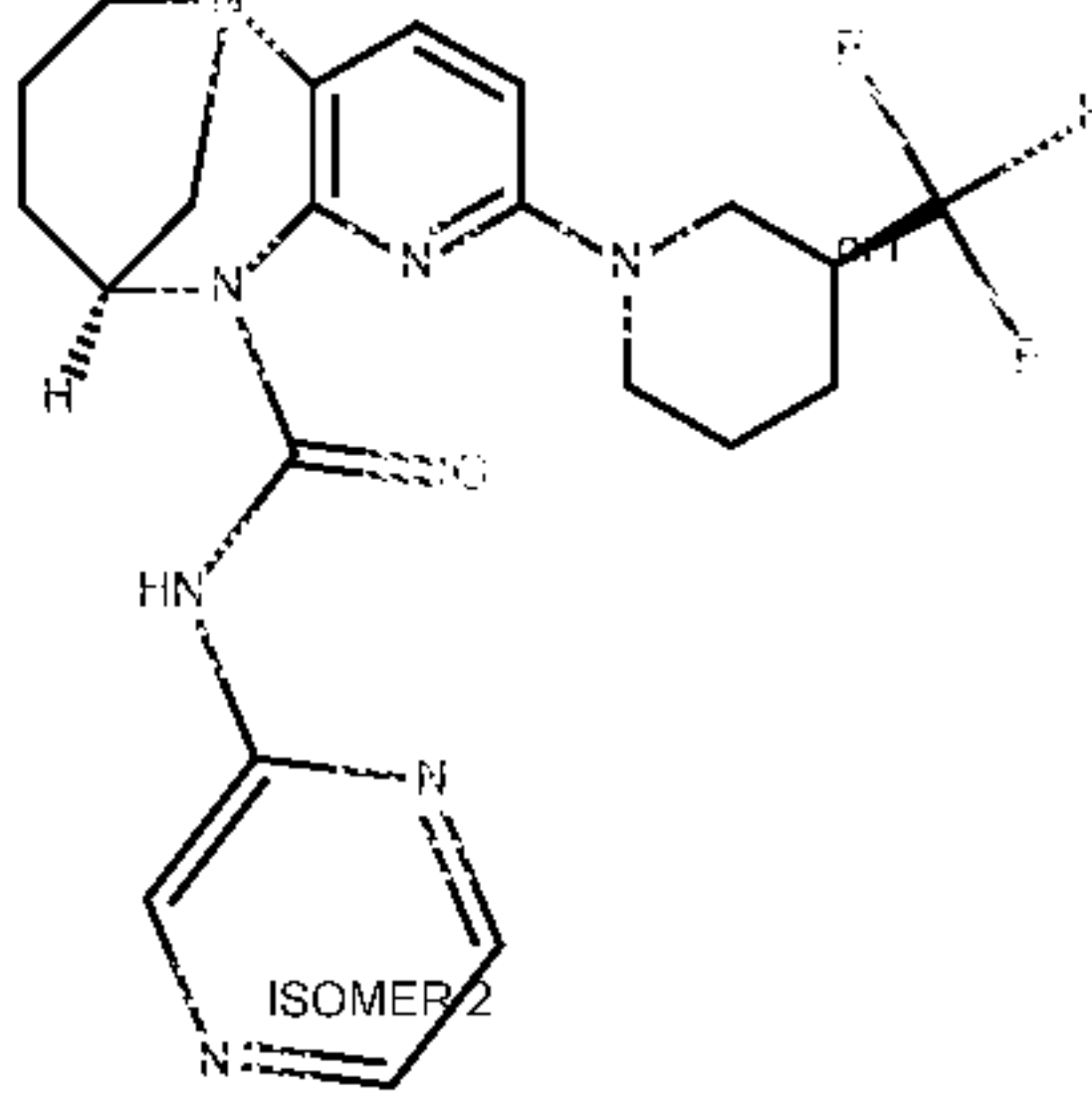
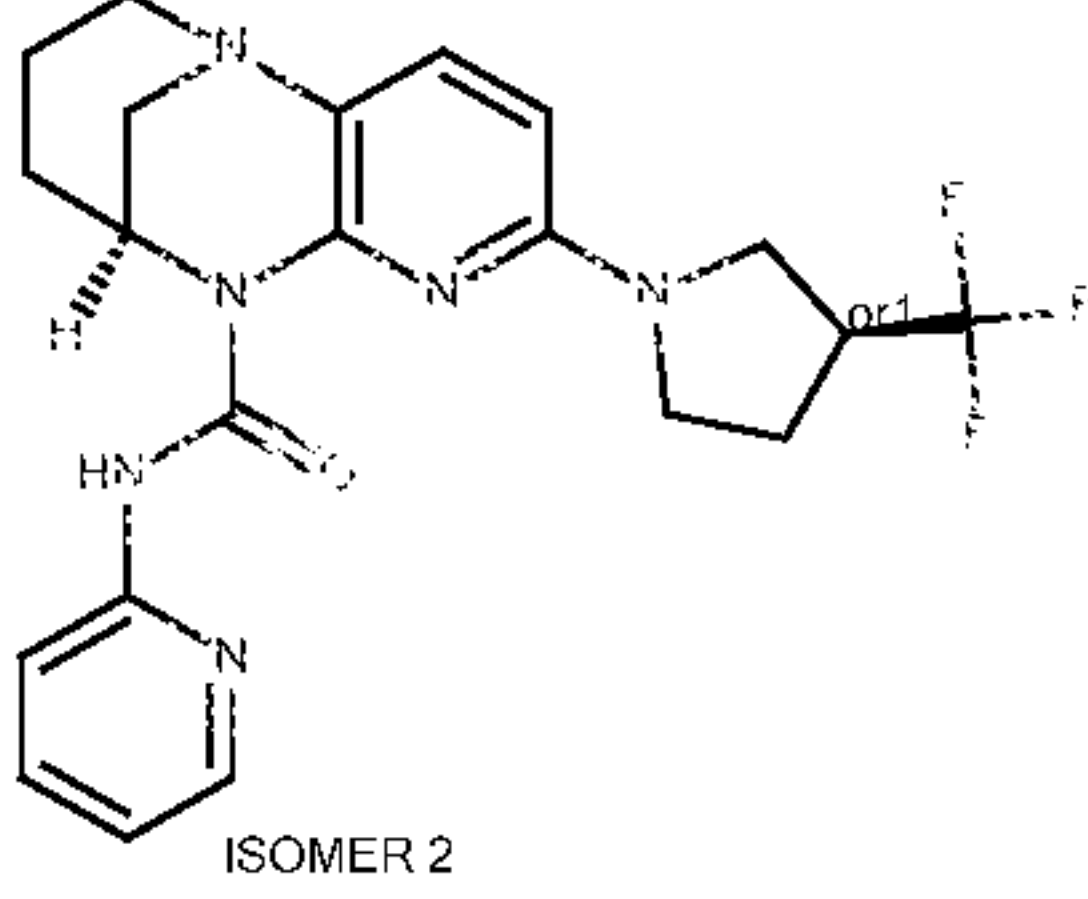
Structure	Chemical Name: Generated by ChemAxon
	(9S)-N-(pyrimidin-5-yl)-5-[5-(trifluoromethyl)pyridin-3-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
	(9S)-5-(2-methylpyridin-4-yl)-N-(pyridin-2-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
	(9S)-N-(pyridin-2-yl)-5-[(2R)-2-(trifluoromethyl)pyrrolidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
	(9S)-N-(5-fluoropyridin-3-yl)-5-(2-methylpyridin-4-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2,4,6-triene-8-carboxamide
	(9S)-N-(pyridazin-3-yl)-5-[2-(trifluoromethyl)pyridin-4-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide

Structure	Chemical Name: Generated by ChemAxon
	(9S)-N-(pyridin-2-yl)-5-[2-(trifluoromethyl)pyridin-4-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
	(9S)-N-(pyridin-2-yl)-5-[(3S)-3-(trifluoromethyl)pyrrolidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
	(9S)-N-(pyrimidin-4-yl)-5-[2-(trifluoromethyl)pyridin-4-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
	(9S)-N-(pyrimidin-5-yl)-5-[2-(trifluoromethyl)pyridin-4-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
	(9S)-N-(pyridin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide

Structure	Chemical Name: Generated by ChemAxon
	(9S)-5-(2-methylpyridin-4-yl)-N-(pyrimidin-4-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
	(9S)-5-(2-methylpyridin-4-yl)-N-(pyrazin-2-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
	(9S)-5-(2-methylpyridin-4-yl)-N-(pyridazin-3-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
	(9S)-N-(2-methyl-2H-indazol-5-yl)-5-(2-methylpyridin-4-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2,4,6-triene-8-carboxamide
	(9S)-5-(2-methylpyridin-4-yl)-N-(pyrimidin-5-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide

Structure	Chemical Name: Generated by ChemAxon
	(9S)-N-(pyrazin-2-yl)-5-[2-(trifluoromethyl)pyridin-4-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
	(9S)-N-(pyrimidin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
	(9S)-N-(dimethyl-1,3-thiazol-2-yl)-5-(2-methylpyridin-4-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
	(9S)-N-(6-methoxypyrazin-2-yl)-5-(2-methylpyridin-4-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
	(9S)-N-(6-methylpyrazin-2-yl)-5-(2-methylpyridin-4-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide

Structure	Chemical Name: Generated by ChemAxon
 ISOMER 2	(9S)-N-(pyrimidin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
 ISOMER 2	(9S)-N-(pyridin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
 ISOMER 1	(9S)-N-(5-fluoropyridin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
 ISOMER 1	(9S)-N-(pyridin-3-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
	(9S)-N-(1-methyl-1H-pyrazol-4-yl)-5-(2-methylpyridin-4-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide

Structure	Chemical Name: Generated by ChemAxon
 ISOMER 1	(9S)-N-(pyrazin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
 ISOMER 2	(9S)-N-(pyridin-3-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
 ISOMER 2	(9S)-N-(5-fluoropyridin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
 ISOMER 2	(9S)-N-(pyrazin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide
 ISOMER 2	(9S)-N-(pyridin-2-yl)-5-[(3S)-3-(trifluoromethyl)pyrrolidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide hydrochloride

TERMS AND DEFINITIONS

Section 1

Certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including
5 *cis*- and *trans*-isomers, (*R*)- and (*S*)-enantiomers, diastereomers, (*D*)-isomers, (*L*)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

10 The compounds and salts thereof described herein can also be present as the corresponding hydrates (e.g., hemihydrate, monohydrate, dihydrate, trihydrate, tetrahydrate) or solvates. Suitable solvents for preparation of solvates and hydrates can generally be selected by a skilled artisan.

The compounds and salts thereof can be present in amorphous or crystalline
15 (including co-crystalline and polymorph) forms.

Sirtuin-modulating compounds of the invention advantageously modulate the level and/or activity of a sirtuin protein, particularly the deacetylase activity of the sirtuin protein.

Separately or in addition to the above properties, certain sirtuin-modulating
20 compounds of the invention do not substantially have one or more of the following activities: inhibition of PI3-kinase, inhibition of aldoreductase, inhibition of tyrosine kinase, transactivation of EGFR tyrosine kinase, coronary dilation, or spasmolytic activity, at concentrations of the compound that are effective for modulating the deacetylation activity of a sirtuin protein (e.g., such as a SIRT1 and/or a SIRT3 protein).

25 An "alkyl" group or "alkane" is a straight chained or branched non-aromatic hydrocarbon which is completely saturated. Typically, a straight chained or branched alkyl group has from 1 to about 20 carbon atoms, preferably from 1 to about 10 unless otherwise defined. Examples of straight chained and branched alkyl groups include methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, *tert*-butyl, pentyl, hexyl, pentyl and
30 octyl. A C₁-C₄ straight chained or branched alkyl group is also referred to as a "lower alkyl" group.

In any of the preceding embodiments, a C₁-C₄ alkoxy-substituted group may include one or more alkoxy substituents such as one, two or three methoxy groups or a methoxy group

and an ethoxy group, for example. Exemplary C₁-C₄ alkoxy substituents include methoxy, ethoxy, isopropoxy, and *tert*-butoxy.

In any of the preceding embodiments, a hydroxy-substituted group may include one or more hydroxy substituents, such as two or three hydroxy groups.

5 A “halogen” refers to F, Cl, Br or I.

A “halogen-substitution” or “halo” substitution designates replacement of one or more hydrogens with F, Cl, Br or I.

In one aspect, the term haloalkyl is defined as any alkyl radical having one or more hydrogen atoms replaced by a halogen atom. In any of the preceding embodiments, a
10 “halo-substituted” group includes from one halo substituent up to perhalo substitution. Exemplary halo-substituted C₁-C₄ alkyl includes CFH₂, CClH₂, CBrH₂, CF₂H, CCl₂H, CBr₂H, CF₃, CCl₃, CBr₃, CH₂CH₂F, CH₂CH₂Cl, CH₂CH₂Br, CH₂CHF₂, CHFCH₃, CHClCH₃, CHBrCH₃, CF₂CHF₂, CF₂CHCl₂, CF₂CHBr₂, CH(CF₃)₂, and C(CF₃)₃. Perhalo-substituted C₁-C₄ alkyl, for example, includes CF₃, CCl₃, CBr₃, CF₂CF₃, CCl₂CF₃ and
15 CBr₂CF₃.

The terms “alkenyl” (“alkene”) and “alkynyl” (“alkyne”) refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyl groups described above, but that contain at least one double or triple bond respectively.

In any of the preceding embodiments, a “carbocycle” group may refer to a monocyclic
20 carbocycle embodiment and/or a polycyclic carbocycle embodiment, such as a fused, bridged or bicyclic carbocycle embodiment. “Carbocycle” groups of the invention may further refer to an aromatic carbocycle embodiment and/or a non-aromatic carbocycle embodiment, or, in the case of polycyclic embodiments, a carbocycle having both one or more aromatic rings and/or one or more non-aromatic rings. Polycyclic carbocycle
25 embodiments may be a bicyclic ring, a fused ring or a bridged bicycle. Non-limiting exemplary carbocycles include phenyl, cyclohexane, cyclopentane, or cyclohexene, amantadine, cyclopentane, cyclohexane, bicyclo[2.2.1]heptane, 1,5-cyclooctadiene, 1,2,3,4-tetrahydronaphthalene, bicyclo[4.2.0]oct-3-ene, naphthalene, adamantane, decalin, naphthalene, 1,2,3,4-tetrahydronaphthalene, norbornane, decalin, spiropentane,
30 memantine, biperiden, rimantadine, camphor, cholesterol, 4-phenylcyclohexanol, bicyclo[4.2.0]octane, memantine and 4,5,6,7-tetrahydro-1H-indene and bicyclo[4.1.0]hept-3-ene.

In any of the preceding embodiments, a “heterocycle” group may refer to a monocyclic heterocycle embodiment and/or a polycyclic heterocyclic embodiment, such as a fused, bridged or bicyclic heterocycle embodiment. “Heterocycle” groups of the invention may further refer to an aromatic heterocycle embodiment and/or a non-aromatic heterocycle embodiment, or, in the case of polycyclic embodiments, a heterocycle having both one or more aromatic rings and/or one or more non-aromatic rings. Polycyclic heterocycle embodiments may be a bicyclic ring, a fused ring or a bridged bicycle. Non-limiting exemplary heterocycles include pyridyl, pyrrolidine, piperidine, piperazine, pyrrolidine, morpholine, pyrimidine, benzofuran, indole, quinoline, lactones, lactams, benzodiazepine, indole, quinoline, purine, adenine, guanine, 4,5,6,7-tetrahydrobenzo[d]thiazole, hexamine and methenamine.

“Alkenyl” refers to an unsaturated hydrocarbon chain having the specified number of member carbon atoms and having one or more carbon-carbon double bonds within the chain. For example, C2-C6 alkenyl refers to an alkenyl group having from 2 to 6 member carbon atoms. In certain embodiments, alkenyl groups have one carbon-carbon double bond within the chain. In other embodiments, alkenyl groups have more than one carbon-carbon double bond within the chain. Alkenyl groups may be optionally substituted with one or more substituents as defined herein. Alkenyl groups may be straight or branched. Representative branched alkenyl groups have one, two, or three branches. Alkenyl includes ethylenyl, propenyl, butenyl, pentenyl, and hexenyl.

“Alkoxy” refers to an alkyl moiety attached through an oxygen bridge (i.e. a –O-C1-C6 alkyl group wherein C1-C6 is defined herein). Examples of such groups include methoxy, ethoxy, propoxy, butoxy, pentoxy and hexoxy.

“Alkynyl” refers to an unsaturated hydrocarbon chain having the specified number of member carbon atoms and having one or more carbon-carbon triple bonds within the chain. For example, C2-C6 alkynyl refers to an alkynyl group having from 2 to 6 member atoms. In certain embodiments alkynyl groups have one carbon-carbon triple bond within the chain. In other embodiments, alkynyl groups have more than one carbon-carbon triple bond within the chain. For the sake of clarity, unsaturated hydrocarbon chains having one or more carbon-carbon triple bond within the chain and one or more carbon-carbon double bond within the chain are referred to as alkynyl groups. Alkynyl groups may be optionally substituted with one or more substituents as

defined herein. Representative branched alkynyl groups have one, two, or three branches. Alkynyl includes ethynyl, propynyl, butynyl, pentynyl, and hexynyl.

The term “aromatic carbocycle” refers to an aromatic hydrocarbon ring system containing at least one aromatic ring. The ring may be fused or otherwise attached to other aromatic carbocyclic rings or non-aromatic carbocyclic rings. Examples of aromatic carbocycle groups include carbocyclic aromatic groups such as phenyl, naphthyl, and anthracyl.

“Azabicyclo” refers to a bicyclic molecule that contains a nitrogen atom in the ring skeleton. The two rings of the bicycle may be fused at two mutually bonded atoms, e.g., indole, across a sequence of atoms, e.g., azabicyclo[2.2.1]heptane, or joined at a single atom, e.g., spirocycle.

“Bicycle” or “bicyclic” refers to a two-ring system in which one, two or three or more atoms are shared between the two rings. Bicycle includes fused bicycles in which two adjacent atoms are shared by each of the two rings, e.g., decalin, indole. Bicycle also includes spiro bicycles in which two rings share a single atom, e.g., spiro[2.2]pentane, 1-oxa-6-azaspiro[3.4]octane. Bicycle further includes bridged bicycles in which at least three atoms are shared between two rings, e.g., norbornane.

“Bridged bicycle” compounds are bicyclic ring systems in which at least three atoms are shared by both rings of the system, i.e., they include at least one bridge of one or more atoms connecting two bridgehead atoms. Bridged azabicyclo refers to a bridged bicyclic molecule that contains a nitrogen atom in at least one of the rings.

The term “Boc” refers to a tert-butyloxycarbonyl group (a common amine protecting group).

The terms “carbocycle”, and “carbocyclic”, as used herein, refers to a saturated or unsaturated ring in which each atom of the ring is carbon. The term carbocycle includes both aromatic carbocycles and non-aromatic carbocycles. Non-aromatic carbocycles include both cycloalkane rings, in which all carbon atoms are saturated, and cycloalkene rings, which contain at least one double bond. “Carbocycle” includes 5-7 membered monocyclic and 8-12 membered bicyclic rings. Each ring of a bicyclic carbocycle may be selected from non-aromatic and aromatic rings. Carbocycle includes bicyclic molecules in which one, two or three or more atoms are shared between the two rings. The term “fused carbocycle” refers to a bicyclic carbocycle in which each of the rings shares two adjacent atoms with the other ring. Each ring of a fused carbocycle may be selected from non-

aromaticaromatic rings. In an exemplary embodiment, an aromatic ring, e.g., phenyl, may be fused to a non-aromatic or aromatic ring, e.g., cyclohexane, cyclopentane, or cyclohexene. Any combination of non-aromatic and aromatic bicyclic rings, as valence permits, is included in the definition of carbocyclic. Exemplary “carbocycles” include
5 cyclopentane, cyclohexane, bicyclo[2.2.1]heptane, 1,5-cyclooctadiene, 1,2,3,4-tetrahydronaphthalene, bicyclo[4.2.0]oct-3-ene, naphthalene and adamantane. Exemplary fused carbocycles include decalin, naphthalene, 1,2,3,4-tetrahydronaphthalene, bicyclo[4.2.0]octane, 4,5,6,7-tetrahydro-1H-indene and bicyclo[4.1.0]hept-3-ene. “Carbocycles” may be substituted at any one or more positions capable of bearing a
10 hydrogen atom.

A “cycloalkyl” group is a cyclic hydrocarbon ring having the specified number of member carbon atoms which is completely saturated (non-aromatic). Typically, a cycloalkyl group has from 3 to about 10 carbon atoms, more typically 3 to 8 carbon atoms unless otherwise defined. Cycloalkyl groups are monocyclic ring systems. For
15 example, C3-C6 cycloalkyl refers to a cycloalkyl group having from 3 to 6 member atoms. Cycloalkyl groups may be optionally substituted with one or more substituents as defined herein. Cycloalkyl includes cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

A “cycloalkenyl” group is a cyclic hydrocarbon ring containing one or more double
20 bonds within the ring. For example, C3-C6 cycloalkenyl refers to a cycloalkenyl group having from 3 to 6 member carbon atoms. In certain embodiments, cycloalkenyl groups have one carbon-carbon double bond within the ring. In other embodiments, cycloalkenyl groups have more than one carbon-carbon double bonds within the ring. Cycloalkenyl rings are not aromatic. Cycloalkenyl groups are monocyclic ring systems. Cycloalkenyl
25 groups may be optionally substituted with one or more substituents as defined herein. Cycloalkenyl includes cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, and cyclohexadienyl.

“Aryl” refers to an aromatic hydrocarbon ring system. Aryl groups are monocyclic ring systems or bicyclic ring systems. Monocyclic aryl ring refers to phenyl. Bicyclic aryl
30 rings refer to naphthyl and to rings wherein phenyl is fused to a cycloalkyl or cycloalkenyl ring having 5, 6, or 7 member carbon atoms. Aryl groups may be optionally substituted with one or more substituents as defined herein.

The term “heteroaryl” or “aromatic heterocycle” includes substituted or unsubstituted aromatic single ring structures, preferably 5- to 7-membered rings, more preferably 5- to 6-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The term “heteroaryl” also includes ring systems having one or two rings wherein at least one of the rings is heteroaromatic, e.g., the other cyclic rings can be cycloalkyl, cycloalkenyl, cycloalkynyl, aromatic carbocycle, heteroaryl, and/or heterocyclyl. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, and pyrimidine.

The terms “heterocycle”, and “heterocyclic”, as used herein, refers to a non-aromatic or aromatic ring comprising one or more heteroatoms selected from, for example, N, O, B and S atoms, preferably N, O, or S. The term “heterocycle” includes both “aromatic heterocycles” and “non-aromatic heterocycles.” Heterocycles include 4-7 membered monocyclic and 8-12 membered bicyclic rings. Heterocycle includes bicyclic molecules in which one, two or three or more atoms are shared between the two rings. Each ring of a bicyclic heterocycle may be selected from non-aromatic and aromatic rings. The term “fused heterocycle” refers to a bicyclic heterocycle in which each of the rings shares two adjacent atoms with the other ring. Each ring of a fused heterocycle may be selected from non-aromatic and aromatic rings. In an exemplary embodiment, an aromatic ring, e.g., pyridyl, may be fused to a non-aromatic or aromatic ring, e.g., cyclohexane, cyclopentane, pyrrolidine, 2,3-dihydrofuran or cyclohexene. “Heterocycle” groups include, for example, piperidine, piperazine, pyrrolidine, morpholine, pyrimidine, benzofuran, indole, quinoline, lactones, and lactams. Exemplary “fused heterocycles” include benzodiazepine, indole, quinoline, purine, and 4,5,6,7-tetrahydrobenzo[d]thiazole. “Heterocycles” may be substituted at any one or more positions capable of bearing a hydrogen atom.

“Monocyclic rings” include 5-7 membered aromatic carbocycle or heteroaryl, 3-7 membered cycloalkyl or cycloalkenyl, and 5-7 membered non-aromatic heterocyclyl. Exemplary monocyclic groups include substituted or unsubstituted heterocycles or carbocycles such as thiazolyl, oxazolyl, oxazinyl, thiazinyl, dithianyl, dioxanyl, isoxazolyl, isothiazolyl, triazolyl, furanyl, tetrahydrofuranyl, dihydrofuranyl, pyranal, tetrazolyl, pyrazolyl, pyrazinyl, pyridazinyl, imidazolyl, pyridinyl, pyrrolyl, dihydropyrrolyl, pyrrolidinyl, piperidinyl, piperazinyl, pyrimidinyl, morpholinyl, tetrahydrothiophenyl,

thiophenyl, cyclohexyl, cyclopentyl, cyclopropyl, cyclobutyl, cycloheptanyl, azetidiny, oxetanyl, thiiranyl, oxiranyl, aziridiny, and thiomorpholinyl.

"Member atoms" refers to the atom or atoms that form a chain or ring. Where more than one member atom is present in a chain and within a ring, each member atom is covalently bound to an adjacent member atom in the chain or ring. Atoms that make up a substituent group on a chain or ring are not member atoms in the chain or ring.

"Optionally substituted" indicates that a group, such as alkyl, alkenyl, alkynyl, aryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heteroaryl, may be unsubstituted, or the group may be substituted with one or more substituents as defined herein.

10

As used herein, "substituted" means substituting a hydrogen atom in a structure with an atom or molecule other than hydrogen. "Substituted" in reference to a group indicates that one or more hydrogen atoms attached to a member atom within the group is replaced with a substituent selected from the group of defined substituents. A substitutable atom such as a "substitutable nitrogen" is an atom that bears a hydrogen atom in at least one resonance form. The hydrogen atom may be substituted for another atom or group such as a CH₃ or an OH group. For example, the nitrogen in a piperidine molecule is substitutable if the nitrogen is bound to a hydrogen atom. If, for example, the nitrogen of a piperidine is bound to an atom other than hydrogen, the nitrogen is not substitutable. An atom that is not capable of bearing a hydrogen atom in any resonance form is not substitutable. . It should be understood that the term "substituted" includes the implicit provision that such substitution be in accordance with the permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound (i.e. one that does not spontaneously undergo transformation such as by hydrolysis, rearrangement, cyclization, or elimination, and that is sufficiently robust to survive isolation from a reaction mixture). When it is stated that a group may contain one or more substituents, one or more (as appropriate) member atom within the group may be substituted. In addition, a single member atom within the group may be substituted with more than one substituent as long as such substitution is in accordance with the permitted valence of the atom. Suitable substituents are defined herein for each substituted or optionally substituted group.

30

Combinations of substituents and variables envisioned by this invention are only those that result in the formation of stable compounds. As used herein, the term "stable"

refers to compounds that possess stability sufficient to allow manufacture and that maintain the integrity of the compound for a sufficient period of time to be useful for the purposes detailed herein.

Deuterated Compounds

5 The compounds disclosed herein also include partially and fully deuterated variants. In certain embodiments, deuterated variants may be used for kinetic studies. One of skill in the art can select the sites at which such deuterium atoms are present.

 The invention also includes various deuterated forms of the compounds of Formulas (I) or pharmaceutically acceptable salts thereof. Each available hydrogen
10 atom attached to a carbon atom may be independently replaced with a deuterium atom. A person of ordinary skill in the art will know how to synthesize deuterated forms of the compounds of Formulas(I) to (II) of the present invention. For example, deuterated materials, such as alkyl groups may be prepared by conventional techniques (see for example: methyl-*d*₃-amine available from Aldrich Chemical Co., Milwaukee, WI, Cat.
15 No.489,689-2).

ISOTOPES

 The subject invention also includes isotopically-labeled compounds which are identical to those recited in Formulas (I) and (II) but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the
20 atomic mass or mass number most commonly found in nature. Examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, fluorine, iodine and chlorine such as ³H, ¹¹C, ¹⁴C, ¹⁸F, ¹²³I or ¹²⁵I.

 Compounds of the present invention and pharmaceutically acceptable salts of said compounds that contain the aforementioned isotopes and/or other isotopes of other atoms are within the scope of the present invention. Isotopically labeled compounds of the present invention, for example those into which radioactive isotopes such as ³H or ¹⁴C have been incorporated, are useful in drug and/or substrate tissue distribution assays. Tritiated, ie. ³H, and carbon-14, ie. ¹⁴C, isotopes are particularly preferred for their ease
30 of preparation and detectability. ¹¹C and ¹⁸F isotopes are particularly useful in PET (positron emission tomography).

PURITY

Because the compounds of the present invention are intended for use in pharmaceutical compositions it will readily be understood that they are each preferably provided in substantially pure form, for example at least 60% pure, more suitably at least 75% pure and preferably at least 85%, especially at least 98% pure (% are on a weight
5 for weight basis). Impure preparations of the compounds may be used for preparing the more pure forms used in the pharmaceutical compositions.

SALTS

In certain embodiments, compounds according to Formula I or a pharmaceutically acceptable salt thereof may contain an acidic functional group. In
10 certain other embodiments, compounds according to Formula I may contain a basic functional group. Thus, the skilled artisan will appreciate that salts of the compounds according to Formula I may be prepared. Indeed, in certain embodiments of the invention, salts of the compounds according to Formula I may be preferred over the respective free base or free acid because, for example, such salts may impart greater
15 stability or solubility to the molecule thereby facilitating formulation into a dosage form.

Because of their potential use in medicine, the salts of the compounds of Formulas (I) are suitably pharmaceutically acceptable salts. Suitable pharmaceutically acceptable salts include those described by Berge, Bighley and Monkhouse J.Pharm.Sci (1977) 66, pp 1-19.

20 Also included in the present invention are salts, particularly pharmaceutically acceptable salts, of the compounds described herein. The compounds of the present invention that possess a sufficiently acidic, a sufficiently basic, or both functional groups, can react with any of a number of inorganic bases, and inorganic and organic acids, to form a salt. Alternatively, compounds that are inherently charged, such as those with
25 quaternary nitrogen, can form a salt with an appropriate counterion (e.g., a halide such as bromide, chloride, or fluoride, particularly bromide).

Acids commonly employed to form acid addition salts are inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid, and the like, and organic acids such as p-toluenesulfonic acid, methanesulfonic acid, oxalic
30 acid, p-bromophenyl-sulfonic acid, carbonic acid, succinic acid, citric acid, benzoic acid, acetic acid, and the like. Examples of such salts include the sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate,

caprylate, acrylate, formate, isobutyrate, caproate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, sulfonate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, gamma-hydroxybutyrate, glycolate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate, and the like.

Base addition salts include those derived from inorganic bases, such as ammonium or alkali or alkaline earth metal hydroxides, carbonates, bicarbonates, and the like. Such bases useful in preparing the salts of this invention thus include sodium hydroxide, potassium hydroxide, ammonium hydroxide, potassium carbonate, and the like.

"Enantiomeric excess" or "ee" is the excess of one enantiomer over the other expressed as a percentage. As a result, since both enantiomers are present in equal amounts in a racemic mixture, the enantiomeric excess is zero (0% ee). However, if one enantiomer was enriched such that it constitutes 95% of the product, then the enantiomeric excess would be 90% ee (the amount of the enriched enantiomer, 95%, minus the amount of the other enantiomer, 5%).

"Enantiomerically enriched" refers to products whose enantiomeric excess is greater than zero. For example, enantiomerically enriched refers to products whose enantiomeric excess is greater than 50% ee, greater than 75% ee, or greater than 90% ee.

"Enantiomerically pure" refers to products whose enantiomeric excess is 99% ee or greater.

"Pharmaceutically acceptable" refers to those compounds, materials, compositions, and dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings or animals without excessive toxicity, irritation, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The compounds according to Formula (I) or a pharmaceutically acceptable salt thereof, may contain one or more asymmetric centers (also referred to as a chiral center) and may, therefore, exist as individual enantiomers, diastereomers, or other stereoisomeric forms, or as mixtures thereof.

Chiral centers, such as chiral carbon atoms, may also be present in a substituent such as an alkyl group. Where the stereochemistry of a chiral center present in Formula I, or in any chemical

structure illustrated herein, is not specified, the structure is intended to encompass all individual stereoisomers and all mixtures thereof.

Thus, compounds according to Formula (I) or pharmaceutically acceptable salts thereof, containing one or more chiral centers may be used as racemic mixtures, diastereomeric mixtures, 5 enantiomerically enriched mixtures, diastereomerically enriched mixtures, or as enantiomerically and diastereomerically pure individual stereoisomers.

Individual stereoisomers of a compound according to Formula (I) or a pharmaceutically acceptable salt thereof which contain one or more asymmetric centers may be resolved by methods known to those skilled in the art. For example, such 10 resolution may be carried out (1) by formation of diastereoisomeric salts, complexes or other derivatives; (2) by selective reaction with a stereoisomer-specific reagent, for example by enzymatic oxidation or reduction; or (3) by gas-liquid or liquid chromatography in a chiral environment, for example, on a chiral support such as silica with a bound chiral ligand or in the presence of a chiral solvent. The skilled artisan will 15 appreciate that where the desired stereoisomer is converted into a diastereomeric salt, complex or derivative, a further step is required to liberate the desired form. Alternatively, specific stereoisomers may be synthesized by asymmetric synthesis using optically active reagents, substrates, catalysts or solvents, or by converting one enantiomer to the other by asymmetric transformation.

20 When a disclosed compound or its salt is named or depicted by structure, it is to be understood that the compound or salt, including solvates (particularly, hydrates) thereof, may exist in crystalline forms, non-crystalline forms or a mixture thereof. The compound or salt, or solvates (particularly, hydrates) thereof, may also exhibit polymorphism (i.e. the capacity to occur in different crystalline forms). These different 25 crystalline forms are typically known as "polymorphs."

In light of this, salt forms of the present invention (i.e., which may include different polymorphs, anhydrous forms, solvates, or hydrates thereof) may exhibit characteristic polymorphism. As conventionally understood in the art, polymorphism is defined as an ability of a compound to crystallize as more than one distinct crystalline or "polymorphic" species. A 30 polymorph is defined as a solid crystalline phase of a compound with at least two different arrangements or polymorphic forms of that compound molecule in the solid state.

Polymorphic forms of any given compound, including those of the present invention, are defined by the same chemical formula or composition and are as distinct in chemical structure as crystalline structures of two different chemical compounds. Such

compounds may differ in packing, geometrical arrangement of respective crystalline lattices, etc.

It is to be understood that when named or depicted by structure, the disclosed compound, or solvates (particularly, hydrates) thereof, also include all polymorphs thereof.

5 Polymorphs have the same chemical composition but differ in packing, geometrical arrangement, and other descriptive properties of the crystalline solid state.

In light of the foregoing, chemical and/or physical properties or characteristics vary with each distinct polymorphic form, which may include variations in solubility, melting point, density, hardness, crystal shape, optical and electrical properties, vapor pressure,
10 stability, etc.

Solvates and/or hydrates of crystalline salt forms of the present invention also may be formed when solvent molecules are incorporated into the crystalline lattice structure of the compound molecule during the crystallization process. For example, solvate forms of the present invention may incorporate nonaqueous solvents such as methanol and the like
15 as described herein below. Hydrate forms are solvate forms, which incorporate water as a solvent into a crystalline lattice.

Anhydrous with respect to solid state polymorphism refers to a crystalline structure that does not contain a repeating, crystalline solvent in the lattice. However, crystalline materials can be porous and may exhibit reversible surface adsorption of water.

20 **TERMS AND DEFINITIONS**

Section 2

1. Definitions

As used herein, the following terms and phrases shall have the meanings set forth below. Unless defined otherwise, all technical and scientific terms used herein have the
25 same meaning as commonly understood to one of ordinary skill in the art.

The term “agent” is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule (such as a nucleic acid, an antibody, a protein or portion thereof, e.g., a peptide), or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian) cells or tissues.

30 The term “bioavailable”, when referring to a compound, is art-recognized and refers to a form of a compound that allows for all or a portion of the amount of compound

administered to be absorbed by, incorporated into, or otherwise physiologically available to a subject or patient to whom it is administered.

“Biologically active portion of a sirtuin” refers to a portion of a sirtuin protein having a biological activity, such as the ability to deacetylate (“catalytically active”).

5 Catalytically active portions of a sirtuin may comprise the core domain of sirtuins.

Catalytically active portions of SIRT1 having GenBank Accession No. NP_036370 that encompass the NAD⁺ binding domain and the substrate binding domain, for example, may include without limitation, amino acids 240-664 or 240-505 of GenBank Accession No. NP_036370, which are encoded by the polynucleotide of GenBank Accession No.

10 NM_012238. Therefore, this region is sometimes referred to as the core domain. Other catalytically active portions of SIRT1, also sometimes referred to as core domains, include about amino acids 261 to 447 of GenBank Accession No. NP_036370, which are encoded by nucleotides 834 to 1394 of GenBank Accession No. NM_012238; about amino acids 242 to 493 of GenBank Accession No. NP_036370, which are encoded by
15 nucleotides 777 to 1532 of GenBank Accession No. NM_012238; or about amino acids 254 to 495 of GenBank Accession No. NP_036370, which are encoded by nucleotides 813 to 1538 of GenBank Accession No. NM_012238. Another “biologically active” portion of SIRT1 is amino acids 62-293 or 183-225 of GenBank Accession No. NP_036370, which comprise a domain N-terminal to the core domain that is important to
20 the compound binding site.

The term “companion animals” refers to cats and dogs. As used herein, the term “dog(s)” denotes any member of the species *Canis familiaris*, of which there are a large number of different breeds. The term “cat(s)” refers to a feline animal including domestic cats and other members of the family *Felidae*, genus *Felis*.

25 “Diabetes” refers to high blood sugar or ketoacidosis, as well as chronic, general metabolic abnormalities arising from a prolonged high blood sugar status or a decrease in glucose tolerance. “Diabetes” encompasses both the type I and type II (Non Insulin Dependent Diabetes Mellitus or NIDDM) forms of the disease. The risk factors for diabetes include the following factors: waistline of more than 40 inches for men or 35
30 inches for women, blood pressure of 130/85 mmHg or higher, triglycerides above 150 mg/dl, fasting blood glucose greater than 100 mg/dl or high-density lipoprotein of less than 40 mg/dl in men or 50 mg/dl in women.

The term “ED₅₀” refers to the art-recognized measure of effective dose. In certain embodiments, ED₅₀ means the dose of a drug which produces 50% of its maximum response or effect, or alternatively, the dose which produces a pre-determined response in 50% of test subjects or preparations, such as isolated tissue or cells. The term “LD₅₀” refers to the art-recognized measure of lethal dose. In certain embodiments, LD₅₀ means the dose of a drug which is lethal in 50% of test subjects. The term “therapeutic index” is an art-recognized term which refers to the therapeutic index of a drug, defined as LD₅₀/ED₅₀.

The term “hyperinsulinemia” refers to a state in an individual in which the level of insulin in the blood is higher than normal.

The term “insulin resistance” refers to a state in which a normal amount of insulin produces a subnormal biologic response relative to the biological response in a subject that does not have insulin resistance.

An “insulin resistance disorder,” as discussed herein, refers to any disease or condition that is caused by or contributed to by insulin resistance. Examples include: diabetes, obesity, metabolic syndrome, insulin-resistance syndromes, syndrome X, insulin resistance, high blood pressure, hypertension, high blood cholesterol, dyslipidemia, hyperlipidemia, atherosclerotic disease including stroke, coronary artery disease or myocardial infarction, hyperglycemia, hyperinsulinemia and/or hyperproinsulinemia, impaired glucose tolerance, delayed insulin release, diabetic complications, including coronary heart disease, angina pectoris, congestive heart failure, stroke, cognitive functions in dementia, retinopathy, peripheral neuropathy, nephropathy, glomerulonephritis, glomerulosclerosis, nephrotic syndrome, hypertensive nephrosclerosis, some types of cancer (such as endometrial, breast, prostate, and colon), complications of pregnancy, poor female reproductive health (such as menstrual irregularities, infertility, irregular ovulation, polycystic ovarian syndrome (PCOS)), lipodystrophy, cholesterol-related disorders, such as gallstones, cholecystitis and cholelithiasis, gout, obstructive sleep apnea and respiratory problems, osteoarthritis, and bone loss, e.g., osteoporosis in particular.

The term “livestock animals” refers to domesticated quadrupeds, which includes those being raised for meat and various byproducts, e.g., a bovine animal including cattle and other members of the genus *Bos*, a porcine animal including domestic swine and other members of the genus *Sus*, an ovine animal including sheep and other members of the

genus *Ovis*, domestic goats and other members of the genus *Capra*; domesticated quadrupeds being raised for specialized tasks such as use as a beast of burden, e.g., an equine animal including domestic horses and other members of the family *Equidae*, genus *Equus*.

5 The term “mammal” is known in the art, and exemplary mammals include humans, primates, livestock animals (including bovines, porcines, etc.), companion animals (e.g., canines, felines, etc.) and rodents (e.g., mice and rats).

 “Obese” individuals or individuals suffering from obesity are generally individuals having a body mass index (BMI) of at least 25 or greater. Obesity may or
10 may not be associated with insulin resistance.

 The terms “parenteral administration” and “administered parenterally” are art-recognized and refer to modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac,
15 intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intra-articular, subcapsular, subarachnoid, intraspinal, and intrasternal injection and infusion.

 A “patient”, “subject”, “individual” or “host” refers to either a human or a non-human animal.

 The term “pharmaceutically acceptable carrier” is art-recognized and refers to a
20 pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting any subject composition or component thereof. Each carrier must be “acceptable” in the sense of being compatible with the subject composition and its components and not injurious to the patient. Some examples of materials which may serve
25 as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame
30 oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide

and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

5 The term "preventing" is art-recognized, and when used in relation to a condition, such as a local recurrence (e.g., pain), a disease such as cancer, a syndrome complex such as heart failure or any other medical condition, is well understood in the art, and includes administration of a composition which reduces the frequency of, or delays the onset of, symptoms of a medical condition in a subject relative to a subject which does not receive the composition. Thus, prevention of cancer includes, for example, reducing the number
10 of detectable cancerous growths in a population of patients receiving a prophylactic treatment relative to an untreated control population, and/or delaying the appearance of detectable cancerous growths in a treated population versus an untreated control population, e.g., by a statistically and/or clinically significant amount. Prevention of an infection includes, for example, reducing the number of diagnoses of the infection in a
15 treated population versus an untreated control population, and/or delaying the onset of symptoms of the infection in a treated population versus an untreated control population. Prevention of pain includes, for example, reducing the magnitude of, or alternatively delaying, pain sensations experienced by subjects in a treated population versus an untreated control population.

20 The term "prophylactic" or "therapeutic" treatment is art-recognized and refers to administration of a drug to a host. If it is administered prior to clinical manifestation of the unwanted condition (e.g., disease or other unwanted state of the host animal) then the treatment is prophylactic, i.e., it protects the host against developing the unwanted condition, whereas if administered after manifestation of the unwanted condition, the
25 treatment is therapeutic (i.e., it is intended to diminish, ameliorate or maintain the existing unwanted condition or side effects therefrom).

The term "pyrogen-free", with reference to a composition, refers to a composition that does not contain a pyrogen in an amount that would lead to an adverse effect (e.g., irritation, fever, inflammation, diarrhea, respiratory distress, endotoxic shock, etc.) in a
30 subject to which the composition has been administered. For example, the term is meant to encompass compositions that are free of, or substantially free of, an endotoxin such as, for example, a lipopolysaccharide (LPS).

“Replicative lifespan” of a cell refers to the number of daughter cells produced by an individual “mother cell.” “Chronological aging” or “chronological lifespan,” on the other hand, refers to the length of time a population of non-dividing cells remains viable when deprived of nutrients. “Increasing the lifespan of a cell” or “extending the lifespan of a cell,” as applied to cells or organisms, refers to increasing the number of daughter cells produced by one cell; increasing the ability of cells or organisms to cope with stresses and combat damage, e.g., to DNA, proteins; and/or increasing the ability of cells or organisms to survive and exist in a living state for longer under a particular condition, e.g., stress (for example, heatshock, osmotic stress, high energy radiation, chemically-induced stress, DNA damage, inadequate salt level, inadequate nitrogen level, or inadequate nutrient level). Lifespan can be increased by at least about 10%, 20%, 30%, 40%, 50%, 60% or between 20% and 70%, 30% and 60%, 40% and 60% or more using methods described herein.

“Sirtuin-modulating compound” refers to a compound that increases the level of a sirtuin protein and/or increases at least one activity of a sirtuin protein. In an exemplary embodiment, a sirtuin-modulating compound may increase at least one biological activity of a sirtuin protein by at least about 10%, 25%, 50%, 75%, 100%, or more. Exemplary biological activities of sirtuin proteins include deacetylation, e.g., of histones and p53; extending lifespan; increasing genomic stability; silencing transcription; and controlling the segregation of oxidized proteins between mother and daughter cells.

proteins include deacetylation, e.g., of an acetylated peptide substrate.

“Sirtuin protein” refers to a member of the sirtuin deacetylase protein family, or preferably to the sir2 family, which include yeast Sir2 (GenBank Accession No. P53685), *C. elegans* Sir-2.1 (GenBank Accession No. NP_501912), and human SIRT1 (GenBank Accession No. NM_012238 and NP_036370 (or AF083106)) and SIRT2 (GenBank Accession No. NM_012237, NM_030593, NP_036369, NP_085096, and AF083107) proteins. Other family members include the four additional yeast Sir2-like genes termed “HST genes” (homologues of Sir two) HST1, HST2, HST3 and HST4, and the five other human homologues hSIRT3, hSIRT4, hSIRT5, hSIRT6 and hSIRT7 (Brachmann et al. (1995) Genes Dev. 9:2888 and Frye et al. (1999) BBRC 260:273).

“SIRT1 protein” refers to a member of the sir2 family of sirtuin deacetylases. In certain embodiments, a SIRT1 protein includes yeast Sir2 (GenBank Accession No. P53685), *C. elegans* Sir-2.1 (GenBank Accession No. NP_501912), human SIRT1

(GenBank Accession No. NM_012238 or NP_036370 (or AF083106)), mouse SIRT1 (GenBank Accession No. NM_019812 or NP_062786), and equivalents and fragments thereof. In another embodiment, a SIRT1 protein includes a polypeptide comprising a sequence consisting of, or consisting essentially of, the amino acid sequence set forth in

5 GenBank Accession Nos. NP_036370, NP_501912, NP_085096, NP_036369, or P53685. SIRT1 proteins include polypeptides comprising all or a portion of the amino acid sequence set forth in GenBank Accession Nos. NP_036370, NP_501912, NP_085096, NP_036369, or P53685; the amino acid sequence set forth in GenBank Accession Nos. NP_036370, NP_501912, NP_085096, NP_036369, or P53685 with 1 to about 2, 3, 5, 7,

10 10, 15, 20, 30, 50, 75 or more conservative amino acid substitutions; an amino acid sequence that is at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to GenBank Accession Nos. NP_036370, NP_501912, NP_085096, NP_036369, or P53685, and functional fragments thereof. Polypeptides of the invention also include homologs (e.g., orthologs and paralogs), variants, or fragments, of GenBank Accession Nos.

15 NP_036370, NP_501912, NP_085096, NP_036369, or P53685.

As used herein “SIRT2 protein”, “SIRT3 protein”, “SIRT4 protein”, SIRT5 protein”, “SIRT6 protein”, and “SIRT7 protein” refer to other mammalian, e.g. human, sirtuin deacetylase proteins that are homologous to SIRT1 protein, particularly in the approximately 275 amino acid conserved catalytic domain. For example, “SIRT3 protein”

20 refers to a member of the sirtuin deacetylase protein family that is homologous to SIRT1 protein. In certain embodiments, a SIRT3 protein includes human SIRT3 (GenBank Accession No. AAH01042, NP_036371, or NP_001017524) and mouse SIRT3 (GenBank Accession No. NP_071878) proteins, and equivalents and fragments thereof. In certain embodiments, a SIRT4 protein includes human SIRT4 (GenBank Accession No.

25 NM_012240 or NP_036372). In certain embodiments, a SIRT5 protein includes human SIRT5 (GenBank Accession No. NM_012241 or NP_036373). In certain embodiments, a SIRT6 protein includes human SIRT6 (GenBank Accession No. NM_016539 or NP_057623). In another embodiment, a SIRT3 protein includes a polypeptide comprising a sequence consisting of, or consisting essentially of, the amino acid sequence set forth in

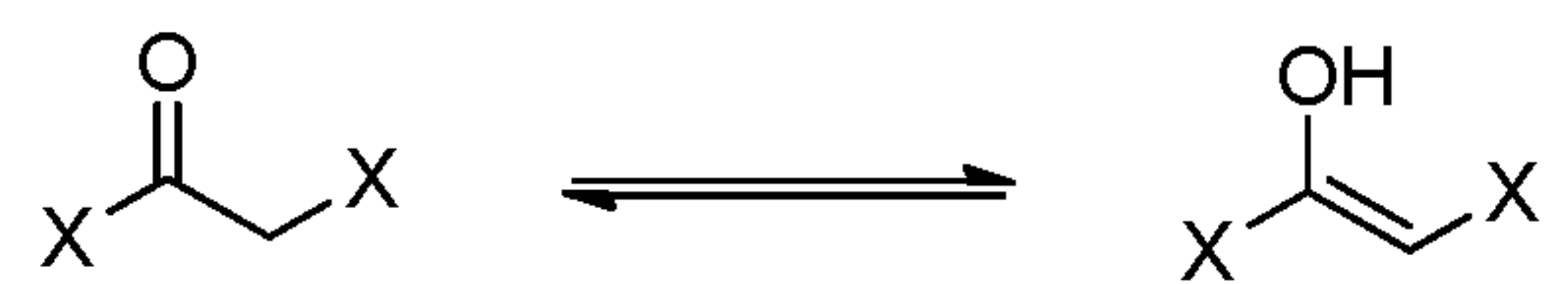
30 GenBank Accession Nos. AAH01042, NP_036371, NP_001017524, or NP_071878. SIRT3 proteins include polypeptides comprising all or a portion of the amino acid sequence set forth in GenBank Accession AAH01042, NP_036371, NP_001017524, or NP_071878; the amino acid sequence set forth in GenBank Accession Nos. AAH01042,

NP_036371, NP_001017524, or NP_071878 with 1 to about 2, 3, 5, 7, 10, 15, 20, 30, 50, 75 or more conservative amino acid substitutions; an amino acid sequence that is at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to GenBank Accession Nos. AAH01042, NP_036371, NP_001017524, or NP_071878, and functional fragments thereof. Polypeptides of the invention also include homologs (e.g., orthologs and paralog), variants, or fragments, of GenBank Accession Nos. AAH01042, NP_036371, NP_001017524, or NP_071878. In certain embodiments, a SIRT3 protein includes a fragment of SIRT3 protein that is produced by cleavage with a mitochondrial matrix processing peptidase (MPP) and/or a mitochondrial intermediate peptidase (MIP).

The term “stereoisomer” as used herein is art-recognized and refers to any of two or more isomers that have the same molecular constitution and differ only in the three-dimensional arrangement of their atomic groupings in space. When used herein to describe a compound or genus of compounds, stereoisomer includes any portion of the compound or the compound in its entirety. For example, diastereomers and enantiomers are stereoisomers.

The terms “systemic administration” and “administered systemically,” are art-recognized and refer to the administration of a subject composition, therapeutic or other material enterally or parenterally.

The term “tautomer” as used herein is art-recognized and refers to any one of the possible alternative structures that may exist as a result of tautomerism, which refers to a form of constitutional isomerism in which a structure may exist in two or more constitutional arrangements, particularly with respect to the position of hydrogens bonded to oxygen. When used herein to describe a compound or genus of compounds, it is further understood that a “tautomer” is readily interconvertible and exists in equilibrium. For example, keto and enol tautomers exist in proportions determined by the equilibrium position for any given condition, or set of conditions:



The term “therapeutic agent” is art-recognized and refers to any biologically, physiologically, or pharmacologically active substance that acts locally or systemically in a subject. The term also means any substance intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease or in the enhancement of desirable physical or mental development and/or conditions in an animal or human.

The term “therapeutic effect” is art-recognized and refers to a beneficial local or systemic effect in animals, particularly mammals, and more particularly humans, caused by a pharmacologically active substance. The phrase “therapeutically-effective amount” means that amount of such a substance that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. The therapeutically effective amount of such substance will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of skill in the art. For example, certain compositions described herein may be administered in a sufficient amount to produce a desired effect at a reasonable benefit/risk ratio applicable to such treatment.

“Treating” a condition or disease refers to curing as well as ameliorating at least one symptom of the condition or disease.

The term “vision impairment” refers to diminished vision, which is often only partially reversible or irreversible upon treatment (e.g., surgery). Particularly severe vision impairment is termed “blindness” or “vision loss”, which refers to a complete loss of vision, vision worse than 20/200 that cannot be improved with corrective lenses, or a visual field of less than 20 degrees diameter (10 degrees radius).

ABBREVIATIONS AND SYMBOLS

In describing the present invention, chemical elements are identified in accordance with the Periodic Table of the Elements. Abbreviations and symbols utilized herein are in accordance with the common usage of such abbreviations and symbols by those skilled in the chemical and biological arts.

Specifically, the following abbreviations may be used in the examples and throughout the specification:

g (grams);	mg (milligrams);
kg (kilograms);	μg (micrograms);
L (liters);	mL (milliliters);
μL (microliters);	psi (pounds per square inch);
M (molar);	mM (millimolar);
μM (micromolar);	nM (nanomolar);
pM (picomolar);	nm (nanometers);

	mm (millimeters);	wt (weight);
	N (Normal);	CFU (colony forming units);
	I. V. (intravenous);	Hz (Hertz);
	MHz (megahertz);	mol (moles);
5	mmol (millimoles);	RT (room temperature);
	min (minutes);	h (hours);
	b.p. (boiling point);	TLC (thin layer chromatography);
	T _r (retention time);	RP (reverse phase);
	MeOH (methanol);	<i>i</i> -PrOH (isopropanol);
10	TEA (triethylamine);	TFA (trifluoroacetic acid);
	TFAA (trifluoroacetic anhydride);	THF (tetrahydrofuran);
	DMSO (dimethylsulfoxide);	EtOAc (ethyl acetate);
	DME (1,2-dimethoxyethane);	DCM (dichloromethane);
	DCE (dichloroethane);	DMF (<i>N,N</i> -dimethylformamide);
15	DMPU (<i>N,N'</i> -dimethylpropyleneurea);	CDI (1,1-carbonyldiimidazole);
	IBCF (isobutyl chloroformate);	AcOH (acetic acid);
	HOAt (1-hydroxy-7-azabenzotriazole);	
	THP (tetrahydropyran);	NMM (<i>N</i> -methylmorpholine);
	Pd/C (Palladium on Carbon);	MTBE (<i>tert</i> -butyl methyl ether);
20	HOBt (1-hydroxybenzotriazole);	mCPBA (meta-chloroperbenzoic acid);
	EDC (1-[3-dimethylamino) propyl]-3-ethylcarbodiimide hydrochloride);	
	Boc (<i>tert</i> -butoxycarbonyl);	Fmoc (9-fluorenylmethoxycarbonyl);
	DCC (dicyclohexylcarbodiimide);	CBZ (benzyloxycarbonyl);
	Ac (acetyl);	atm (atmosphere);
25	TMSE (2-(trimethylsilyl)ethyl);	TMS (trimethylsilyl);
	TIPS (triisopropylsilyl);	TBS (<i>t</i> -butyldimethylsilyl);
	DMAP (4-dimethylaminopyridine);	BSA (bovine serum albumin)
	NAD (nicotinamide adenine dinucleotide);	
	HPLC (high pressure liquid chromatography);	
30	LC/MS (liquid chromatography/mass spectrometry);	
	BOP (bis(2-oxo-3-oxazolidinyl)phosphinic chloride);	
	TBAF (tetra- <i>n</i> -butylammonium fluoride);	
	HBTU(O-Benzotriazole-1-yl- <i>N,N,N',N'</i> -tetramethyluroniumhexafluoro phosphate).	
	HEPES (4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid);	

DPPA (diphenylphosphoryl azide); LAH (Lithium aluminum hydride);
 fHNO₃ (fuming HNO₃); NaOMe (sodium methoxide);
 EDTA (ethylenediaminetetraacetic acid);
 TMEDA (N,N,N',N'-tetramethyl-1,2-ethanediamine);
 5 NBS (N-bromosuccinimide); DIPEA (diisopropylethylamine);
 dppf (1,1'-bis(diphenylphosphino)ferrocene); and
 NIS (N-iodosuccinimide).

All references to ether are to diethyl ether and brine refers to a saturated aqueous solution of NaCl.

10 **SYNTHETIC SCHEMES AND GENERAL METHODS OF PREPARATION**

The present invention also relates to processes for making compounds of Formulas (I) to (IV), corresponding analogs (i.e., with hydrogen substitution at the R² position), and/or intermediate compounds thereof, respectively.

15 The compounds of Formulas (I) to (IV), corresponding analogs (i.e., with hydrogen substitution at the R² position) and/or intermediate compounds thereof, or pharmaceutically acceptable salts thereof, may be obtained by using synthetic procedures illustrated in the Schemes below or by drawing on the knowledge of a skilled organic chemist.

20 The synthesis provided in these Schemes (I) to (VI) are applicable for producing compounds of the invention having a variety of different functional groups employing appropriate precursors, which are suitably protected if needed, to achieve compatibility with the reactions outlined herein. Subsequent deprotection, where needed, affords compounds of the nature generally disclosed. While the Schemes are shown with compounds, they are illustrative of processes that may be used to make the compounds of
 25 the invention.

Intermediates (compounds used in the preparation of the compounds of the invention) may also be present as salts. Thus, in reference to intermediates, the phrase “compound(s) of formula (number)” means a compound having that structural formula or a pharmaceutically acceptable salt thereof.

30 The present invention also relates to processes for making compounds of Formulas (I) to (IV), corresponding analogs (i.e., with hydrogen substitution at the R² position), and/or intermediate compounds thereof, respectively, or pharmaceutically acceptable salts thereof.

The compounds according to Formulas (I) to (II), respectively, The present invention also relates to processes for making compounds of Formulas (I) to (IV), corresponding analogs (i.e., with hydrogen substitution at the R² position), and/or intermediate compounds thereof, respectively, or pharmaceutically acceptable salts thereof
5 are prepared using conventional organic syntheses.

The compounds of the present invention may be obtained by using synthetic procedures illustrated in Schemes below or by drawing on the knowledge of a skilled organic chemist.

Suitable synthetic routes are depicted below in the following general reaction
10 schemes.

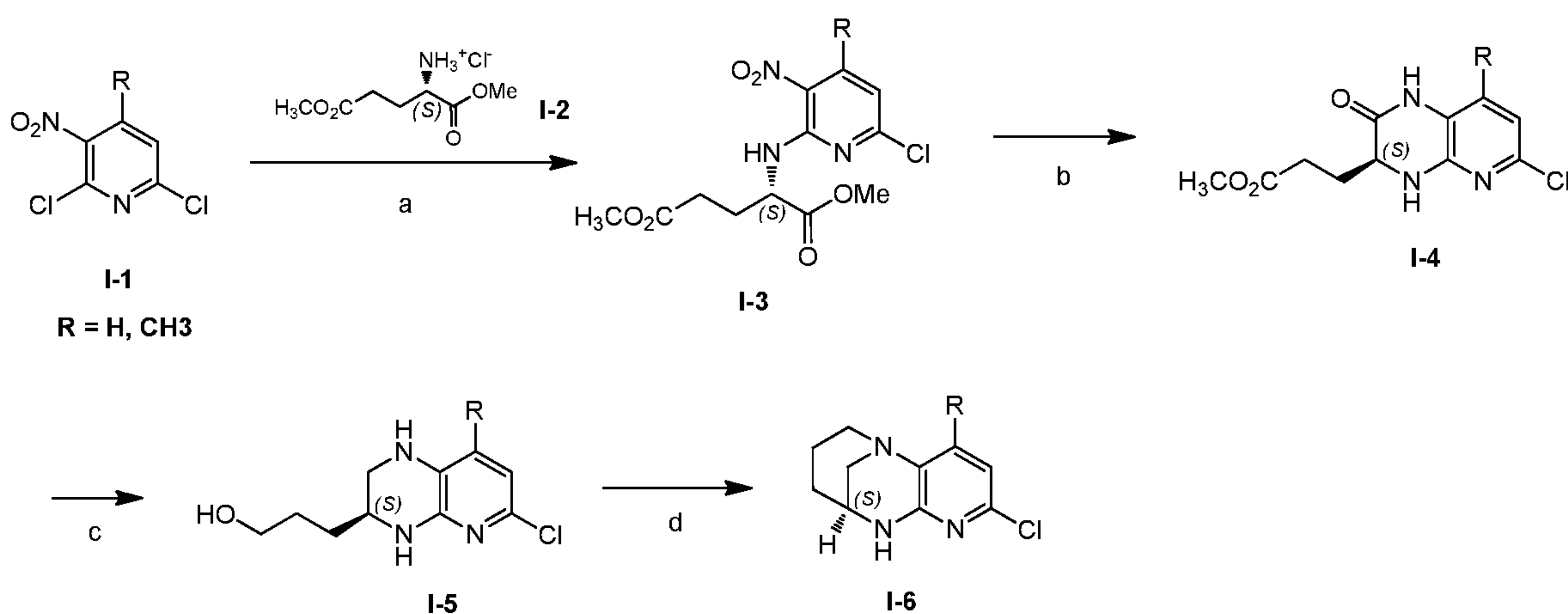
COMPOUND PREPARATION

According to another embodiment, the present invention provides methods of producing the above-defined compounds. The compounds may be synthesized using conventional techniques. Advantageously, these compounds are conveniently synthesized
15 from readily available starting materials.

Synthetic chemistry transformations and methodologies useful in synthesizing the compounds described herein are known in the art and include, for example, those described in R. Larock, *Comprehensive Organic Transformations* (1989); T. W. Greene and P. G. M. Wuts, *Protective Groups in Organic Synthesis*, 2d. Ed. (1991); L. Fieser
20 and M. Fieser, *Fieser and Fieser's Reagents for Organic Synthesis* (1994); and L. Paquette, ed., *Encyclopedia of Reagents for Organic Synthesis* (1995).

General Procedures

Scheme I

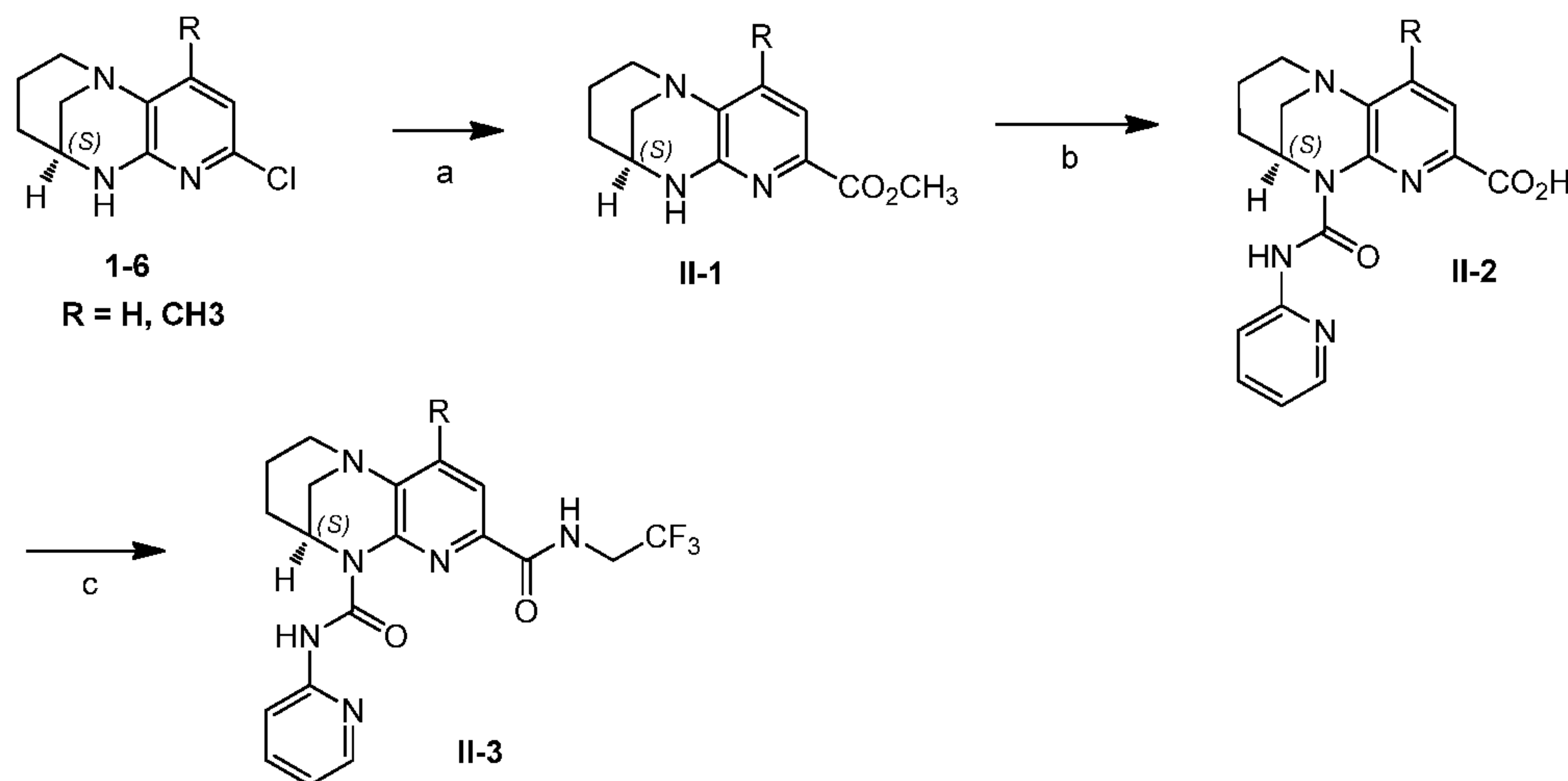


Reagents: (a) THF, NaHCO₃, 60°C; (b) Fe, i-PrOH, HOAc, 80°C; (c) AlCl₃, LiAlH₄, THF, RT; (d) POCl₃, DEA, DCM, 0°C.

The commercial chloropyridine (**I-1**) was reacted with a nucleophilic amine (**I-2**) in the presence of a base (to scavenge HCl) in an aprotic solvent (eg., THF, DMF, dioxane) to provide the regioselective addition product (**I-3**). The nitro functionality of species (**I-3**) was reduced using Fe(0), (see, Bechamp reduction, *Org React.* **2**, 428, **1944**) in the presence of a Bronstead acid (HCl, HOAc) and a protic solvent. Other metals may be used such as Sn to effect this reduction. The resulting intermediate amine species formed in situ reacted with the ester functionality under elevated temperatures to form the cyclic amide **I-4**. A strong hydride reducing agent, such as LiAlH₄, was reacted with compound **I-4** resulting in the reduction of the ester to the corresponding alcohol and simultaneous reduction of the lactam to a cyclic amine. Reductions of this type are well-known to those instructed in the art, see H.C. Brown and S.Krishnamurthy, *Tetrahedron*, **1979**, 35, 567.

Reaction of the alcohol (**I-5**) with an activating group (such as POCl₃), capable of forming facile leaving group, provided the bicyclic amine compound (**I-6**).

Scheme II



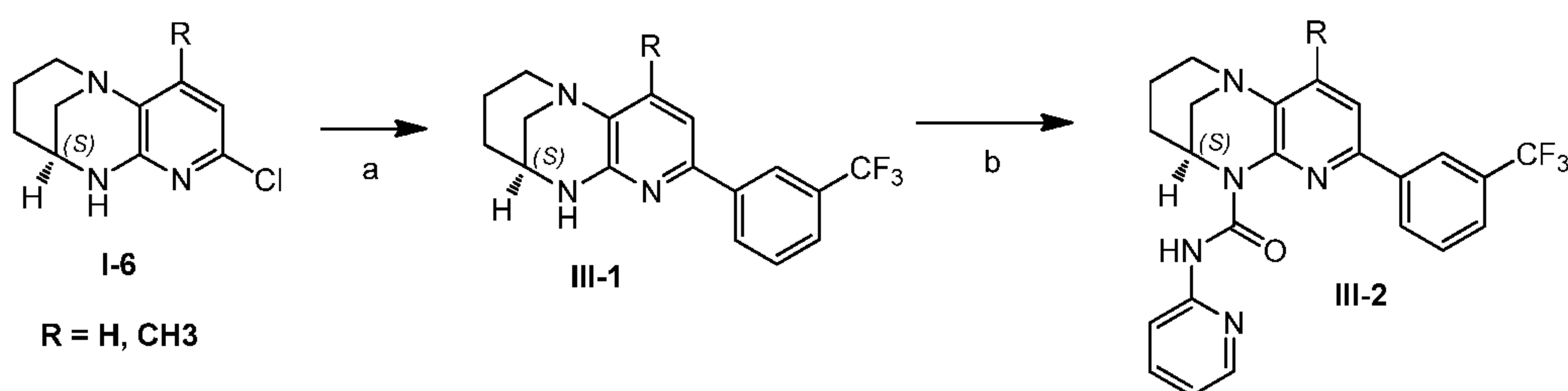
Reagents: (a) TEA, CH₃OH, 120°C, 300 psi CO, PdCl₂(dppf); (b) NaH, THF, 3-(pyridin-2-yl)-2H-pyrido[1,2a][1,3,5]triazine-2,4(3H)-dione, 65°C, then H₂O; (c) DIPEA, DMF, HATU, alkyl amine, RT.

Aryl chloride (**I-6**) was reacted with CO under pressure and elevated temperature in the presence of an alcohol to produce the ester (**II-1**). Carbonylation reactions are described in the literature (see, Principles and Applications of Organotransition Metal Chemistry.

Sausalito, CA: University Science Books; 1987) and are well known to those skilled in the art. Amine (**II-1**) was reacted with an acylating reagent, such as triphosgene or carbonyl diimidazole in an aprotic solvent (DCM, CHCl_3 , THF, etc.) followed by treatment *in situ* with an aniline compound or alkyl amine in the presence of a tertiary alkyl amine base.

- 5 Concomitant hydrolysis of the ester functionality occurred via *in situ* generated NaOH to form the urea species (**II-2**). Carboxylic acid (**II-2**) was reacted with an alkyl amine in the presense of a coupling reagent (HATU) in a polar aprotic solvent to give the corresponding amide (**II-3**). A variety of amide coupling reagents such as EDC, PyBrop, etc. are commercially available. Amide coupling reactions are generally run in solvents such as
- 10 DCM or DMF, utilizing an organic base like Et_3N or $(i\text{-Pr})_2\text{NEt}$.

Scheme III

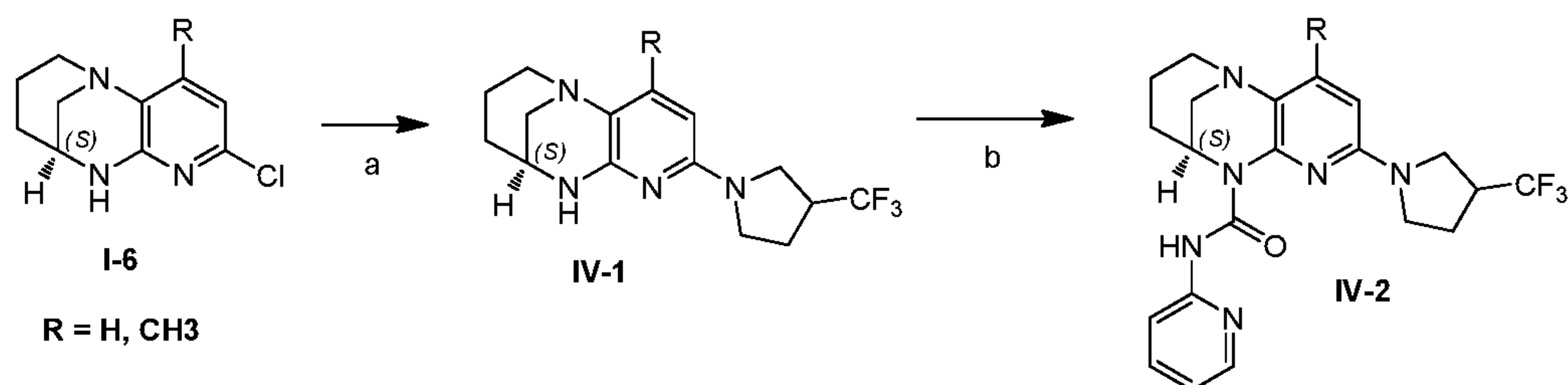


- 15 **Reagents:** (a) $\text{Pd}_2(\text{dba})_3$, X-Phos, Cs_2CO_3 , dioxane/ H_2O , 90°C ; (b) TEA, triphosgene, THF; then 2-aminopyridine, 65°C .

The chloro functionality of compound **I-6** was coupled with a boronic acid using Suzuki coupling chemistry to give **III-1**. Suzuki-like couplings are typically run using a

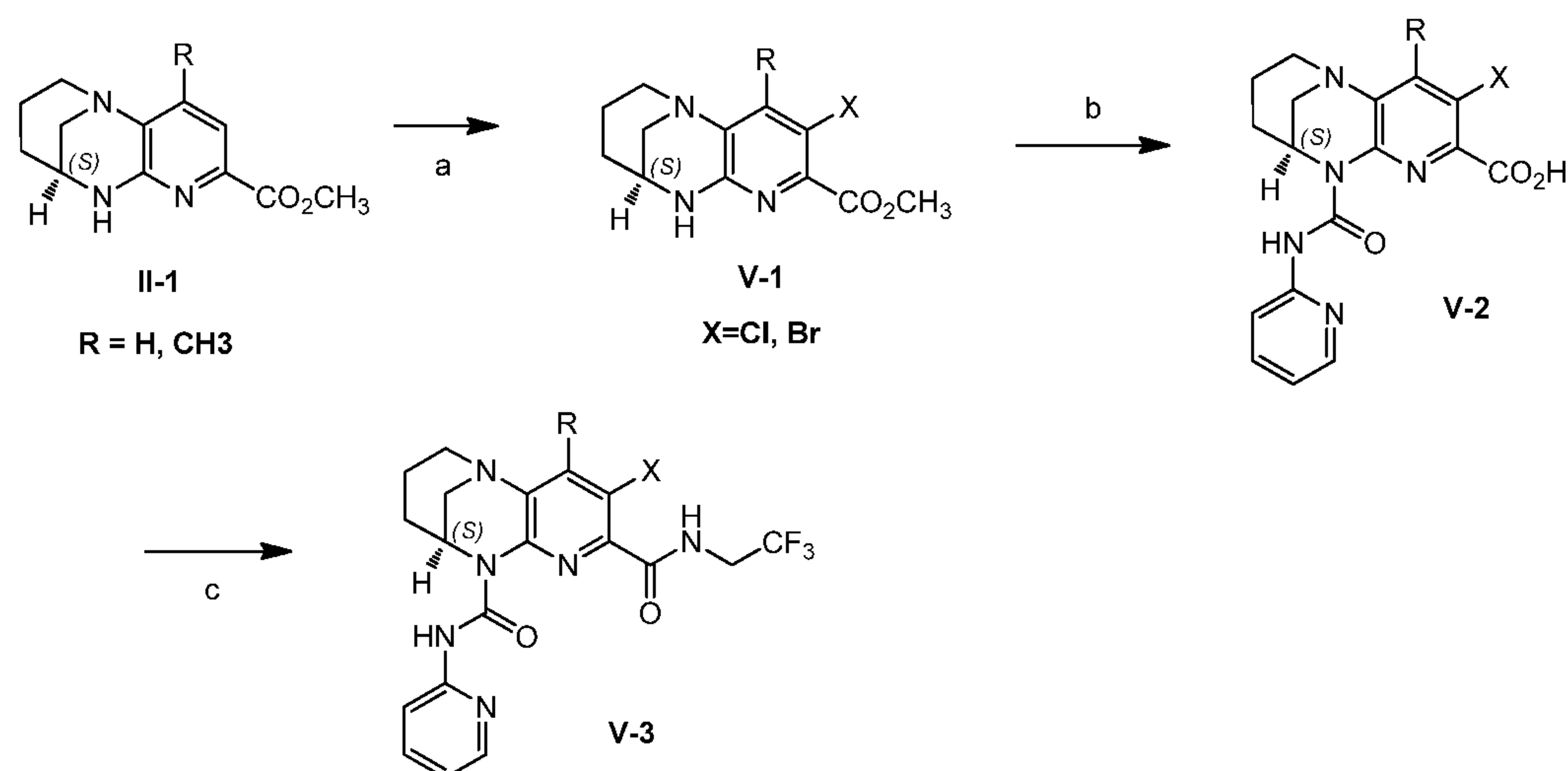
20 palladium(0) catalyst such as $\text{Pd}(\text{PPh}_3)_4$ with an inorganic base, for example K_2CO_3 , Na_2CO_3 or K_3PO_4 , in an aqueous mixture containing ethereal solvents such as DME, dioxane, or THF. Methods for palladium-mediated couplings are described in standard reference volumes, such as Schlosser "Organometallics in Synthesis" (published by Wiley and sons). Compound **III-1** was reacted with an acylating reagent, such as triphosgene or

25 carbonyl diimidazole in an aprotic solvent (DCM, CHCl_3 , THF, etc.) to give a reactive acyl intermediate species which was treated *in situ* with an aniline compound or alkyl amine in the presence of a tertiary alkyl amine base to form the urea species (**III-2**).

Scheme IV

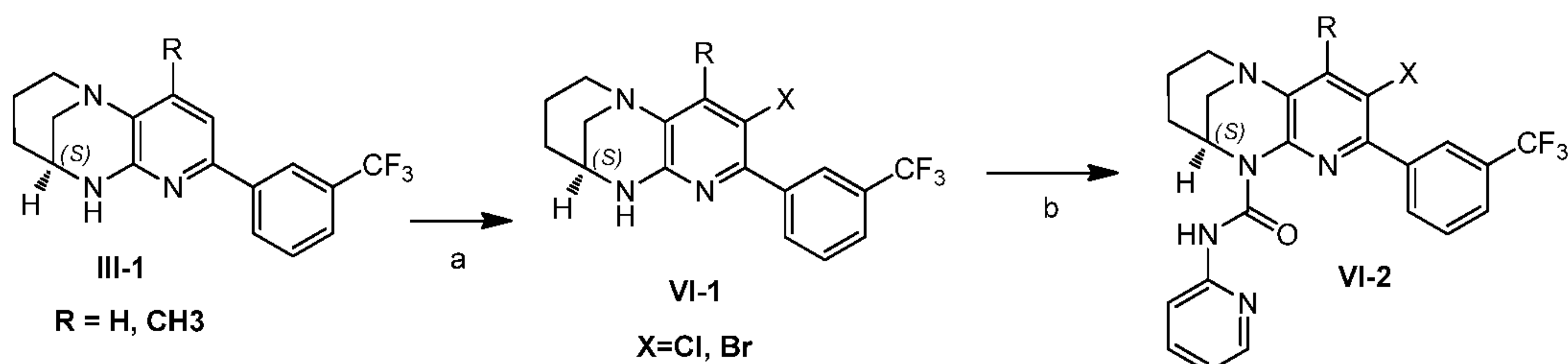
- 5 **Reagents:** (a) 3-trifluoromethylpyrrolidine, (1,3-Bis(2,6-di-isopropylphenyl)-4,5-dihydroimidazol-2-ylidene)chloro) (3-phenylallyl)palladium, KO*t*-Bu, DME, 80°C; (b) TEA, triphosgene, THF; then 2-aminopyridine, 65°C.

The chloro functionality of urea **I-6** was selectively displaced by an alkyl amine using
 10 Buchwald-Hartwig amination conditions to give (**IV-1**). Buchwald-Hartwig reactions are typically run using a palladium(0) catalyst such as Pd(PPh₃)₄ with an bulky Bronstead base, for example KO*t*-Bu or KHMDS, containing ethereal solvents such as DME, dioxane, or THF. Methods for palladium-mediated amine couplings are described in Hartwig, J.F. (1998), *Transition Metal Catalyzed Synthesis of Arylamines and Aryl Ethers from Aryl Halides and Triflates: Scope and Mechanism*, *Angew. Chem. Int. Ed.* **37**: 2046–
 15 2067. Compound **IV-1** was reacted with an acylating reagent, such as triphosgene or carbonyl diimidazole in an aprotic solvent (DCM, CHCl₃, THF, etc.) to give a reactive acyl intermediate species which was treated *in situ* with an aniline compound in the presence of a tertiary alkyl amine base to form the urea species (**IV-2**).

Scheme V

Reagents: (a) NBS or NCS, CHCl₃, 60°C; (b) NaH, THF, 3-(pyridin-2-yl)-2H-pyrido[1,2a][1,3,5]triazine-2,4(3H)-dione, 65°C, then H₂O; (c) DIPEA, DMF, HATU, alkyl amine, RT.

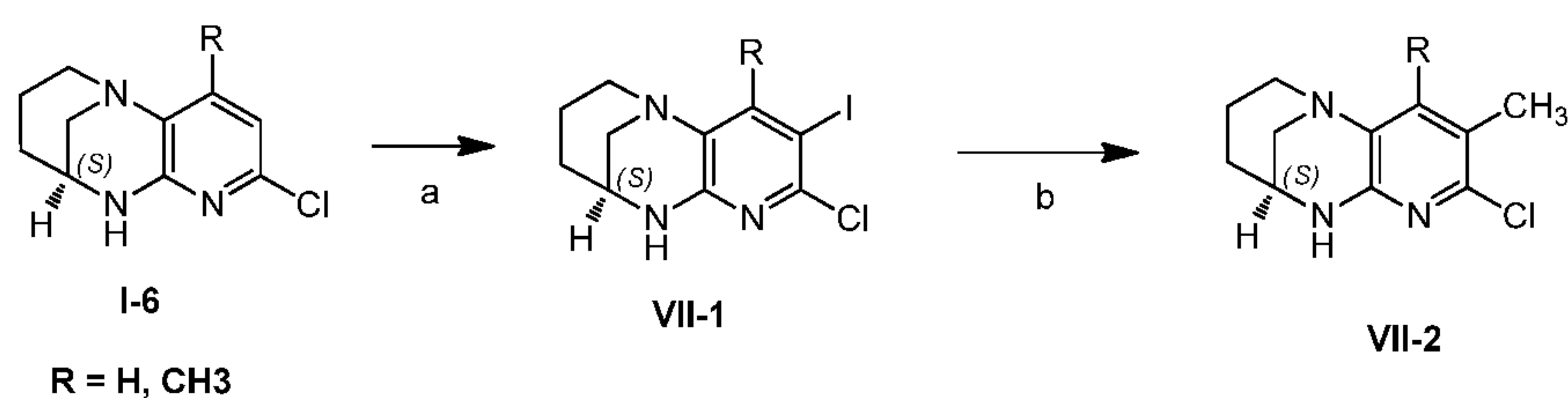
Amine (II-1) was reacted with an electrophilic halogenating reagent, such as NCS or NBS, in an appropriate solvent to give the corresponding halogenated species V-1 in a regioselective manner. Many methods exist to effect the halogenation of an aromatic ring and are well-known to those skilled in the art. Ester (V-1) was reacted with an acylating reagent, such as triphosgene or carbonyl diimidazole in an aprotic solvent (DCM, CHCl₃, THF, etc.) followed by treatment with an aniline compound or alkyl amine in the presence of a tertiary alkyl amine base. Concomitant hydrolysis of the ester functionality occurred via *in situ* generated NaOH to form the urea species (V-2). Carboxylic acid (V-2) was reacted with an alkyl amine in the presence of a coupling reagent (HATU) in a polar aprotic solvent to give the corresponding amide (V-3). A variety of amide coupling reagents such as EDC, PyBrop, etc. are commercially available. Amide coupling reactions are generally run in solvents such as DCM or DMF, utilizing an organic base like Et₃N or (i-Pr)₂NEt.

Scheme VI

Reagents: (a) NBS or NCS, CHCl_3 , 60°C ; (b) TEA, triphosgene, THF; then 2-aminopyridine, 65°C .

Compound **III-1** was reacted with an electrophilic halogenating reagent, such as NCS or NBS, in an appropriate solvent to give the corresponding halogenated species **VI-1** in a regioselective manner. Many methods exist to effect the halogenation of an aromatic ring and are well-known to those skilled in the art. Compound **VI-1** was reacted with an acylating reagent, such as triphosgene or carbonyl diimidazole in an aprotic solvent (DCM , CHCl_3 , THF, etc.) to give a reactive acyl intermediate species which was treated *in situ* with an aniline compound or alkyl amine in the presence of a tertiary alkyl amine base to form the urea species (**VI-2**).

Scheme VII



Reagents: (a) NIS, CHCl_3 , 60°C ; (b) trimethylboroxine, $\text{Pd}(\text{PPh}_3)_4$, K_2CO_3 , dioxane/ H_2O , 90°C ;

Compound **I-6** was reacted with an electrophilic halogenating reagent (NIS) in an appropriate solvent to give the corresponding halogenated species **VII-1** in a regioselective manner. Many methods exist to effect the halogenation of an aromatic ring and are well-known to those skilled in the art. The iodo functionality of compound **VII-1** was coupled with an alkylboronic acid using Suzuki coupling chemistry to give **VII-2**. Suzuki-like couplings are typically run using a palladium(0) catalyst such as $\text{Pd}(\text{PPh}_3)_4$ with an inorganic base, for example K_2CO_3 , Na_2CO_3 or K_3PO_4 , in an aqueous mixture containing ethereal solvents such as DME, dioxane, or THF. Methods for palladium-mediated couplings are described in standard reference volumes, such as Schlosser "Organometallics in Synthesis" (published by Wiley and sons). The methylated species (**VII-2**) was used in schemes II and III as a surrogate for compound **I-6** to produce the analogous methylated products.

Compound Characteristics and Properties

In an exemplary embodiment, a therapeutic compound may traverse the cytoplasmic membrane of a cell. For example, a compound may have a cell-permeability of at least about 20%, 50%, 75%, 80%, 90% or 95%.

5 Compounds described herein may also have one or more of the following characteristics: the compound may be essentially non-toxic to a cell or subject; the compound may be an organic molecule or a small molecule of 2000 amu or less, 1000 amu or less; a compound may have a half-life under normal atmospheric conditions of at least about 30 days, 60 days, 120 days, 6 months or 1 year; the compound may have a
10 half-life in solution of at least about 30 days, 60 days, 120 days, 6 months or 1 year; a compound may be more stable in solution than resveratrol by at least a factor of about 50%, 2 fold, 5 fold, 10 fold, 30 fold, 50 fold or 100 fold; a compound may promote deacetylation of the DNA repair factor Ku70; a compound may promote deacetylation of RelA/p65; a compound may increase general turnover rates and enhance the sensitivity of
15 cells to TNF-induced apoptosis.

 In certain embodiments, a sirtuin-modulating compound does not have any substantial ability to inhibit a histone deacetylase (HDAC) class I, and/or an HDAC class II at concentrations (e.g., *in vivo*) effective for modulating the deacetylase activity of the sirtuin. For instance, in preferred embodiments, the sirtuin-modulating compound is a
20 sirtuin-modulating compound and is chosen to have an EC₅₀ for activating sirtuin deacetylase activity that is at least 5 fold less than the EC₅₀ for inhibition of an HDAC I and/or HDAC II, and even more preferably at least 10 fold, 100 fold or even 1000 fold less. Methods for assaying HDAC I and/or HDAC II activity are well known in the art and kits to perform such assays may be purchased commercially. See e.g., BioVision,
25 Inc. (Mountain View, CA; world wide web at biovision.com) and Thomas Scientific (Swedesboro, NJ; world wide web at tomassci.com).

 In certain embodiments, a sirtuin-modulating compound does not have any substantial ability to modulate sirtuin homologs. In certain embodiments, an activator of a human sirtuin protein may not have any substantial ability to activate a sirtuin protein
30 from lower eukaryotes, particularly yeast or human pathogens, at concentrations (e.g., *in vivo*) effective for activating the deacetylase activity of human sirtuin. For example, a sirtuin-modulating compound may be chosen to have an EC₅₀ for activating a human sirtuin, such as SIRT1 and/or SIRT3, deacetylase activity that is at least 5 fold less than

the EC₅₀ for activating a yeast sirtuin, such as Sir2 (such as *Candida*, *S. cerevisiae*, etc.), and even more preferably at least 10 fold, 100 fold or even 1000 fold less. In another embodiment, an inhibitor of a sirtuin protein from lower eukaryotes, particularly yeast or human pathogens, does not have any substantial ability to inhibit a sirtuin protein from humans at concentrations (e.g., *in vivo*) effective for inhibiting the deacetylase activity of a sirtuin protein from a lower eukaryote. For example, a sirtuin-inhibiting compound may be chosen to have an IC₅₀ for inhibiting a human sirtuin, such as SIRT1 and/or SIRT3, deacetylase activity that is at least 5 fold less than the IC₅₀ for inhibiting a yeast sirtuin, such as Sir2 (such as *Candida*, *S. cerevisiae*, etc.), and even more preferably at least 10 fold, 100 fold or even 1000 fold less.

In certain embodiments, a sirtuin-modulating compound may have the ability to modulate one or more sirtuin protein homologs, such as, for example, one or more of human SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, or SIRT7. In some embodiments, a sirtuin-modulating compound has the ability to modulate both a SIRT1 and a SIRT3 protein.

In other embodiments, a SIRT1 modulator does not have any substantial ability to modulate other sirtuin protein homologs, such as, for example, one or more of human SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, or SIRT7, at concentrations (e.g., *in vivo*) effective for modulating the deacetylase activity of human SIRT1. For example, a sirtuin-modulating compound may be chosen to have an ED₅₀ for modulating human SIRT1 deacetylase activity that is at least 5 fold less than the ED₅₀ for modulating one or more of human SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, or SIRT7, and even more preferably at least 10 fold, 100 fold or even 1000 fold less. In some embodiments, a SIRT1 modulator does not have any substantial ability to modulate a SIRT3 protein.

In other embodiments, a SIRT3 modulator does not have any substantial ability to modulate other sirtuin protein homologs, such as, for example, one or more of human SIRT1, SIRT2, SIRT4, SIRT5, SIRT6, or SIRT7, at concentrations (e.g., *in vivo*) effective for modulating the deacetylase activity of human SIRT3. For example, a sirtuin-modulating compound may be chosen to have an ED₅₀ for modulating human SIRT3 deacetylase activity that is at least 5 fold less than the ED₅₀ for modulating one or more of human SIRT1, SIRT2, SIRT4, SIRT5, SIRT6, or SIRT7, and even more preferably at least 10 fold, 100 fold or even 1000 fold less. In some embodiments, a SIRT3 modulator does not have any substantial ability to modulate a SIRT1 protein.

In certain embodiments, a sirtuin-modulating compound may have a binding affinity for a sirtuin protein of about 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M or less. A sirtuin-modulating compound may reduce (activator) or increase (inhibitor) the apparent K_m of a sirtuin protein for its substrate or NAD^+ (or other cofactor) by a factor of at least about 2, 3, 4, 5, 10, 20, 30, 50 or 100. In certain embodiments, K_m values are determined using the mass spectrometry assay described herein. Preferred activating compounds reduce the K_m of a sirtuin for its substrate or cofactor to a greater extent than caused by resveratrol at a similar concentration or reduce the K_m of a sirtuin for its substrate or cofactor similar to that caused by resveratrol at a lower concentration. A sirtuin-modulating compound may increase the V_{max} of a sirtuin protein by a factor of at least about 2, 3, 4, 5, 10, 20, 30, 50 or 100. A sirtuin-modulating compound may have an ED_{50} for modulating the deacetylase activity of a SIRT1 and/or SIRT3 protein of less than about 1 nM, less than about 10 nM, less than about 100 nM, less than about 1 μ M, less than about 10 μ M, less than about 100 μ M, or from about 1-10 nM, from about 10-100 nM, from about 0.1-1 μ M, from about 1-10 μ M or from about 10-100 μ M. A sirtuin-modulating compound may modulate the deacetylase activity of a SIRT1 and/or SIRT3 protein by a factor of at least about 5, 10, 20, 30, 50, or 100, as measured in a cellular assay or in a cell based assay. A sirtuin-modulating compound may cause at least about 10%, 30%, 50%, 80%, 2 fold, 5 fold, 10 fold, 50 fold or 100 fold greater induction of the deacetylase activity of a sirtuin protein relative to the same concentration of resveratrol. A sirtuin-modulating compound may have an ED_{50} for modulating SIRT5 that is at least about 10 fold, 20 fold, 30 fold, 50 fold greater than that for modulating SIRT1 and/or SIRT3.

Exemplary Uses

In certain aspects, the invention provides methods or uses for modulating the level and/or activity of a sirtuin protein and methods of use thereof.

In certain embodiments, the invention provides methods or uses for using sirtuin-modulating compounds wherein the sirtuin-modulating compounds activate a sirtuin protein, e.g., increase the level and/or activity of a sirtuin protein. Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be useful for a variety of therapeutic applications including, for example, increasing the lifespan of a cell, and treating and/or preventing a wide variety of diseases and disorders including, for example, diseases or disorders related to aging or stress, diabetes, obesity,

neurodegenerative diseases, cardiovascular disease, blood clotting disorders, inflammation, cancer, and/or flushing, etc. The methods or uses comprise administering to a subject in need thereof a pharmaceutically effective amount of a sirtuin-modulating compound, e.g., a sirtuin-modulating compound.

5 Without wishing to be bound by theory, it is believed that activators of the instant invention may interact with a sirtuin at the same location within the sirtuin protein (e.g., active site or site affecting the K_m or V_{max} of the active site). It is believed that this is the reason why certain classes of sirtuin activators and inhibitors can have substantial structural similarity.

10 In certain embodiments, the sirtuin-modulating compounds described herein may be taken alone or in combination with other compounds. In certain embodiments, a mixture of two or more sirtuin-modulating compounds may be administered to a subject in need thereof.

In another embodiment, a sirtuin-modulating compound that increases the level
15 and/or activity of a sirtuin protein may be administered with one or more of the following compounds: resveratrol, butein, fisetin, piceatannol, or quercetin.

In an exemplary embodiment, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be administered in combination with nicotinic acid or nicotinamide riboside. In another embodiment, a sirtuin-modulating compound
20 that decreases the level and/or activity of a sirtuin protein may be administered with one or more of the following compounds: nicotinamide (NAM), suramin; NF023 (a G-protein antagonist); NF279 (a purinergic receptor antagonist); Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid); (-)-epigallocatechin (hydroxy on sites 3,5,7,3',4', 5'); (-)-epigallocatechin gallate (Hydroxy sites 5,7,3',4',5' and gallate ester on 3);
25 cyanidin chloride (3,5,7,3',4'-pentahydroxyflavylium chloride); delphinidin chloride (3,5,7,3',4',5'-hexahydroxyflavylium chloride); myricetin (cannabiscetin; 3,5,7,3',4',5'-hexahydroxyflavone); 3,7,3',4',5'-pentahydroxyflavone; gossypetin (3,5,7,8,3',4'-hexahydroxyflavone), sirtinol; and splitomicin.

In yet another embodiment, one or more sirtuin-modulating compounds may be
30 administered with one or more therapeutic agents for the treatment or prevention of various diseases, including, for example, cancer, diabetes, neurodegenerative diseases, cardiovascular disease, blood clotting, inflammation, flushing, obesity, aging, stress, etc. In various embodiments, combination therapies comprising a sirtuin-modulating

compound may refer to (1) pharmaceutical compositions that comprise one or more sirtuin-modulating compounds in combination with one or more therapeutic agents (e.g., one or more therapeutic agents described herein); and (2) co-administration of one or more sirtuin-modulating compounds with one or more therapeutic agents wherein the sirtuin-modulating compound and therapeutic agent have not been formulated in the same compositions (but may be present within the same kit or package, such as a blister pack or other multi-chamber package; connected, separately sealed containers (e.g., foil pouches) that can be separated by the user; or a kit where the compound(s) and other therapeutic agent(s) are in separate vessels). When using separate formulations, the sirtuin-modulating compound may be administered simultaneous with, intermittent with, staggered with, prior to, subsequent to, or combinations thereof, the administration of another therapeutic agent.

In certain embodiments, methods or uses for reducing, preventing or treating diseases or disorders using a compound described herein may also comprise increasing the protein level of a sirtuin, such as human SIRT1, SIRT2 and/or SIRT3, or homologs thereof. Increasing protein levels can be achieved by introducing into a cell one or more copies of a nucleic acid that encodes a sirtuin. For example, the level of a sirtuin can be increased in a mammalian cell by introducing into the mammalian cell a nucleic acid encoding the sirtuin, e.g., increasing the level of SIRT1 by introducing a nucleic acid encoding the amino acid sequence set forth in GenBank Accession No. NP_036370 and/or increasing the level of SIRT3 by introducing a nucleic acid encoding the amino acid sequence set forth in GenBank Accession No. AAH01042.

A nucleic acid that is introduced into a cell to increase the protein level of a sirtuin may encode a protein that is at least about 80%, 85%, 90%, 95%, 98%, or 99% identical to the sequence of a sirtuin, e.g., SIRT1 and/or SIRT3 protein. For example, the nucleic acid encoding the protein may be at least about 80%, 85%, 90%, 95%, 98%, or 99% identical to a nucleic acid encoding a SIRT1 (e.g. GenBank Accession No. NM_012238) and/or SIRT3 (e.g., GenBank Accession No. BC001042) protein. The nucleic acid may also be a nucleic acid that hybridizes, preferably under stringent hybridization conditions, to a nucleic acid encoding a wild-type sirtuin, e.g., SIRT1 and/or SIRT3 protein. Stringent hybridization conditions may include hybridization and a wash in 0.2 x SSC at 65 °C. When using a nucleic acid that encodes a protein that is different from a wild-type sirtuin protein, such as a protein that is a fragment of a wild-type sirtuin, the protein is preferably biologically active, e.g., is capable of deacetylation. It is only necessary to express in a

cell a portion of the sirtuin that is biologically active. For example, a protein that differs from wild-type SIRT1 having GenBank Accession No. NP_036370, preferably contains the core structure thereof. The core structure sometimes refers to amino acids 62-293 of GenBank Accession No. NP_036370, which are encoded by nucleotides 237 to 932 of

5 GenBank Accession No. NM_012238, which encompasses the NAD binding as well as the substrate binding domains. The core domain of SIRT1 may also refer to about amino acids 261 to 447 of GenBank Accession No. NP_036370, which are encoded by nucleotides 834 to 1394 of GenBank Accession No. NM_012238; to about amino acids 242 to 493 of GenBank Accession No. NP_036370, which are encoded by nucleotides 777

10 to 1532 of GenBank Accession No. NM_012238; or to about amino acids 254 to 495 of GenBank Accession No. NP_036370, which are encoded by nucleotides 813 to 1538 of GenBank Accession No. NM_012238. Whether a protein retains a biological function, e.g., deacetylation capabilities, can be determined according to methods known in the art.

In certain embodiments, methods or uses for reducing, preventing or treating

15 diseases or disorders using a sirtuin-modulating compound may also comprise decreasing the protein level of a sirtuin, such as human SIRT1, SIRT2 and/or SIRT3, or homologs thereof. Decreasing a sirtuin protein level can be achieved according to methods known in the art. For example, an siRNA, an antisense nucleic acid, or a ribozyme targeted to the sirtuin can be expressed in the cell. A dominant negative sirtuin mutant, e.g., a mutant that

20 is not capable of deacetylating, may also be used. For example, mutant H363Y of SIRT1, described, e.g., in Luo et al. (2001) Cell 107:137 can be used. Alternatively, agents that inhibit transcription can be used.

Methods or uses for modulating sirtuin protein levels also include methods or uses for modulating the transcription of genes encoding sirtuins, methods for

25 stabilizing/destabilizing the corresponding mRNAs, and other methods known in the art.

Aging/Stress

In one aspect, the invention provides a method extending the lifespan of a cell, extending the proliferative capacity of a cell, slowing aging of a cell, promoting the survival of a cell, delaying cellular senescence in a cell, mimicking the effects of calorie

30 restriction, increasing the resistance of a cell to stress, or preventing apoptosis of a cell, by contacting the cell with a sirtuin-modulating compound of the invention that increases the level and/or activity of a sirtuin protein. In an exemplary embodiment, the methods or uses comprise contacting the cell with a sirtuin-modulating compound.

The methods or uses described herein may be used to increase the amount of time that cells, particularly primary cells (i.e., cells obtained from an organism, e.g., a human), may be kept alive in a cell culture. Embryonic stem (ES) cells and pluripotent cells, and cells differentiated therefrom, may also be treated with a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein to keep the cells, or progeny thereof, in culture for longer periods of time. Such cells can also be used for transplantation into a subject, e.g., after *ex vivo* modification.

In one aspect, cells that are intended to be preserved for long periods of time may be treated with a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein. The cells may be in suspension (e.g., blood cells, serum, biological growth media, etc.) or in tissues or organs. For example, blood collected from an individual for purposes of transfusion may be treated with a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein to preserve the blood cells for longer periods of time. Additionally, blood to be used for forensic purposes may also be preserved using a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein. Other cells that may be treated to extend their lifespan or protect against apoptosis include cells for consumption, e.g., cells from non-human mammals (such as meat) or plant cells (such as vegetables).

Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may also be applied during developmental and growth phases in mammals, plants, insects or microorganisms, in order to, e.g., alter, retard or accelerate the developmental and/or growth process.

In another aspect, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used to treat cells useful for transplantation or cell therapy, including, for example, solid tissue grafts, organ transplants, cell suspensions, stem cells, bone marrow cells, etc. The cells or tissue may be an autograft, an allograft, a syngraft or a xenograft. The cells or tissue may be treated with the sirtuin-modulating compound prior to administration/implantation, concurrently with administration/implantation, and/or post administration/implantation into a subject. The cells or tissue may be treated prior to removal of the cells from the donor individual, *ex vivo* after removal of the cells or tissue from the donor individual, or post implantation into the recipient. For example, the donor or recipient individual may be treated systemically with a sirtuin-modulating compound or may have a subset of cells/tissue

treated locally with a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein. In certain embodiments, the cells or tissue (or donor/recipient individuals) may additionally be treated with another therapeutic agent useful for prolonging graft survival, such as, for example, an immunosuppressive agent, a cytokine,
5 an angiogenic factor, etc.

In yet other embodiments, cells may be treated with a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein *in vivo*, e.g., to increase their lifespan or prevent apoptosis. For example, skin can be protected from aging (e.g., developing wrinkles, loss of elasticity, etc.) by treating skin or epithelial cells
10 with a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein. In an exemplary embodiment, skin is contacted with a pharmaceutical or cosmetic composition comprising a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein. Exemplary skin afflictions or skin conditions that may be treated in accordance with the methods or uses described herein include disorders or
15 diseases associated with or caused by inflammation, sun damage or natural aging. For example, the compositions find utility in the prevention or treatment of contact dermatitis (including irritant contact dermatitis and allergic contact dermatitis), atopic dermatitis (also known as allergic eczema), actinic keratosis, keratinization disorders (including eczema), epidermolysis bullosa diseases (including pemphigus), exfoliative dermatitis, seborrheic dermatitis, erythemas (including erythema multiforme and erythema
20 nodosum), damage caused by the sun or other light sources, discoid lupus erythematosus, dermatomyositis, psoriasis, skin cancer and the effects of natural aging. In another embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used for the treatment of wounds and/or burns to promote healing, including, for example, first-, second- or third-degree burns and/or thermal, chemical or
25 electrical burns. The formulations may be administered topically, to the skin or mucosal tissue.

Topical formulations comprising one or more sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may also be used as preventive, e.g.,
30 chemopreventive, compositions. When used in a chemopreventive method, susceptible skin is treated prior to any visible condition in a particular individual.

Sirtuin-modulating compounds may be delivered locally or systemically to a subject. In certain embodiments, a sirtuin-modulating compound is delivered locally to a tissue or organ of a subject by injection, topical formulation, etc.

5 In another embodiment, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be used for treating or preventing a disease or condition induced or exacerbated by cellular senescence in a subject; methods or uses for decreasing the rate of senescence of a subject, e.g., after onset of senescence; methods for extending the lifespan of a subject; methods or uses for treating or preventing a disease or condition relating to lifespan; methods or uses for treating or preventing a disease or
10 condition relating to the proliferative capacity of cells; and methods or uses for treating or preventing a disease or condition resulting from cell damage or death. In certain embodiments, the method does not act by decreasing the rate of occurrence of diseases that shorten the lifespan of a subject. In certain embodiments, a method does not act by reducing the lethality caused by a disease, such as cancer.

15 In yet another embodiment, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be administered to a subject in order to generally increase the lifespan of its cells and to protect its cells against stress and/or against apoptosis. It is believed that treating a subject with a compound described herein is similar to subjecting the subject to hormesis, i.e., mild stress that is beneficial to
20 organisms and may extend their lifespan.

Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be administered to a subject to prevent aging and aging-related consequences or diseases, such as stroke, heart disease, heart failure, arthritis, high blood pressure, and Alzheimer's disease. Other conditions that can be treated include ocular disorders, e.g.,
25 associated with the aging of the eye, such as cataracts, glaucoma, and macular degeneration. Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein can also be administered to subjects for treatment of diseases, e.g., chronic diseases, associated with cell death, in order to protect the cells from cell death. Exemplary diseases include those associated with neural cell death, neuronal dysfunction,
30 or muscular cell death or dysfunction, such as Parkinson's disease, Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, and muscular dystrophy; AIDS; fulminant hepatitis; diseases linked to degeneration of the brain, such as Creutzfeld-Jakob disease, retinitis pigmentosa and cerebellar degeneration; myelodysplasia such as aplastic

anemia; ischemic diseases such as myocardial infarction and stroke; hepatic diseases such as alcoholic hepatitis, hepatitis B and hepatitis C; joint-diseases such as osteoarthritis; atherosclerosis; alopecia; damage to the skin due to UV light; lichen planus; atrophy of the skin; cataract; and graft rejections. Cell death can also be caused by surgery, drug
5 therapy, chemical exposure or radiation exposure.

Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein can also be administered to a subject suffering from an acute disease, e.g., damage to an organ or tissue, e.g., a subject suffering from stroke or myocardial infarction or a subject suffering from a spinal cord injury. Sirtuin-modulating compounds that increase
10 the level and/or activity of a sirtuin protein may also be used to repair an alcoholic's liver.

Cardiovascular Disease

In another embodiment, the invention provides a method for treating and/or preventing a cardiovascular disease by administering to a subject in need thereof a sirtuin-
15 modulating compound that increases the level and/or activity of a sirtuin protein.

Cardiovascular diseases that can be treated or prevented using the sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein include cardiomyopathy or myocarditis; such as idiopathic cardiomyopathy, metabolic cardiomyopathy, alcoholic cardiomyopathy, drug-induced cardiomyopathy, ischemic
20 cardiomyopathy, and hypertensive cardiomyopathy. Also treatable or preventable using compounds and methods or uses described herein are atheromatous disorders of the major blood vessels (macrovascular disease) such as the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the femoral arteries, and the popliteal arteries. Other vascular diseases that can be treated or
25 prevented include those related to platelet aggregation, the retinal arterioles, the glomerular arterioles, the vasa nervorum, cardiac arterioles, and associated capillary beds of the eye, the kidney, the heart, and the central and peripheral nervous systems. The sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may also be used for increasing HDL levels in plasma of an individual.

30 Yet other disorders that may be treated with sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein include restenosis, e.g., following coronary intervention, and disorders relating to an abnormal level of high density and low density cholesterol.

In certain embodiments, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be administered as part of a combination therapy with another cardiovascular agent. In certain embodiments, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be administered
5 as part of a combination therapy with an anti-arrhythmia agent. In another embodiment, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be administered as part of a combination therapy with another cardiovascular agent.

Cell Death/Cancer

Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin
10 protein may be administered to subjects who have recently received or are likely to receive a dose of radiation or toxin. In certain embodiments, the dose of radiation or toxin is received as part of a work-related or medical procedure, e.g., administered as a prophylactic measure. In another embodiment, the radiation or toxin exposure is received unintentionally. In such a case, the compound is preferably administered as soon as
15 possible after the exposure to inhibit apoptosis and the subsequent development of acute radiation syndrome.

Sirtuin-modulating compounds may also be used for treating and/or preventing cancer. In certain embodiments, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used for treating and/or preventing cancer.
20 Calorie restriction has been linked to a reduction in the incidence of age-related disorders including cancer. Accordingly, an increase in the level and/or activity of a sirtuin protein may be useful for treating and/or preventing the incidence of age-related disorders, such as, for example, cancer. Exemplary cancers that may be treated using a sirtuin-modulating compound are those of the brain and kidney; hormone-dependent cancers
25 including breast, prostate, testicular, and ovarian cancers; lymphomas, and leukemias. In cancers associated with solid tumors, a modulating compound may be administered directly into the tumor. Cancer of blood cells, e.g., leukemia, can be treated by administering a modulating compound into the blood stream or into the bone marrow. Benign cell growth, e.g., warts, can also be treated. Other diseases that can be treated
30 include autoimmune diseases, e.g., systemic lupus erythematosus, scleroderma, and arthritis, in which autoimmune cells should be removed. Viral infections such as herpes, HIV, adenovirus, and HTLV-1 associated malignant and benign disorders can also be treated by administration of sirtuin-modulating compound. Alternatively, cells can be

obtained from a subject, treated *ex vivo* to remove certain undesirable cells, e.g., cancer cells, and administered back to the same or a different subject.

Chemotherapeutic agents may be co-administered with modulating compounds described herein as having anti-cancer activity, e.g., compounds that induce apoptosis,
5 compounds that reduce lifespan or compounds that render cells sensitive to stress.

Chemotherapeutic agents may be used by themselves with a sirtuin-modulating compound described herein as inducing cell death or reducing lifespan or increasing sensitivity to stress and/or in combination with other chemotherapeutics agents. In addition to
conventional chemotherapeutics, the sirtuin-modulating compounds described herein may
10 also be used with antisense RNA, RNAi or other polynucleotides to inhibit the expression of the cellular components that contribute to unwanted cellular proliferation.

Combination therapies comprising sirtuin-modulating compounds and a conventional chemotherapeutic agent may be advantageous over combination therapies known in the art because the combination allows the conventional chemotherapeutic
15 agent to exert greater effect at lower dosage. In a preferred embodiment, the effective dose (ED₅₀) for a chemotherapeutic agent, or combination of conventional chemotherapeutic agents, when used in combination with a sirtuin-modulating compound is at least 2 fold less than the ED₅₀ for the chemotherapeutic agent alone, and even more preferably at 5 fold, 10 fold or even 25 fold less. Conversely, the therapeutic index (TI)
20 for such chemotherapeutic agent or combination of such chemotherapeutic agent when used in combination with a sirtuin-modulating compound described herein can be at least 2 fold greater than the TI for conventional chemotherapeutic regimen alone, and even more preferably at 5 fold, 10 fold or even 25 fold greater.

Neuronal Diseases/Disorders

25 In certain aspects, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein can be used to treat patients suffering from neurodegenerative diseases, and traumatic or mechanical injury to the central nervous system (CNS), spinal cord or peripheral nervous system (PNS). Neurodegenerative disease typically involves reductions in the mass and volume of the human brain, which may be due to the atrophy
30 and/or death of brain cells, which are far more profound than those in a healthy person that are attributable to aging. Neurodegenerative diseases can evolve gradually, after a long period of normal brain function, due to progressive degeneration (e.g., nerve cell dysfunction and death) of specific brain regions. Alternatively, neurodegenerative

diseases can have a quick onset, such as those associated with trauma or toxins. The actual onset of brain degeneration may precede clinical expression by many years. Examples of neurodegenerative diseases include, but are not limited to, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS; Lou Gehrig's disease), diffuse Lewy body disease, chorea-acanthocytosis, primary lateral sclerosis, ocular diseases (ocular neuritis), chemotherapy-induced neuropathies (e.g., from vincristine, paclitaxel, bortezomib), diabetes-induced neuropathies and Friedreich's ataxia. Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein can be used to treat these disorders and others as described below.

AD is a CNS disorder that results in memory loss, unusual behavior, personality changes, and a decline in thinking abilities. These losses are related to the death of specific types of brain cells and the breakdown of connections and their supporting network (e.g. glial cells) between them. The earliest symptoms include loss of recent memory, faulty judgment, and changes in personality. PD is a CNS disorder that results in uncontrolled body movements, rigidity, tremor, and dyskinesia, and is associated with the death of brain cells in an area of the brain that produces dopamine. ALS (motor neuron disease) is a CNS disorder that attacks the motor neurons, components of the CNS that connect the brain to the skeletal muscles.

HD is another neurodegenerative disease that causes uncontrolled movements, loss of intellectual faculties, and emotional disturbance. Tay-Sachs disease and Sandhoff disease are glycolipid storage diseases where GM2 ganglioside and related glycolipids substrates for β -hexosaminidase accumulate in the nervous system and trigger acute neurodegeneration.

It is well-known that apoptosis plays a role in AIDS pathogenesis in the immune system. However, HIV-1 also induces neurological disease, which can be treated with sirtuin-modulating compounds of the invention.

Neuronal loss is also a salient feature of prion diseases, such as Creutzfeldt-Jakob disease in human, BSE in cattle (mad cow disease), Scrapie Disease in sheep and goats, and feline spongiform encephalopathy (FSE) in cats. Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be useful for treating or preventing neuronal loss due to these prior diseases.

In another embodiment, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be used to treat or prevent any disease or disorder

involving axonopathy. Distal axonopathy is a type of peripheral neuropathy that results from some metabolic or toxic derangement of peripheral nervous system (PNS) neurons. It is the most common response of nerves to metabolic or toxic disturbances, and as such may be caused by metabolic diseases such as diabetes, renal failure, deficiency syndromes
5 such as malnutrition and alcoholism, or the effects of toxins or drugs. Those with distal axonopathies usually present with symmetrical glove-stocking sensori-motor disturbances. Deep tendon reflexes and autonomic nervous system (ANS) functions are also lost or diminished in affected areas.

Diabetic neuropathies are neuropathic disorders that are associated with diabetes
10 mellitus. Relatively common conditions which may be associated with diabetic neuropathy include third nerve palsy; mononeuropathy; mononeuritis multiplex; diabetic amyotrophy; a painful polyneuropathy; autonomic neuropathy; and thoracoabdominal neuropathy.

Peripheral neuropathy is the medical term for damage to nerves of the peripheral
15 nervous system, which may be caused either by diseases of the nerve or from the side-effects of systemic illness. Major causes of peripheral neuropathy include seizures, nutritional deficiencies, and HIV, though diabetes is the most likely cause.

In an exemplary embodiment, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be used to treat or prevent multiple sclerosis
20 (MS), including relapsing MS and monosymptomatic MS, and other demyelinating conditions, such as, for example, chronic inflammatory demyelinating polyneuropathy (CIDP), or symptoms associated therewith.

In yet another embodiment, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be used to treat trauma to the nerves, including,
25 trauma due to disease, injury (including surgical intervention), or environmental trauma (e.g., neurotoxins, alcoholism, etc.).

Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may also be useful to prevent, treat, and alleviate symptoms of various PNS disorders. The term “peripheral neuropathy” encompasses a wide range of disorders in
30 which the nerves outside of the brain and spinal cord—peripheral nerves—have been damaged. Peripheral neuropathy may also be referred to as peripheral neuritis, or if many nerves are involved, the terms polyneuropathy or polyneuritis may be used.

PNS diseases treatable with sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein include: diabetes, leprosy, Charcot-Marie-Tooth disease, Guillain-Barré syndrome and Brachial Plexus Neuropathies (diseases of the cervical and first thoracic roots, nerve trunks, cords, and peripheral nerve components of the brachial plexus.

In another embodiment, a sirtuin-modulating compound may be used to treat or prevent a polyglutamine disease. Exemplary polyglutamine diseases include Spinobulbar muscular atrophy (Kennedy disease), Huntington's Disease (HD), Dentatorubral-pallidoluysian atrophy (Haw River syndrome), Spinocerebellar ataxia type 1, Spinocerebellar ataxia type 2, Spinocerebellar ataxia type 3 (Machado-Joseph disease), Spinocerebellar ataxia type 6, Spinocerebellar ataxia type 7, and Spinocerebellar ataxia type 17.

In certain embodiments, the invention provides a method to treat a central nervous system cell to prevent damage in response to a decrease in blood flow to the cell. Typically the severity of damage that may be prevented will depend in large part on the degree of reduction in blood flow to the cell and the duration of the reduction. In certain embodiments, apoptotic or necrotic cell death may be prevented. In still a further embodiment, ischemic-mediated damage, such as cytotoxic edema or central nervous system tissue anoxemia, may be prevented. In each embodiment, the central nervous system cell may be a spinal cell or a brain cell.

Another aspect encompasses administering a sirtuin-modulating compound to a subject to treat a central nervous system ischemic condition. A number of central nervous system ischemic conditions may be treated by the sirtuin-modulating compounds described herein. In certain embodiments, the ischemic condition is a stroke that results in any type of ischemic central nervous system damage, such as apoptotic or necrotic cell death, cytotoxic edema or central nervous system tissue anoxia. The stroke may impact any area of the brain or be caused by any etiology commonly known to result in the occurrence of a stroke. In one alternative of this embodiment, the stroke is a brain stem stroke. In another alternative of this embodiment, the stroke is a cerebellar stroke. In still another embodiment, the stroke is an embolic stroke. In yet another alternative, the stroke may be a hemorrhagic stroke. In a further embodiment, the stroke is a thrombotic stroke.

In yet another aspect, a sirtuin-modulating compound may be administered to reduce infarct size of the ischemic core following a central nervous system ischemic

condition. Moreover, a sirtuin-modulating compound may also be beneficially administered to reduce the size of the ischemic penumbra or transitional zone following a central nervous system ischemic condition.

5 In certain embodiments, a combination drug regimen may include drugs or compounds for the treatment or prevention of neurodegenerative disorders or secondary conditions associated with these conditions. Thus, a combination drug regimen may include one or more sirtuin activators and one or more anti-neurodegeneration agents.

Blood Coagulation Disorders

10 In other aspects, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein can be used to treat or prevent blood coagulation disorders (or hemostatic disorders). As used interchangeably herein, the terms “hemostasis”, “blood coagulation,” and “blood clotting” refer to the control of bleeding, including the physiological properties of vasoconstriction and coagulation. Blood coagulation assists in maintaining the integrity of mammalian circulation after injury, inflammation, disease,
15 congenital defect, dysfunction or other disruption. Further, the formation of blood clots does not only limit bleeding in case of an injury (hemostasis), but may lead to serious organ damage and death in the context of atherosclerotic diseases by occlusion of an important artery or vein. Thrombosis is thus blood clot formation at the wrong time and place.

20 Accordingly, the present invention provides anticoagulation and antithrombotic treatments aiming at inhibiting the formation of blood clots in order to prevent or treat blood coagulation disorders, such as myocardial infarction, stroke, loss of a limb by peripheral artery disease or pulmonary embolism.

25 As used interchangeably herein, “modulating or modulation of hemostasis” and “regulating or regulation of hemostasis” includes the induction (e.g., stimulation or increase) of hemostasis, as well as the inhibition (e.g., reduction or decrease) of hemostasis.

30 In one aspect, the invention provides a method for reducing or inhibiting hemostasis in a subject by administering a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein. The compositions and methods or uses disclosed herein are useful for the treatment or prevention of thrombotic disorders. As used herein, the term “thrombotic disorder” includes any disorder or condition characterized by excessive or unwanted coagulation or hemostatic activity, or a hypercoagulable state.

Thrombotic disorders include diseases or disorders involving platelet adhesion and thrombus formation, and may manifest as an increased propensity to form thromboses, e.g., an increased number of thromboses, thrombosis at an early age, a familial tendency towards thrombosis, and thrombosis at unusual sites.

5 In another embodiment, a combination drug regimen may include drugs or compounds for the treatment or prevention of blood coagulation disorders or secondary conditions associated with these conditions. Thus, a combination drug regimen may include one or more sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein and one or more anti-coagulation or anti-thrombosis agents.

10 **Weight Control**

 In another aspect, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used for treating or preventing weight gain or obesity in a subject. For example, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used, for example, to treat or prevent hereditary
15 obesity, dietary obesity, hormone related obesity, obesity related to the administration of medication, to reduce the weight of a subject, or to reduce or prevent weight gain in a subject. A subject in need of such a treatment may be a subject who is obese, likely to become obese, overweight, or likely to become overweight. Subjects who are likely to become obese or overweight can be identified, for example, based on family history,
20 genetics, diet, activity level, medication intake, or various combinations thereof.

 In yet other embodiments, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be administered to subjects suffering from a variety of other diseases and conditions that may be treated or prevented by promoting weight loss in the subject. Such diseases include, for example, high blood pressure, hypertension, high
25 blood cholesterol, dyslipidemia, type 2 diabetes, insulin resistance, glucose intolerance, hyperinsulinemia, coronary heart disease, angina pectoris, congestive heart failure, stroke, gallstones, cholecystitis and cholelithiasis, gout, osteoarthritis, obstructive sleep apnea and respiratory problems, some types of cancer (such as endometrial, breast, prostate, and colon), complications of pregnancy, poor female reproductive health (such as menstrual
30 irregularities, infertility, irregular ovulation), bladder control problems (such as stress incontinence); uric acid nephrolithiasis; psychological disorders (such as depression, eating disorders, distorted body image, and low self-esteem). Finally, patients with AIDS

can develop lipodystrophy or insulin resistance in response to combination therapies for AIDS.

In another embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used for inhibiting adipogenesis or fat cell differentiation, whether *in vitro* or *in vivo*. Such methods or uses may be used for treating or preventing obesity.

In other embodiments, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used for reducing appetite and/or increasing satiety, thereby causing weight loss or avoidance of weight gain. A subject in need of such a treatment may be a subject who is overweight, obese or a subject likely to become overweight or obese. The method may comprise administering daily or, every other day, or once a week, a dose, e.g., in the form of a pill, to a subject. The dose may be an “appetite reducing dose.”

In an exemplary embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be administered as a combination therapy for treating or preventing weight gain or obesity. For example, one or more sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be administered in combination with one or more anti-obesity agents.

In another embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be administered to reduce drug-induced weight gain. For example, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be administered as a combination therapy with medications that may stimulate appetite or cause weight gain, in particular, weight gain due to factors other than water retention.

25 **Metabolic Disorders/Diabetes**

In another aspect, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used for treating or preventing a metabolic disorder, such as insulin-resistance, a pre-diabetic state, type II diabetes, and/or complications thereof. Administration of a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may increase insulin sensitivity and/or decrease insulin levels in a subject. A subject in need of such a treatment may be a subject who has insulin resistance or other precursor symptom of type II diabetes, who has type II diabetes, or who is likely to develop any of these conditions. For example, the subject may be a subject

having insulin resistance, e.g., having high circulating levels of insulin and/or associated conditions, such as hyperlipidemia, dyslipogenesis, hypercholesterolemia, impaired glucose tolerance, high blood glucose sugar level, other manifestations of syndrome X, hypertension, atherosclerosis and lipodystrophy.

5 In an exemplary embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be administered as a combination therapy for treating or preventing a metabolic disorder. For example, one or more sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be administered in combination with one or more anti-diabetic agents.

10 **Inflammatory Diseases**

 In other aspects, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein can be used to treat or prevent a disease or disorder associated with inflammation. Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be administered prior to the onset of, at, or after the initiation of
15 inflammation. When used prophylactically, the compounds are preferably provided in advance of any inflammatory response or symptom. Administration of the compounds may prevent or attenuate inflammatory responses or symptoms.

 In another embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used to treat or prevent allergies and respiratory
20 conditions, including asthma, bronchitis, pulmonary fibrosis, allergic rhinitis, oxygen toxicity, emphysema, chronic bronchitis, acute respiratory distress syndrome, and any chronic obstructive pulmonary disease (COPD). The compounds may be used to treat chronic hepatitis infection, including hepatitis B and hepatitis C.

 Additionally, sirtuin-modulating compounds that increase the level and/or activity
25 of a sirtuin protein may be used to treat autoimmune diseases, and/or inflammation associated with autoimmune diseases, such as arthritis, including rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis, as well as organ-tissue autoimmune diseases (e.g., Raynaud's syndrome), ulcerative colitis, Crohn's disease, oral mucositis, scleroderma, myasthenia gravis, transplant rejection, endotoxin shock, sepsis, psoriasis,
30 eczema, dermatitis, multiple sclerosis, autoimmune thyroiditis, uveitis, systemic lupus erythematosus, Addison's disease, autoimmune polyglandular disease (also known as autoimmune polyglandular syndrome), and Grave's disease.

In certain embodiments, one or more sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be taken alone or in combination with other compounds useful for treating or preventing inflammation.

Flushing

5 In another aspect, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used for reducing the incidence or severity of flushing and/or hot flashes which are symptoms of a disorder. For instance, the subject method includes the use of sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein, alone or in combination with other agents, for reducing incidence or
10 severity of flushing and/or hot flashes in cancer patients. In other embodiments, the method provides for the use of sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein to reduce the incidence or severity of flushing and/or hot flashes in menopausal and post-menopausal woman.

In another aspect, sirtuin-modulating compounds that increase the level and/or
15 activity of a sirtuin protein may be used as a therapy for reducing the incidence or severity of flushing and/or hot flashes which are side-effects of another drug therapy, e.g., drug-induced flushing. In certain embodiments, a method for treating and/or preventing drug-induced flushing comprises administering to a patient in need thereof a formulation comprising at least one flushing inducing compound and at least one sirtuin-modulating
20 compound that increases the level and/or activity of a sirtuin protein. In other embodiments, a method for treating drug induced flushing comprises separately administering one or more compounds that induce flushing and one or more sirtuin-modulating compounds, e.g., wherein the sirtuin-modulating compound and flushing inducing agent have not been formulated in the same compositions. When using separate
25 formulations, the sirtuin-modulating compound may be administered (1) at the same as administration of the flushing inducing agent, (2) intermittently with the flushing inducing agent, (3) staggered relative to administration of the flushing inducing agent, (4) prior to administration of the flushing inducing agent, (5) subsequent to administration of the flushing inducing agent, and (6) various combination thereof. Exemplary flushing
30 inducing agents include, for example, niacin, raloxifene, antidepressants, anti-psychotics, chemotherapeutics, calcium channel blockers, and antibiotics.

In certain embodiments, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used to reduce flushing side effects of a

vasodilator or an antilipemic agent (including anticholesteremic agents and lipotropic agents). In an exemplary embodiment, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be used to reduce flushing associated with the administration of niacin.

5 In another embodiment, the invention provides a method for treating and/or preventing hyperlipidemia with reduced flushing side effects. In another representative embodiment, the method involves the use of sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein to reduce flushing side effects of raloxifene. In another representative embodiment, the method involves the use of sirtuin-modulating
10 compounds that increase the level and/or activity of a sirtuin protein to reduce flushing side effects of antidepressants or anti-psychotic agent. For instance, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein can be used in conjunction (administered separately or together) with a serotonin reuptake inhibitor, or a 5HT2 receptor antagonist.

15 In certain embodiments, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used as part of a treatment with a serotonin reuptake inhibitor (SRI) to reduce flushing. In still another representative embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used to reduce flushing side effects of chemotherapeutic agents, such as
20 cyclophosphamide and tamoxifen.

In another embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used to reduce flushing side effects of calcium channel blockers, such as amlodipine.

In another embodiment, sirtuin-modulating compounds that increase the level
25 and/or activity of a sirtuin protein may be used to reduce flushing side effects of antibiotics. For example, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein can be used in combination with levofloxacin.

Ocular Disorders

One aspect of the present invention is a method for inhibiting, reducing or
30 otherwise treating vision impairment by administering to a patient a therapeutic dosage of sirtuin modulator selected from a compound disclosed herein, or a pharmaceutically acceptable salt, prodrug or a metabolic derivative thereof.

In certain aspects of the invention, the vision impairment is caused by damage to the optic nerve or central nervous system. In particular embodiments, optic nerve damage is caused by high intraocular pressure, such as that created by glaucoma. In other particular embodiments, optic nerve damage is caused by swelling of the nerve, which is often associated with an infection or an immune (e.g., autoimmune) response such as in optic neuritis.

In certain aspects of the invention, the vision impairment is caused by retinal damage. In particular embodiments, retinal damage is caused by disturbances in blood flow to the eye (e.g., arteriosclerosis, vasculitis). In particular embodiments, retinal damage is caused by disruption of the macula (e.g., exudative or non-exudative macular degeneration).

Exemplary retinal diseases include Exudative Age Related Macular Degeneration, Nonexudative Age Related Macular Degeneration, Retinal Electronic Prosthesis and RPE Transplantation Age Related Macular Degeneration, Acute Multifocal Placoid Pigment Epitheliopathy, Acute Retinal Necrosis, Best Disease, Branch Retinal Artery Occlusion, Branch Retinal Vein Occlusion, Cancer Associated and Related Autoimmune Retinopathies, Central Retinal Artery Occlusion, Central Retinal Vein Occlusion, Central Serous Chorioretinopathy, Eales Disease, Epimacular Membrane, Lattice Degeneration, Macroaneurysm, Diabetic Macular Edema, Irvine-Gass Macular Edema, Macular Hole, Subretinal Neovascular Membranes, Diffuse Unilateral Subacute Neuroretinitis, Nonpseudophakic Cystoid Macular Edema, Presumed Ocular Histoplasmosis Syndrome, Exudative Retinal Detachment, Postoperative Retinal Detachment, Proliferative Retinal Detachment, Rhegmatogenous Retinal Detachment, Tractional Retinal Detachment, Retinitis Pigmentosa, CMV Retinitis, Retinoblastoma, Retinopathy of Prematurity, Birdshot Retinopathy, Background Diabetic Retinopathy, Proliferative Diabetic Retinopathy, Hemoglobinopathies Retinopathy, Purtscher Retinopathy, Valsalva Retinopathy, Juvenile Retinoschisis, Senile Retinoschisis, Terson Syndrome and White Dot Syndromes.

Other exemplary diseases include ocular bacterial infections (e.g. conjunctivitis, keratitis, tuberculosis, syphilis, gonorrhea), viral infections (e.g., Ocular Herpes Simplex Virus, Varicella Zoster Virus, Cytomegalovirus retinitis, Human Immunodeficiency Virus (HIV)) as well as progressive outer retinal necrosis secondary to HIV or other HIV-associated and other immunodeficiency-associated ocular diseases. In addition, ocular

diseases include fungal infections (e.g., Candida choroiditis, histoplasmosis), protozoal infections (e.g., toxoplasmosis) and others such as ocular toxocariasis and sarcoidosis.

One aspect of the invention is a method for inhibiting, reducing or treating vision impairment in a subject undergoing treatment with a chemotherapeutic drug (e.g., a
5 neurotoxic drug, or a drug that raises intraocular pressure, such as a steroid), by administering to the subject in need of such treatment a therapeutic dosage of a sirtuin modulator disclosed herein.

Another aspect of the invention is a method for inhibiting, reducing or treating vision impairment in a subject undergoing surgery, including ocular or other surgeries
10 performed in the prone position such as spinal cord surgery, by administering to the subject in need of such treatment a therapeutic dosage of a sirtuin modulator disclosed herein. Ocular surgeries include cataract, iridotomy and lens replacements.

Another aspect of the invention is the treatment, including inhibition and prophylactic treatment, of age related ocular diseases include cataracts, dry eye, age-
15 related macular degeneration (AMD), retinal damage and the like, by administering to the subject in need of such treatment a therapeutic dosage of a sirtuin modulator disclosed herein.

Another aspect of the invention is the prevention or treatment of damage to the eye caused by stress, chemical insult or radiation, by administering to the subject in need of
20 such treatment a therapeutic dosage of a sirtuin modulator disclosed herein. Radiation or electromagnetic damage to the eye can include that caused by CRT's or exposure to sunlight or UV.

In certain embodiments, a combination drug regimen may include drugs or compounds for the treatment or prevention of ocular disorders or secondary conditions
25 associated with these conditions. Thus, a combination drug regimen may include one or more sirtuin activators and one or more therapeutic agents for the treatment of an ocular disorder.

In certain embodiments, a sirtuin modulator can be administered in conjunction with a therapy for reducing intraocular pressure. In another embodiment, a sirtuin
30 modulator can be administered in conjunction with a therapy for treating and/or preventing glaucoma. In yet another embodiment, a sirtuin modulator can be administered in conjunction with a therapy for treating and/or preventing optic neuritis. In certain embodiments, a sirtuin modulator can be administered in conjunction with a therapy for

treating and/or preventing CMV Retinopathy. In another embodiment, a sirtuin modulator can be administered in conjunction with a therapy for treating and/or preventing multiple sclerosis.

Mitochondrial-Associated Diseases and Disorders

5 In certain embodiments, the invention provides methods or uses for treating diseases or disorders that would benefit from increased mitochondrial activity. The methods or uses involve administering to a subject in need thereof a therapeutically effective amount of a sirtuin-modulating compound. Increased mitochondrial activity refers to increasing activity of the mitochondria while maintaining the overall numbers of
10 mitochondria (e.g., mitochondrial mass), increasing the numbers of mitochondria thereby increasing mitochondrial activity (e.g., by stimulating mitochondrial biogenesis), or combinations thereof. In certain embodiments, diseases and disorders that would benefit from increased mitochondrial activity include diseases or disorders associated with mitochondrial dysfunction.

15 In certain embodiments, methods or uses for treating diseases or disorders that would benefit from increased mitochondrial activity may comprise identifying a subject suffering from a mitochondrial dysfunction. Methods or uses for diagnosing a mitochondrial dysfunction may involve molecular genetics, pathologic and/or biochemical analyses. Diseases and disorders associated with mitochondrial dysfunction include
20 diseases and disorders in which deficits in mitochondrial respiratory chain activity contribute to the development of pathophysiology of such diseases or disorders in a mammal. Diseases or disorders that would benefit from increased mitochondrial activity generally include for example, diseases in which free radical mediated oxidative injury leads to tissue degeneration, diseases in which cells inappropriately undergo apoptosis, and
25 diseases in which cells fail to undergo apoptosis.

In certain embodiments, the invention provides methods or uses for treating a disease or disorder that would benefit from increased mitochondrial activity that involves administering to a subject in need thereof one or more sirtuin-modulating compounds in combination with another therapeutic agent such as, for example, an agent useful for
30 treating mitochondrial dysfunction or an agent useful for reducing a symptom associated with a disease or disorder involving mitochondrial dysfunction.

In exemplary embodiments, the invention provides methods or uses for treating diseases or disorders that would benefit from increased mitochondrial activity by

administering to a subject a therapeutically effective amount of a sirtuin-modulating compound. Exemplary diseases or disorders include, for example, neuromuscular disorders (e.g., Friedreich's Ataxia, muscular dystrophy, multiple sclerosis, etc.), disorders of neuronal instability (e.g., seizure disorders, migraine, etc.), developmental delay, neurodegenerative disorders (e.g., Alzheimer's Disease, Parkinson's Disease, amyotrophic lateral sclerosis, etc.), ischemia, renal tubular acidosis, age-related neurodegeneration and cognitive decline, chemotherapy fatigue, age-related or chemotherapy-induced menopause or irregularities of menstrual cycling or ovulation, mitochondrial myopathies, mitochondrial damage (e.g., calcium accumulation, excitotoxicity, nitric oxide exposure, hypoxia, etc.), and mitochondrial deregulation.

Muscular dystrophy refers to a family of diseases involving deterioration of neuromuscular structure and function, often resulting in atrophy of skeletal muscle and myocardial dysfunction, such as Duchenne muscular dystrophy. In certain embodiments, sirtuin-modulating compounds may be used for reducing the rate of decline in muscular functional capacities and for improving muscular functional status in patients with muscular dystrophy.

In certain embodiments, sirtuin-modulating compounds may be useful for treatment mitochondrial myopathies. Mitochondrial myopathies range from mild, slowly progressive weakness of the extraocular muscles to severe, fatal infantile myopathies and multisystem encephalomyopathies. Some syndromes have been defined, with some overlap between them. Established syndromes affecting muscle include progressive external ophthalmoplegia, the Kearns-Sayre syndrome (with ophthalmoplegia, pigmentary retinopathy, cardiac conduction defects, cerebellar ataxia, and sensorineural deafness), the MELAS syndrome (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes), the MERFF syndrome (myoclonic epilepsy and ragged red fibers), limb-girdle distribution weakness, and infantile myopathy (benign or severe and fatal).

In certain embodiments, sirtuin-modulating compounds may be useful for treating patients suffering from toxic damage to mitochondria, such as, toxic damage due to calcium accumulation, excitotoxicity, nitric oxide exposure, drug induced toxic damage, or hypoxia.

In certain embodiments, sirtuin-modulating compounds may be useful for treating diseases or disorders associated with mitochondrial deregulation.

Muscle Performance

In other embodiments, the invention provides methods or uses for enhancing muscle performance by administering a therapeutically effective amount of a sirtuin-modulating compound. For example, sirtuin-modulating compounds may be useful for improving physical endurance (e.g., ability to perform a physical task such as exercise, physical labor, sports activities, etc.), inhibiting or retarding physical fatigues, enhancing blood oxygen levels, enhancing energy in healthy individuals, enhance working capacity and endurance, reducing muscle fatigue, reducing stress, enhancing cardiac and cardiovascular function, improving sexual ability, increasing muscle ATP levels, and/or reducing lactic acid in blood. In certain embodiments, the methods or uses involve administering an amount of a sirtuin-modulating compound that increase mitochondrial activity, increase mitochondrial biogenesis, and/or increase mitochondrial mass.

Sports performance refers to the ability of the athlete's muscles to perform when participating in sports activities. Enhanced sports performance, strength, speed and endurance are measured by an increase in muscular contraction strength, increase in amplitude of muscle contraction, shortening of muscle reaction time between stimulation and contraction. Athlete refers to an individual who participates in sports at any level and who seeks to achieve an improved level of strength, speed and endurance in their performance, such as, for example, body builders, bicyclists, long distance runners, short distance runners, etc. Enhanced sports performance is manifested by the ability to overcome muscle fatigue, ability to maintain activity for longer periods of time, and have a more effective workout.

In the arena of athlete muscle performance, it is desirable to create conditions that permit competition or training at higher levels of resistance for a prolonged period of time.

It is contemplated that the methods or uses of the present invention will also be effective in the treatment of muscle related pathological conditions, including acute sarcopenia, for example, muscle atrophy and/or cachexia associated with burns, bed rest, limb immobilization, or major thoracic, abdominal, and/or orthopedic surgery.

In certain embodiments, the invention provides novel dietary compositions comprising sirtuin modulators, a method for their preparation, and a method of using the compositions for improvement of sports performance. Accordingly, provided are therapeutic compositions, foods and beverages that have actions of improving physical endurance and/or inhibiting physical fatigues for those people involved in broadly-defined exercises including sports requiring endurance and labors requiring repeated muscle

exertions. Such dietary compositions may additionally comprise electrolytes, caffeine, vitamins, carbohydrates, etc.

Other Uses

Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used for treating or preventing viral infections (such as infections by influenza, herpes or papilloma virus) or as antifungal agents. In certain embodiments, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be administered as part of a combination drug therapy with another therapeutic agent for the treatment of viral diseases. In another embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be administered as part of a combination drug therapy with another anti-fungal agent.

Subjects that may be treated as described herein include eukaryotes, such as mammals, e.g., humans, ovines, bovines, equines, porcines, canines, felines, non-human primate, mice, and rats. Cells that may be treated include eukaryotic cells, e.g., from a subject described above, or plant cells, yeast cells and prokaryotic cells, e.g., bacterial cells. For example, modulating compounds may be administered to farm animals to improve their ability to withstand farming conditions longer.

Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may also be used to increase lifespan, stress resistance, and resistance to apoptosis in plants. In certain embodiments, a compound is applied to plants, e.g., on a periodic basis, or to fungi. In another embodiment, plants are genetically modified to produce a compound. In another embodiment, plants and fruits are treated with a compound prior to picking and shipping to increase resistance to damage during shipping. Plant seeds may also be contacted with compounds described herein, e.g., to preserve them.

In other embodiments, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used for modulating lifespan in yeast cells. Situations in which it may be desirable to extend the lifespan of yeast cells include any process in which yeast is used, e.g., the making of beer, yogurt, and bakery items, e.g., bread. Use of yeast having an extended lifespan can result in using less yeast or in having the yeast be active for longer periods of time. Yeast or other mammalian cells used for recombinantly producing proteins may also be treated as described herein.

Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may also be used to increase lifespan, stress resistance and resistance to apoptosis

in insects. In this embodiment, compounds would be applied to useful insects, e.g., bees and other insects that are involved in pollination of plants. In a specific embodiment, a compound would be applied to bees involved in the production of honey. Generally, the methods or uses described herein may be applied to any organism, e.g., eukaryote, which
5 may have commercial importance. For example, they can be applied to fish (aquaculture) and birds (e.g., chicken and fowl).

Higher doses of sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may also be used as a pesticide by interfering with the regulation of silenced genes and the regulation of apoptosis during development. In this
10 embodiment, a compound may be applied to plants using a method known in the art that ensures the compound is bio-available to insect larvae, and not to plants.

At least in view of the link between reproduction and longevity, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein can be applied to affect the reproduction of organisms such as insects, animals and
15 microorganisms.

Additional Embodiments

In one aspect, the present invention relates to a method of increasing sirtuin-1 activity in a cell comprising the step of contacting the cell with a compound of Formula (I) or a pharmaceutically acceptable salt or corresponding pharmaceutical composition,
20 respectively, thereof.

In one aspect, the present invention relates to a method for treating insulin resistance, a metabolic syndrome, diabetes, or complications thereof, or for increasing insulin sensitivity, comprising administering a compound or a pharmaceutically acceptable salt or corresponding pharmaceutical composition, respectively, thereof, to a subject in
25 need thereof.

In one aspect, the present invention relates to a method for treating metabolic dysfunctions comprising administering a compound or a pharmaceutically acceptable salt or corresponding pharmaceutical composition, respectively, thereof, to a subject in need thereof.

30 In one aspect, the present invention relates to a method for treating diseases or disorders resulting from diminished SIRT1 expression or activity, which comprises administering a compound or a pharmaceutically acceptable salt or corresponding pharmaceutical composition, respectively, thereof, to a subject in need thereof.

In one aspect, the present invention relates to a method where the diseases or disorders resulting from diminished SIRT1 expression or activity are selected from, but not limited to aging or stress, diabetes, metabolic dysfunctions, neurodegenerative diseases, cardiovascular disease, cancer or inflammatory disease.

5 In one aspect, the present invention relates to a method, where diseases related to aging or stress, diabetes, metabolic dysfunctions, neurodegenerative diseases, cardiovascular disease, cancer or inflammatory disease are selected from psoriasis, atopic dermatitis, acne, rosacea, inflammatory bowel disease, osteoporosis, sepsis, arthritis, COPD, systemic lupus erythematosus and ophthalmic inflammation.

10 In one aspect, the present invention relates to a method, where diseases related to aging or stress, diabetes, metabolic dysfunctions, neurodegenerative diseases, cardiovascular disease, cancer or inflammatory disease are selected from psoriasis, atopic dermatitis, acne, rosacea, inflammatory bowel disease, osteoporosis, sepsis, arthritis, COPD, systemic lupus erythematosus and ophthalmic inflammation.

15 In one aspect, the present invention relates to a method for treating psoriasis, which comprises administering administering a compound or a pharmaceutically acceptable salt or corresponding pharmaceutical composition, respectively, thereof, to a subject in need thereof.

In one aspect, the present invention relates to administering a compound or a
20 pharmaceutically acceptable salt or corresponding pharmaceutical composition, respectively, thereof, for use in therapy in treating a subject suffering from or susceptible to insulin resistance, a metabolic syndrome, diabetes, or complications thereof, or for increasing insulin sensitivity in a subject.

In one aspect, the present invention relates to a use of administering a compound or
25 a pharmaceutically acceptable salt or corresponding pharmaceutical composition, respectively, thereof, in the manufacture of a medicament for use in the treatment of insulin resistance, a metabolic syndrome, diabetes, or complications thereof, or for increasing insulin sensitivity in a subject.

30 **ASSAYS**

Yet other methods or uses contemplated herein include screening methods for identifying compounds or agents that modulate sirtuins. An agent may be a nucleic acid, such as an aptamer. Assays may be conducted in a cell based or cell free format. For

example, an assay may comprise incubating (or contacting) a sirtuin with a test agent under conditions in which a sirtuin can be modulated by an agent known to modulate the sirtuin, and monitoring or determining the level of modulation of the sirtuin in the presence of the test agent relative to the absence of the test agent. The level of modulation of a sirtuin can be determined by determining its ability to deacetylate a substrate. Exemplary substrates are acetylated peptides which can be obtained from BIOMOL (Plymouth Meeting, PA). Preferred substrates include peptides of p53, such as those comprising an acetylated K382. A particularly preferred substrate is the Fluor de Lys-SIRT1 (BIOMOL), i.e., the acetylated peptide Arg-His-Lys-Lys. Other substrates are peptides from human histones H3 and H4 or an acetylated amino acid. Substrates may be fluorogenic. The sirtuin may be SIRT1, Sir2, SIRT3, or a portion thereof. For example, recombinant SIRT1 can be obtained from BIOMOL. The reaction may be conducted for about 30 minutes and stopped, e.g., with nicotinamide. The HDAC fluorescent activity assay/drug discovery kit (AK-500, BIOMOL Research Laboratories) may be used to determine the level of acetylation. Similar assays are described in Bitterman et al. (2002) J. Biol. Chem. 277:45099. The level of modulation of the sirtuin in an assay may be compared to the level of modulation of the sirtuin in the presence of one or more (separately or simultaneously) compounds described herein, which may serve as positive or negative controls. Sirtuins for use in the assays may be full length sirtuin proteins or portions thereof. Since it has been shown herein that activating compounds appear to interact with the N-terminus of SIRT1, proteins for use in the assays include N-terminal portions of sirtuins, e.g., about amino acids 1-176 or 1-255 of SIRT1; about amino acids 1-174 or 1-252 of Sir2.

In certain embodiments, a screening assay comprises (i) contacting a sirtuin with a test agent and an acetylated substrate under conditions appropriate for the sirtuin to deacetylate the substrate in the absence of the test agent; and (ii) determining the level of acetylation of the substrate, wherein a lower level of acetylation of the substrate in the presence of the test agent relative to the absence of the test agent indicates that the test agent stimulates deacetylation by the sirtuin, whereas a higher level of acetylation of the substrate in the presence of the test agent relative to the absence of the test agent indicates that the test agent inhibits deacetylation by the sirtuin.

In another embodiment, the screening assay may detect the formation of a 2'/3'-O-acetyl-ADP-ribose product of sirtuin-mediated NAD-dependent deacetylation. This O-

acetyl-ADP-ribose product is formed in equimolar quantities with the deacetylated peptide product of the sirtuin deacetylation reaction. Accordingly, the screening assay may include (i) contacting a sirtuin with a test agent and an acetylated substrate under conditions appropriate for the sirtuin to deacetylate the substrate in the absence of the test agent ; and (ii) determining the amount of O-acetyl-ADP-ribose formation, wherein an increase in O-acetyl-ADP-ribose formation in the presence of the test agent relative to the absence of the test agent indicates that the test agent stimulates deacetylation by the sirtuin, while a decrease in O-acetyl-ADP-ribose formation in the presence of the test agent relative to the absence of the test agent indicates that the test agent inhibits deacetylation by the sirtuin.

Methods or uses for identifying an agent that modulates, e.g., stimulates, sirtuins *in vivo* may comprise (i) contacting a cell with a test agent and a substrate that is capable of entering a cell in the presence of an inhibitor of class I and class II HDACs under conditions appropriate for the sirtuin to deacetylate the substrate in the absence of the test agent; and (ii) determining the level of acetylation of the substrate, wherein a lower level of acetylation of the substrate in the presence of the test agent relative to the absence of the test agent indicates that the test agent stimulates deacetylation by the sirtuin, whereas a higher level of acetylation of the substrate in the presence of the test agent relative to the absence of the test agent indicates that the test agent inhibits deacetylation by the sirtuin.

A preferred substrate is an acetylated peptide, which is also preferably fluorogenic, as further described herein. The method may further comprise lysing the cells to determine the level of acetylation of the substrate. Substrates may be added to cells at a concentration ranging from about 1 μ M to about 10mM, preferably from about 10 μ M to 1mM, even more preferably from about 100 μ M to 1mM, such as about 200 μ M. A preferred substrate is an acetylated lysine, e.g., ϵ -acetyl lysine (Fluor de Lys, FdL) or Fluor de Lys-SIRT1. A preferred inhibitor of class I and class II HDACs is trichostatin A (TSA), which may be used at concentrations ranging from about 0.01 to 100 μ M, preferably from about 0.1 to 10 μ M, such as 1 μ M. Incubation of cells with the test compound and the substrate may be conducted for about 10 minutes to 5 hours, preferably for about 1-3 hours. Since TSA inhibits all class I and class II HDACs, and that certain substrates, e.g., Fluor de Lys, is a poor substrate for SIRT2 and even less a substrate for SIRT3-7, such an assay may be used to identify modulators of SIRT1 *in vivo*.

METHODS

The present invention also relates to methods or uses for using Sirtuin Modulator compounds as defined herein in treating and/or preventing a wide variety of diseases and disorders, which include, but are not limited to, for example, diseases or disorders related to aging or stress, diabetes, obesity, neurodegenerative diseases, cardiovascular disease, blood clotting disorders, inflammation, cancer, and/or flushing as well as diseases or disorders that would benefit from increased mitochondrial activity, further which may be selected from or include, but are not limited to psoriasis, atopic dermatitis, acne, rosacea, inflammatory bowel disease, osteoporosis, sepsis, arthritis, COPD, systemic lupus erythematosus and ophthalmic inflammation.

In another aspect, the invention provides methods or uses for using sirtuin-modulating compounds, or compositions comprising sirtuin-modulating compounds. In certain embodiments, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used for a variety of therapeutic applications including, for example, increasing the lifespan of a cell, and treating and/or preventing a wide variety of diseases and disorders including, for example, diseases or disorders related to aging or stress, diabetes, obesity, neurodegenerative diseases, chemotherapeutic-induced neuropathy, neuropathy associated with an ischemic event, ocular diseases and/or disorders, cardiovascular disease, blood clotting disorders, inflammation, and/or flushing, etc. Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may also be used for treating a disease or disorder in a subject that would benefit from increased mitochondrial activity, for enhancing muscle performance, for increasing muscle ATP levels, or for treating or preventing muscle tissue damage associated with hypoxia or ischemia. In other embodiments, sirtuin-modulating compounds that decrease the level and/or activity of a sirtuin protein may be used for a variety of therapeutic applications including, for example, increasing cellular sensitivity to stress, increasing apoptosis, treatment of cancer, stimulation of appetite, and/or stimulation of weight gain, etc. As described further below, the methods or uses comprise administering to a subject in need thereof a pharmaceutically effective amount of a sirtuin-modulating compound.

In certain aspects, the sirtuin-modulating compounds may be administered alone or in combination with other compounds, including other sirtuin-modulating compounds, or other therapeutic agents.

In another aspect, the present invention relates to a method of increasing sirtuin-1 activity in a cell, which comprises the step of contacting the cell with a compound of Formulas (I) to (IV), corresponding analogs or derivatives thereof (i.e., with hydrogen substitution at the R^2 position) or a pharmaceutical acceptable salt thereof of the present invention.

In another aspect, the present invention relates to a method of increasing sirtuin-1 activity in a cell comprising the step of contacting the cell with a pharmaceutical composition of the present invention as defined herein

In another aspect, the present invention relates to a method for treating insulin resistance, a metabolic syndrome, diabetes, or complications thereof, or for increasing insulin sensitivity, which comprises administering a compound a compound of Formulas (I) to (IV), **corresponding analogs or derivatives thereof** (i.e., with hydrogen substitution at the R^2 position) of the present invention to a subject in need thereof.

In another aspect, the present invention relates to a method for treating a subject suffering from or susceptible to insulin resistance, a metabolic syndrome, diabetes, or complications thereof, or for increasing insulin sensitivity in a subject, comprising administering a pharmaceutical composition of the present invention to the subject in need thereof.

In another aspect, the present invention relates to a method for treating insulin resistance, a metabolic syndrome, diabetes, or complications thereof, or for increasing insulin sensitivity, comprising administering a pharmaceutical composition of the present invention to a subject in need thereof.

In another aspect, the present invention relates to a method of increasing sirtuin-1 activity in a cell, which comprises the step of contacting a cell with a compound of Formulas (I) to (IV), corresponding analogs or derivatives thereof (i.e., with hydrogen substitution at the R^2 position) or a pharmaceutical acceptable salt thereof .

In another aspect, the present invention relates to a method of increasing sirtuin-1 activity in a cell, which comprises the step of contacting a cell with a pharmaceutical composition of the present invention

In another aspect, the present invention relates to a method for treating metabolic dysfunctions, which comprises administering a compound of Formulas (I) to (IV), corresponding analogs or derivatives thereof (i.e., with hydrogen substitution at the R^2 position) or a pharmaceutical acceptable salt thereof to a subject in need thereof.

In another aspect, the present invention relates to a method for treating metabolic dysfunctions comprising administering a pharmaceutical composition of the present invention to a subject in need thereof.

5 In another aspect, the present invention relates to a method for treating diseases or disorders resulting from diminished SIRT1 expression or activity, which comprises administering a compound of Formulas (I) to (IV), corresponding anlogs or derivatives thereof (i.e., with hydrogen substitution at the R^2 position) or a pharmaceutical acceptable salt thereof to a subject in need thereof.

10 In another aspect, the present invention relates to method where the diseases or disorders resulting from diminished SIRT1 expression or activity are selected from, but not limited to aging or stress, diabetes, metabolic dysfunctions, neurodegenerative diseases, cardiovascular disease, cancer or inflammatory disease.

In another aspect, the present invention relates to a method where diseases related
15 to aging or stress, diabetes, metabolic dysfunctions, neurodegenerative diseases, cardiovascular disease, cancer or inflammatory disease are selected from psoriasis, atopic dermatitis, acne, rosacea, inflammatory bowel disease, osteoporosis, sepsis, arthritis, COPD, systemic lupus erythematosus and ophthalmic inflammation.

In another aspect, the present invention relates to a method for treating psoriasis,
20 which comprises administering a compound of Formulas (I) to (IV), corresponding anlogs or derivatives thereof (i.e., with hydrogen substitution at the R^2 position) or a pharmaceutical acceptable salt thereof to a subject in need thereof.

In another aspect, the present invention relates to a method for treating psoriasis,
which comprises administering a pharmaceutical composition of the present invention to a
25 subject in need thereof

PHARMACEUTICAL COMPOSITIONS AND FORMULATIONS

In general, the present invention relates to substituted bridged urea analog compounds of Formulas (I) to (IV), corresponding anlogs or derivatives thereof thereof
30 (i.e., with hydrogen substitution at the R^2 position), or pharmaceutically acceptable salts thereof, corresponding pharmaceutical compositions, processes for making and use of such compounds, alone or in combination with other therapeutic agents, as Sirtuin Modulators useful for increasing lifespan of a cell, and in treating and/or preventing a

wide variety of diseases and disorders, which include, but are not limited to, for example, diseases or disorders related to aging or stress, diabetes, obesity, neurodegenerative diseases, cardiovascular disease, blood clotting disorders, inflammation, cancer, and/or flushing as well as diseases or disorders that would benefit
5 from increased mitochondrial activity.

In particular, the present invention relates to novel compounds of Formulas (I) to (IV), corresponding analogs or derivatives thereof (i.e., with hydrogen substitution at the R^2 position) or a pharmaceutical acceptable salt thereof and corresponding pharmaceutical compositions comprising compounds of Formulas (I) to (IV), respectively.

10 In another aspect, the present invention relates to a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and a compound of Formulas (I) to (IV), corresponding analogs or derivatives thereof (i.e., with hydrogen substitution at the R^2 position) or a pharmaceutical acceptable salt thereof.

In another aspect, the present invention relates to a pharmaceutical composition of
15 the present invention, further comprising an additional active agent.

In another aspect, the present invention relates to a pharmaceutical composition comprising a compound of Formulas (I) to (IV), corresponding analogs or derivatives thereof (i.e., with hydrogen substitution at the R^2 position) or a pharmaceutical acceptable salt thereof and at least one pharmaceutically acceptable carrier.

20 The compounds described herein may be formulated in a conventional manner using one or more physiologically or pharmaceutically acceptable carriers or excipients. For example, compounds and their pharmaceutically acceptable salts and solvates may be formulated for administration by, for example, injection (e.g. SubQ, IM, IP), inhalation or insufflation (either through the mouth or the nose) or oral, buccal, sublingual, transdermal,
25 nasal, parenteral or rectal administration. In certain embodiments, a compound may be administered locally, at the site where the target cells are present, i.e., in a specific tissue, organ, or fluid (e.g., blood, cerebrospinal fluid, etc.).

The compounds can be formulated for a variety of modes of administration, including systemic and topical or localized administration. Techniques and formulations
30 generally may be found in Remington's Pharmaceutical Sciences, Meade Publishing Co., Easton, PA. For parenteral administration, injection is preferred, including intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the compounds can be formulated in liquid solutions, preferably in physiologically compatible buffers such as

Hank's solution or Ringer's solution. In addition, the compounds may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets, lozenges, or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For administration by inhalation (e.g., pulmonary delivery), the compounds may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin, for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or

emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

5 The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

 In addition to the formulations described previously, compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by
10 implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt. Controlled release formula also includes patches.

15 In certain embodiments, the compounds described herein can be formulated for delivery to the central nervous system (CNS) (reviewed in Begley, Pharmacology & Therapeutics 104: 29-45 (2004)). Conventional approaches for drug delivery to the CNS include: neurosurgical strategies (e.g., intracerebral injection or intracerebroventricular infusion); molecular manipulation of the agent (e.g., production of a chimeric fusion
20 protein that comprises a transport peptide that has an affinity for an endothelial cell surface molecule in combination with an agent that is itself incapable of crossing the BBB) in an attempt to exploit one of the endogenous transport pathways of the BBB; pharmacological strategies designed to increase the lipid solubility of an agent (e.g., conjugation of water-soluble agents to lipid or cholesterol carriers); and the transitory disruption of the integrity
25 of the BBB by hyperosmotic disruption (resulting from the infusion of a mannitol solution into the carotid artery or the use of a biologically active agent such as an angiotensin peptide).

 Liposomes are a further drug delivery system which is easily injectable. Accordingly, in the method of invention the active compounds can also be administered in
30 the form of a liposome delivery system. Liposomes are well known by those skilled in the art. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines. Liposomes usable for the method of invention

encompass all types of liposomes including, but not limited to, small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles.

Another way to produce a formulation, particularly a solution, of a compound described herein, is through the use of cyclodextrin. By cyclodextrin is meant α -, β -, or γ -cyclodextrin. Cyclodextrins are described in detail in Pitha et al., U.S. Pat. No. 4,727,064. Cyclodextrins are cyclic oligomers of glucose; these compounds form inclusion complexes with any drug whose molecule can fit into the lipophile-seeking cavities of the cyclodextrin molecule.

Rapidly disintegrating or dissolving dosage forms are useful for the rapid absorption, particularly buccal and sublingual absorption, of pharmaceutically active agents. Fast melt dosage forms are beneficial to patients, such as aged and pediatric patients, who have difficulty in swallowing typical solid dosage forms, such as caplets and tablets. Additionally, fast melt dosage forms circumvent drawbacks associated with, for example, chewable dosage forms, wherein the length of time an active agent remains in a patient's mouth plays an important role in determining the amount of taste masking and the extent to which a patient may object to throat grittiness of the active agent.

Pharmaceutical compositions (including cosmetic preparations) may comprise from about 0.00001 to 100% such as from 0.001 to 10% or from 0.1% to 5% by weight of one or more compounds described herein. In other embodiments, the pharmaceutical composition comprises: (i) 0.05 to 1000 mg of the compounds of the invention, or a pharmaceutically acceptable salt thereof, and (ii) 0.1 to 2 grams of one or more pharmaceutically acceptable excipients.

In some embodiments, a compound described herein is incorporated into a topical formulation containing a topical carrier that is generally suited to topical drug administration and comprising any such material known in the art. The topical carrier may be selected so as to provide the composition in the desired form, e.g., as an ointment, lotion, cream, microemulsion, gel, oil, solution, or the like, and may be comprised of a material of either naturally occurring or synthetic origin. It is preferable that the selected carrier not adversely affect the active agent or other components of the topical formulation. Examples of suitable topical carriers for use herein include water, alcohols and other nontoxic organic solvents, glycerin, mineral oil, silicone, petroleum jelly, lanolin, fatty acids, vegetable oils, parabens, waxes, and the like.

Formulations may be colorless, odorless ointments, lotions, creams, microemulsions and gels.

The compounds may be incorporated into ointments, which generally are semisolid preparations which are typically based on petrolatum or other petroleum derivatives. The specific ointment base to be used, as will be appreciated by those skilled in the art, is one that will provide for optimum drug delivery, and, preferably, will provide for other desired characteristics as well, e.g., emolliency or the like. As with other carriers or vehicles, an ointment base should be inert, stable, nonirritating and nonsensitizing.

The compounds may be incorporated into lotions, which generally are preparations to be applied to the skin surface without friction, and are typically liquid or semiliquid preparations in which solid particles, including the active agent, are present in a water or alcohol base. Lotions are usually suspensions of solids, and may comprise a liquid oily emulsion of the oil-in-water type.

The compounds may be incorporated into creams, which generally are viscous liquid or semisolid emulsions, either oil-in-water or water-in-oil. Cream bases are water-washable, and contain an oil phase, an emulsifier and an aqueous phase. The oil phase is generally comprised of petrolatum and a fatty alcohol such as cetyl or stearyl alcohol; the aqueous phase usually, although not necessarily, exceeds the oil phase in volume, and generally contains a humectant. The emulsifier in a cream formulation, as explained in Remington's, *supra*, is generally a nonionic, anionic, cationic or amphoteric surfactant.

The compounds may be incorporated into microemulsions, which generally are thermodynamically stable, isotropically clear dispersions of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant molecules (Encyclopedia of Pharmaceutical Technology (New York: Marcel Dekker, 1992), volume 9).

The compounds may be incorporated into gel formulations, which generally are semisolid systems consisting of either suspensions made up of small inorganic particles (two-phase systems) or large organic molecules distributed substantially uniformly throughout a carrier liquid (single phase gels). Although gels commonly employ aqueous carrier liquid, alcohols and oils can be used as the carrier liquid as well.

Other active agents may also be included in formulations, e.g., other anti-inflammatory agents, analgesics, antimicrobial agents, antifungal agents, antibiotics, vitamins, antioxidants, and sunblock agents commonly found in sunscreen formulations

including, but not limited to, anthranilates, benzophenones (particularly benzophenone-3), camphor derivatives, cinnamates (e.g., octyl methoxycinnamate), dibenzoyl methanes (e.g., butyl methoxydibenzoyl methane), p-aminobenzoic acid (PABA) and derivatives thereof, and salicylates (e.g., octyl salicylate).

5 In certain topical formulations, the active agent is present in an amount in the range of approximately 0.25 wt. % to 75 wt. % of the formulation, preferably in the range of approximately 0.25 wt. % to 30 wt. % of the formulation, more preferably in the range of approximately 0.5 wt. % to 15 wt. % of the formulation, and most preferably in the range of approximately 1.0 wt. % to 10 wt. % of the formulation.

10 Conditions of the eye can be treated or prevented by, e.g., systemic, topical, intraocular injection of a compound, or by insertion of a sustained release device that releases a compound. A compound may be delivered in a pharmaceutically acceptable ophthalmic vehicle, such that the compound is maintained in contact with the ocular surface for a sufficient time period to allow the compound to penetrate the corneal and
15 internal regions of the eye, as for example the anterior chamber, posterior chamber, vitreous body, aqueous humor, vitreous humor, cornea, iris/ciliary, lens, choroid/retina and sclera. The pharmaceutically acceptable ophthalmic vehicle may, for example, be an ointment, vegetable oil or an encapsulating material. Alternatively, the compounds of the invention may be injected directly into the vitreous and aqueous humour. In a further
20 alternative, the compounds may be administered systemically, such as by intravenous infusion or injection, for treatment of the eye.

The compounds described herein may be stored in oxygen free environment. For example, a composition can be prepared in an airtight capsule for oral administration, such as Capsugel from Pfizer, Inc.

25 Cells, e.g., treated *ex vivo* with a compound as described herein, can be administered according to methods or uses for administering a graft to a subject, which may be accompanied, e.g., by administration of an immunosuppressant drug, e.g., cyclosporin A. For general principles in medicinal formulation, the reader is referred to Cell Therapy: Stem Cell Transplantation, Gene Therapy, and Cellular Immunotherapy, by
30 G. Morstyn & W. Sheridan eds, Cambridge University Press, 1996; and Hematopoietic Stem Cell Therapy, E. D. Ball, J. Lister & P. Law, Churchill Livingstone, 2000.

Toxicity and therapeutic efficacy of compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The LD₅₀ is the dose

lethal to 50% of the population. The ED₅₀ is the dose therapeutically effective in 50% of the population. The dose ratio between toxic and therapeutic effects (LD₅₀/ ED₅₀) is the therapeutic index. Compounds that exhibit large therapeutic indexes are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds may lie within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

KITS

Also provided herein are kits, e.g., kits for therapeutic purposes or kits for modulating the lifespan of cells or modulating apoptosis. A kit may comprise one or more compounds as described herein, e.g., in premeasured doses. A kit may optionally comprise devices for contacting cells with the compounds and instructions for use. Devices include syringes, stents and other devices for introducing a compound into a subject (e.g., the blood vessel of a subject) or applying it to the skin of a subject.

In yet another embodiment, the invention provides a composition of matter comprising a compound of this invention and another therapeutic agent (the same ones used in combination therapies and combination compositions) in separate dosage forms, but associated with one another. The term “associated with one another” as used herein means that the separate dosage forms are packaged together or otherwise attached to one another such that it is readily apparent that the separate dosage forms are intended to be sold and administered as part of the same regimen. The compound and the other agent are preferably packaged together in a blister pack or other multi-chamber package, or as

connected, separately sealed containers (such as foil pouches or the like) that can be separated by the user (e.g., by tearing on score lines between the two containers).

In still another embodiment, the invention provides a kit comprising in separate vessels, a) a compound of this invention; and b) another therapeutic agent such as those
5 described elsewhere in the specification.

The practice of the present methods will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example,
10 Molecular Cloning A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989); DNA Cloning, Volumes I and II (D. N. Glover ed., 1985); Oligonucleotide Synthesis (M. J. Gait ed., 1984); Mullis et al. U.S. Patent No: 4,683,195; Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins eds. 1984); Transcription And Translation (B. D. Hames & S. J. Higgins eds. 1984); Culture Of
15 Animal Cells (R. I. Freshney, Alan R. Liss, Inc., 1987); Immobilized Cells And Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide To Molecular Cloning (1984); the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Gene Transfer Vectors For Mammalian Cells (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Methods In Enzymology, Vols. 154 and 155 (Wu et al. eds.),
20 Immunochemical Methods In Cell And Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook Of Experimental Immunology, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). The Examples set forth below are illustrative of the present invention and are not intended to limit, in any way, the
25 scope of the present invention.

The Examples set forth below are illustrative of the present invention and are not intended to limit, in any way, the scope of the present invention.

EXAMPLES

The Examples set forth below are illustrative of the present invention and are not intended to limit, in any way, the scope of the present invention, but rather to provide guidance to the skilled artisan to prepare and use the compounds, compositions, and methods or uses of the present invention. While particular embodiments of the present invention are described, the skilled artisan will appreciate that various changes and modifications can be made without departing from the spirit and scope of the invention.

As used herein the symbols and conventions used in these processes, schemes and examples are consistent with those used in the contemporary scientific literature, for example, the *Journal of the American Chemical Society* or the *Journal of Biological Chemistry*. Standard single-letter or three-letter abbreviations are generally used to designate amino acid residues, which are assumed to be in the L-configuration unless otherwise noted. Unless otherwise noted, all starting materials were obtained from commercial suppliers and used without further purification.

All references to ether are to diethyl ether; brine refers to a saturated aqueous solution of NaCl. Unless otherwise indicated, all temperatures are expressed in °C (degrees Centigrade). All reactions are conducted under an inert atmosphere at room temperature unless otherwise noted, and all solvents are highest available purity unless otherwise indicated.

INSTRUMENTATION USED

LCMS with PDA:

Waters Alliance2695-2996/Quattro micro

Agilent-1200/SQD

Preparative LC with UV Detector (Prep HPLC):

Waters-2545/2998 PDA and 2487 UV

Shimadzu –LC-20AP/20AV-UV

Gilson-333,334/115-UV

Chiral HPLC:

Waters Alliance-2695/2998 & 2996

SFC Purification Systems:

Thar - SFC-80

Waters SFC – 200

NMR (400 MHz):

Varian-400 MHz

¹H-NMR tabulation was generated with 2014 ACD labs software.

¹H NMR (hereinafter also "NMR") spectra were recorded on a Varian-400 MHz spectrometer. Chemical shifts are expressed in parts per million (ppm, δ units). Coupling constants are in units of hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet), br (broad).

LCMS Methods Used

10 Acq. Method Conditions: RND-ABC-6-MIN

Column: XBridge BEH C18 (50mmx4.6mm, 2.5 μ m)

Mobile Phase: A: 5mM Ammonium Bicarbonate in water (PH-10 with Ammonia): ACN

Time (min) /%ACN: 0/5, 0.5/5, 1/15, 3.3/98, 5.2/98, 5.5/5, 6.0/5

Column temp: 35°C, Flow Rate 1.3 ml/min

15 MS Parameters:

Mass Range: 100-1000

Scan Time: 0.5 Sec

Inter-Scan delay: 0.1 sec

Run Time: 6.0 min

20

Acq. Method Conditions: RND-FA-4.5-MIN

Column: Acquity BEH C18 (50mmx2.1mm, 1.7 μ m)

Mobile Phase: A: 0.1% FA in water; B: 0.1% FA in ACN

Time (min) /%B: 0/3, 0.4/3, 3.2/98, 3.8/98, 4.2/3, 4.5/3

25 Column Temp: 35°C, Flow Rate: 0.6mL/min

MS Parameters:

Mass Range: 100-1000

Scan Time: 0.5 Sec

Inter-Scan delay: 0.1 sec

30 Run Time: 4.5 min

Acq. Method Conditions: RND-FA-4.5-MIN

Column: Acquity BEH C18 (50mmx2.1mm, 1.7 μ m)

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Mobile Phase: A: 0.1% FA in water; B: 0.1% FA in ACN

Time (min) /%B: 0/3, 0.4/3, 3.2/98, 3.8/98, 4.2/3, 4.5/3

Column Temp: 35°C, Flow Rate: 0.6mL/min

MS Parameters:

5 Mass Range: 100-1000

Fragmentor: 100

Step Size: 0.1

Run Time: 4.5 min

10 Acq. Method Conditions: RND-ABC-6.5-MIN

Column: XBridge BEH C18 (50mmx4.6mm, 2.5µm)

Mobile Phase: A: 5mM Ammonium Bicarbonate in water (PH-10 with Ammonia): ACN

Time (min) /%ACN: 0/5, 0.5/5, 1/15, 3.3/98, 6.0/98, 6.1/5, 6.5/5

Column temp: 35°C, Flow Rate 1.3 ml/min

15 MS Parameters:

Mass Range: 100-1000

Fragmentor: 100

Step Size: 0.1

Run Time: 6.5 min

20

Acq. Method Conditions: RND-ABC-10-MIN

Column: XBridge BEH C18 (50mmx4.6mm, 2.5µm)

Mobile Phase: A: 5mM Ammonium Bicarbonate in water (PH-10 with Ammonia): ACN

Time (min) /%ACN: 0/5, 0.5/5, 1.5/15, 7/98, 9.0/98, 9.5/5, 10/5

25 Column temp: 35°C, Flow Rate 1.3 ml/min

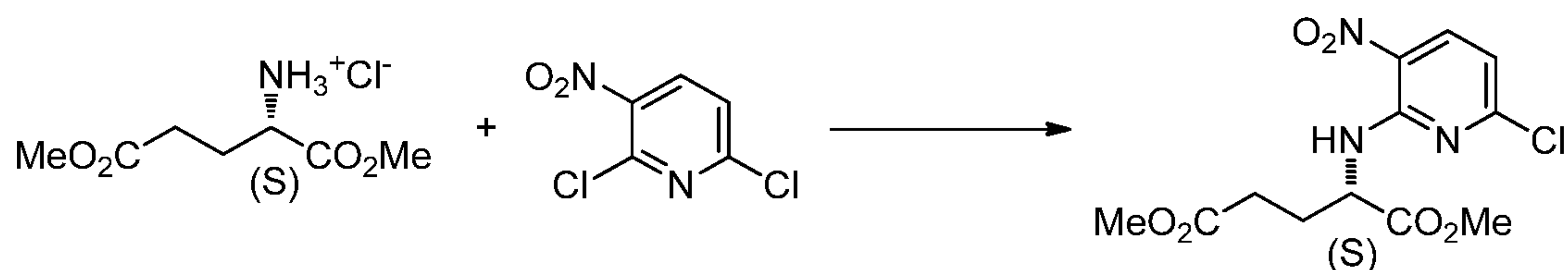
MS Parameters:

Mass Range: 100-1000

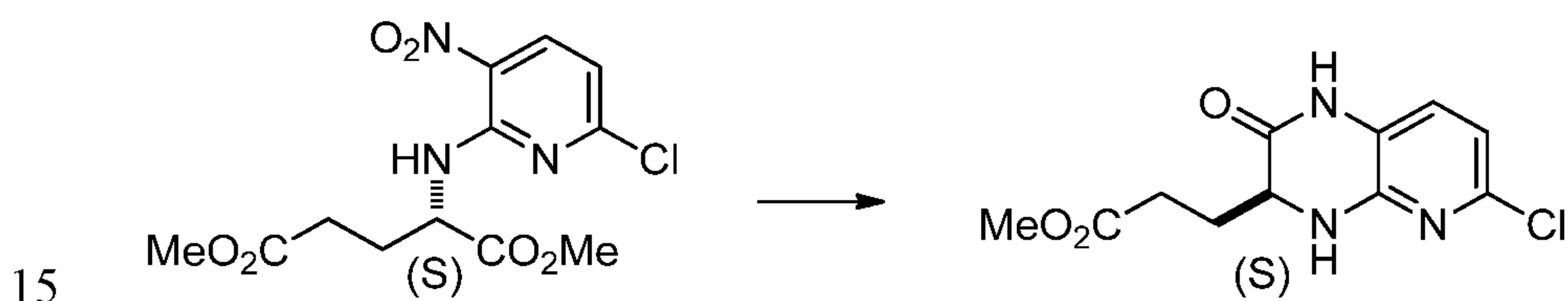
Fragmentor: 100

Step Size: 0.1

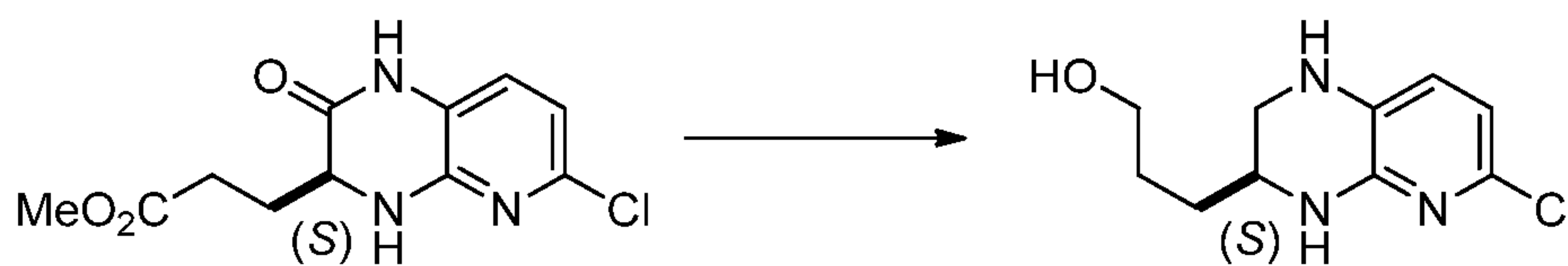
30 Run Time: 10.0 min

INTERMEDIATES**Synthesis of (S)-dimethyl 2-((6-chloro-3-nitropyridin-2-yl)amino)pentanedioate**

To a mixture of 40.0 g (207 mmol) of 2,6-dichloro-3-nitropyridine, 87.7 g (414 mmol) of L-glutamic acid dimethyl ester hydrochloride, and 69.6 g (829 mmol) of NaHCO₃ was added 600 mL of tetrahydrofuran. The mixture was stirred at 40 °C for 24 h, monitoring for the disappearance of 2,6-dichloro-3-nitropyridine by HPLC. After the reaction was complete, the solids were filtered away and washed with ethyl acetate (3 x 100 mL). The combined filtrate and washings were concentrated in vacuo, then the residue was purified via silica gel chromatography, eluting with 10/1 (v/v) hexanes/ethyl acetate, to give (60 g, 87%) of the product as a yellow solid, LCMS (*m/z*) 332.1 [M+H]⁺.

Synthesis of (S)-methyl 3-(6-chloro-2-oxo-1,2,3,4-tetrahydropyrido[2,3-b]pyrazin-3-yl)propanoate

To a mixture of 20 g (60.2 mmol) of (S)-dimethyl 2-((6-chloro-3-nitropyridin-2-yl)amino)pentanedioate and 16.8 g (301 mmol) of iron powder was added 375 mL of 2-propanol, then 125 mL of water. To the stirred mixture was added 5.5 g (90.3 mmol) of acetic acid, then the reaction was stirred at reflux for 1 h. The reaction was monitored for the disappearance of starting material by HPLC. After the reaction was complete, the solids were filtered off and washed with 2-propanol (3 x 50 mL). The combined filtrate and washings were concentrated to dryness, and then the residue was dried in vacuo to give 15 g (81%) of the product as a dark yellow solid. This was used without further purification in the next step, LCMS (*m/z*) 270.1 [M+H]⁺.

Synthesis of (S)-3-(6-chloro-1,2,3,4-tetrahydropyrido[2,3-b]pyrazin-3-yl)propan-1-ol

To a solution of 17.78 g (133.3 mmol) of AlCl_3 in 260 mL of tetrahydrofuran (THF) under N_2 was added 200 mL of 2M LiAlH_4 in THF, dropwise, at a rate to control gas evolution.

- 5 This gave a solution of alane (AlH_3) in THF. In a separate flask, a solution of 26.0 g (96.4 mmol) of (S)-methyl 3-(6-chloro-2-oxo-1,2,3,4-tetrahydropyrido[2,3-b]pyrazin-3-yl)propanoate in 460 mL of THF was prepared under N_2 , then cooled with a dry ice/acetone bath. To this was added the alane solution, dropwise with stirring, over 2 h. When the addition was complete, the cooling bath was removed, and the reaction was
- 10 allowed to warm to ambient temperature. After 1.5 h, LCMS analysis showed that the reaction was complete. Next, a solution of 17.6 g NaOH in 65 mL of water was added slowly to control the evolution of H_2 . The suspension was allowed to stir for 18 h, and then the solids were filtered away. The precipitate was washed with ethyl acetate, then the filtrate and washings were concentrated in vacuo. The product was purified via silica gel
- 15 chromatography eluting with CH_2Cl_2 , followed by a gradient of 0 to 10% methanol in CH_2Cl_2 to give 15.21 g (69%) of a yellow-orange solid, LCMS (m/z) 228.1 $[\text{M}+\text{H}]^+$.

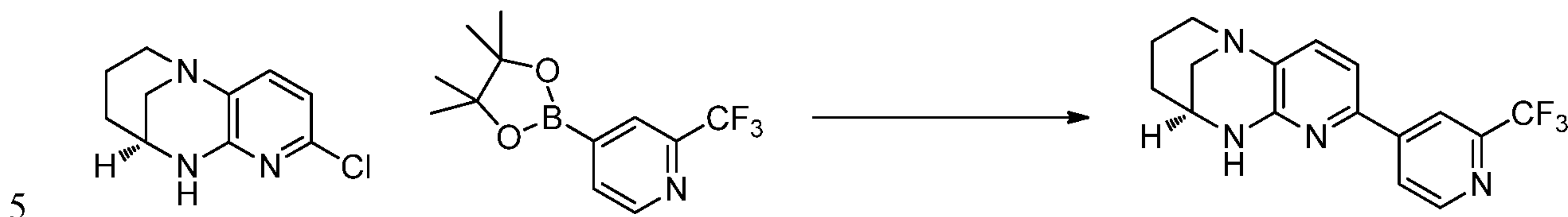
Synthesis of (9S)-2-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine

20

- To 12 g (52.7 mmol) of (S)-3-(6-chloro-1,2,3,4-tetrahydropyrido[2,3-b]pyrazin-3-yl)propan-1-ol was added 160 mL of 48% (w/w) aq. HBr, then the reaction was stirred at 90 °C for 18 h. The reaction was monitored by HPLC for the disappearance of the starting alcohol. After the reaction was complete, it was cooled to ambient temperature, then 1.2
- 25 M aq. NaHCO_3 was added until pH = 8. The mixture was extracted with ethyl acetate (3 x 100 mL), then the combined organic phases were washed with brine (1 x 100 mL), dried over Na_2SO_4 and filtered, and the filtrate was concentrated to dryness. The residue was

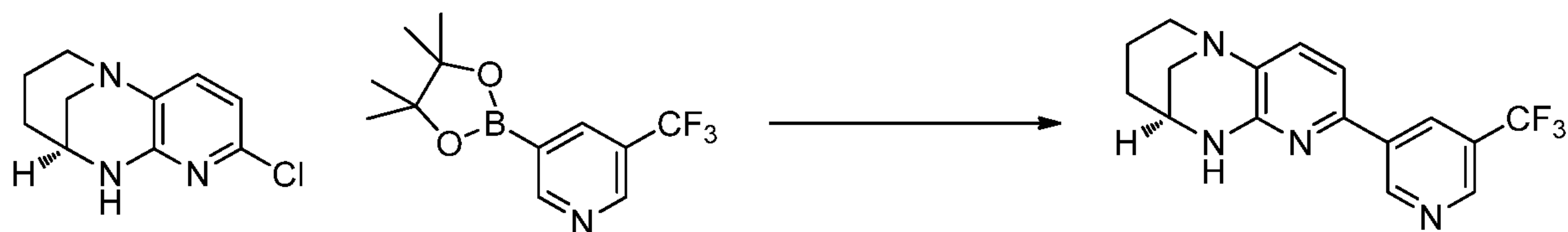
purified via silica gel chromatography, eluting with 2/1 (v/v) hexanes/ethyl acetate to give 6.0 g (55%) of the product as a light yellow solid, LCMS (m/z) 210.1 $[M+H]^+$.

Synthesis of 2-(2-(trifluoromethyl)pyridin-4-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine



To a solution of 2-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (3 g, 14.31 mmol), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2-(trifluoromethyl)pyridine (3.91 g, 14.31 mmol) and cesium carbonate (4.66 g, 14.31 mmol) in 1,4-Dioxane (60 ml) and water (6 ml) at room temp and reaction mass degassed with argon for 20 min. Next, added solid palladium(II) acetate (3.21 g, 14.31 mmol) and x-phos (6.82 g, 14.31 mmol) in to the reaction mass in one charge. The reaction mixture was stirred at 105 °C for 3-4 hrs. The reaction mass was filtered through celite bed and concentrated. The crude material was taken and dissolved in ethyl acetate and washed with sodium bicarbonate solution and water. Organic phase was dried over sodium sulfate and concentrated to get. The residue was triturated with n-pentane (3x 50 mL). The resulting solid was filtered through a Buchner funnel, rinsed with n-pentane, and collected as the desired product (4 g, 86 %), LCMS (m/z) 321.3 $(M+H)^+$.

20 **Synthesis of (9S)-2-(5-(trifluoromethyl)pyridin-3-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine**

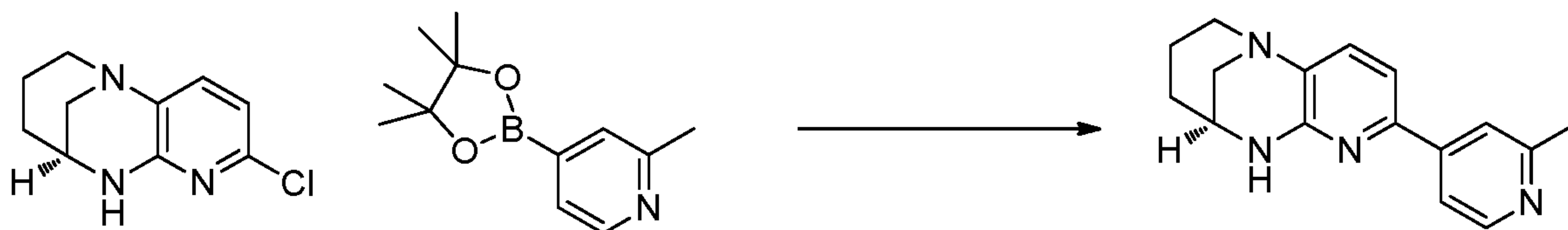


To a solution of (9S)-2-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (3 g, 14.31 mmol), (5-(trifluoromethyl)pyridin-3-yl)boronic acid (5.46 g, 28.6 mmol) and Cs₂CO₃ (13.99 g, 42.9 mmol) in Tetrahydrofuran (THF) (60 ml), water (4 ml) stirred under nitrogen at 25 °C, purged with Argon gas for 20 minutes. Then palladium (II) acetate (0.080 g, 0.358 mmol) and X-Phos (227 mg) were added. The reaction mixture was stirred at 110 °C for 16 hr. Next, the reaction mixture was concentrated and the residue was taken up in DCM (100 mL). The solution was washed

with water and brine, dried over Na₂SO₄, filtered and concentrated. The crude product was added to a silica gel column and was eluted with Hex/EtOAc (1:1) Collected fractions were evaporated to give as a off white solid (3.6g, 11.1mmol 76%), LCMS (*m/z*) 321.2 [M+H]⁺.

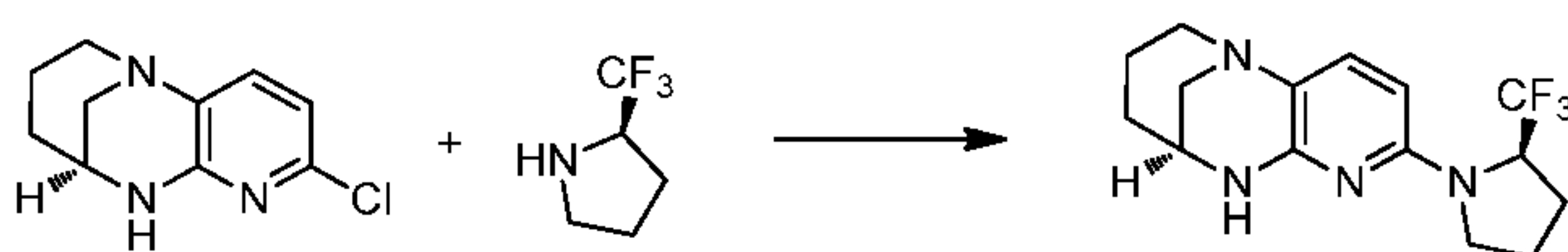
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Synthesis of (9S)-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine



To a solution of (9S)-2-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (10 g, 47.7 mmol), (2-methylpyridin-4-yl)boronic acid (8.49 g, 62.0 mmol) and Potassium phosphate (30.4 g, 143 mmol) in 1-Butanol (300 ml), water(100ml) stirred under nitrogen at 25°C, purged with Argon gas for 20 minutes was added X-Phos (2.274 g, 4.77 mmol), Pd₂(dba)₃ (2.184 g, 2.385 mmol). The reaction mixture was stirred at 120 °C for 16 hr. Before being concentrated and the residue was taken up in DCM (700 mL). The solution was washed with water and brine, dried over Na₂SO₄, filtered and concentrated to get brown semisolid crude product. The crude product was purified by washing with hexane to get off white solid product (9 g, 32.4 mmol, 67.9 % yield), LCMS (*m/z*): 267.3 (M+H)⁺.

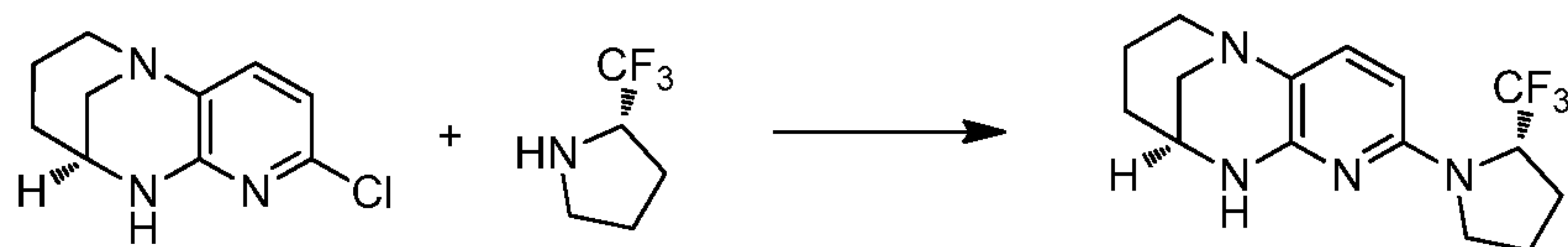
Synthesis of (9S)-2-((R)-2-(trifluoromethyl)pyrrolidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine



To a suspension of (9S)-2-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (0.5 g, 2.385 mmol), (R)-2-(trifluoromethyl)pyrrolidine (0.663 g, 4.77 mmol) and KO^tBu (0.535 g, 4.77 mmol) in 1,2-Dimethoxyethane (DME) (10 mL) under nitrogen atmosphere at room temperature was added solid [1,3-Bis(2,6-diisopropylphenyl)-4,5-dihydroimidazol-2-ylidene]chloro[[3-phenylallyl]palladium(II) (1.549 g, 2.385 mmol) and stirred at 80 °C for 16 h. The reaction mass was cooled down to room temperature and filtered through celite and the solvent was evaporated under

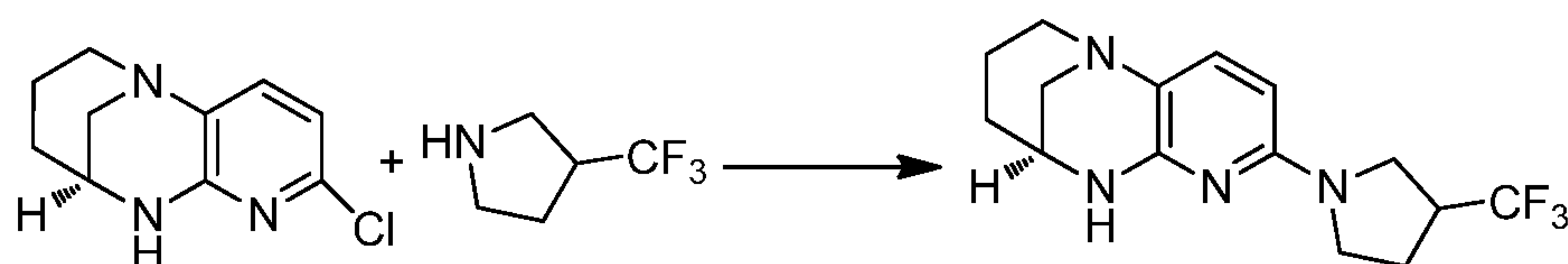
reduced pressure to obtain crude residue. The crude residue was diluted with EtOAc (100 mL) and washed with water (50mL x2) followed by brine solution and dried over anhydrous sodium sulfate, filtered and evaporated to obtain the crude product. The crude product was purified by flash column chromatography (silica gel: 100-200 mesh, eluted with 1:1 Hex/EtOAc) to afford (9*S*)-2-((2*R*)-2-(trifluoromethyl)cyclopentyl)-6,7,8,9,10,10*a*-hexahydro-4*aH*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (500mg, 1.596 mmol, 64.6% yield) as a light green solid (TLC: R_f 0.3, eluent: 80%EtOAc in Hexane), LCMS (m/z) 313.2 $[M+H]^+$.

Synthesis of (9*S*)-2-((*S*)-2-(trifluoromethyl)pyrrolidin-1-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine



A suspension of (9*S*)-2-chloro-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (0.5 g, 2.385 mmol), (*S*)-2-(trifluoromethyl)pyrrolidine (0.663 g, 4.77 mmol) and KOtBu (0.535 g, 4.77 mmol) in 1,2-Dimethoxyethane (DME) (10 mL) was stirred and degassed with argon at room temp for 15 mins. Next, [1,3-Bis(2,6-diisopropylphenyl)-4,5-dihydroimidazol-2-ylidene]chloro[[3-phenylallyl]palladium(II) (1.549 g, 2.385 mmol) added to the reaction mixture. Then the reaction mixture was stirred 16 hr at 80 °C. After completion, the reaction mass filtered through celite and concentrated to dryness. The resulting residue was diluted with EtOAc and washed with water followed by brine solution and dried out with sodium sulfate, filtered and evaporated. The crude product was added to a silica gel column, eluted with Hex/EtOAc (1:1). Collected fractions were evaporated to get (9*S*)-2-((*S*)-2-(trifluoromethyl)pyrrolidin-1-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (0.6 g, 1.868 mmol, 78 % yield) as an off-white solid, LCMS (m/z) 313.3 $[M+H]^+$.

Synthesis of (9*S*)-2-(3-(trifluoromethyl)pyrrolidin-1-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine



KOtBu (0.321 g, 2.86 mmol) was added to a stirred solution of (9S)-2-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (0.3 g, 1.431 mmol), and 3-(trifluoromethyl)pyrrolidine (0.398 g, 2.86 mmol) in 1,2-Dimethoxyethane (DME) (10 mL). The reaction mixture was stirred and degassed with argon at room temp for 15 mins. and then (1,3-Bis(2,6-di-isopropylphenyl)-4,5-dihydroimidazol-2-ylidene)chloro (3-phenylallyl)palladium(2) (0.037 g, 0.057 mmol) added to the reaction mixture. The reaction was stirred for 16 h at 80 °C. The reaction was cooled to room temperature, filtered through celite and evaporated under reduced pressure. The reaction mixture was partitioned between water (20 mL) and EtOAc (50 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid (TLC eluent: 80% EtOAc: R_f-0.4; UV active). The crude was purified by column chromatography using (100-200 mesh) silica gel and was eluted with 50% EtOAc in Hexane to afford (85: 15) mixture and further purified by SFC to afford pure (9S)-2-(3-(trifluoromethyl)pyrrolidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (0.151 g, 0.473 mmol, 33.1 % yield) as a Off-white solid.

Analytical SFC Conditions: Peak-II Column/dimensions:

Chiralpak AD-H (250 X 4.6)mm, 5u

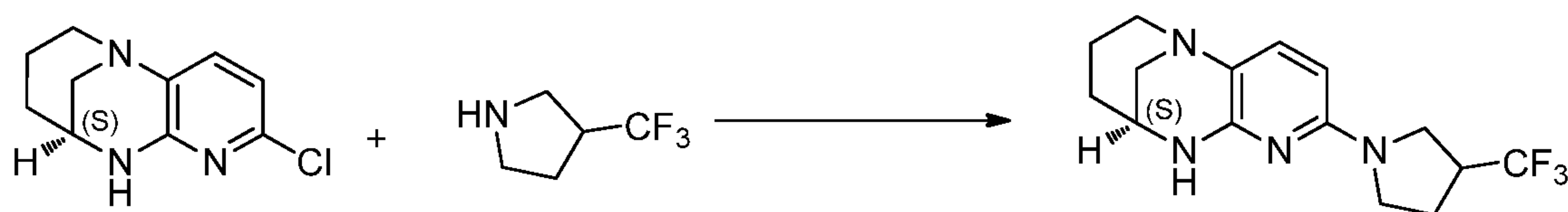
% CO₂ : 70.0%
 % Co solvent : 30.0% (0.5% DEA In MeOH)
 Total Flow : 3.0g/min
 Back Pressure : 100 bar
 Temperature : 26.8oC
 UV : 212nm

Preparative SFC Conditions

Column/dimensions : Chiralpak AD-H (250 X 30)mm
 % CO₂ : 75%
 % Co solvent : 25.0% (MeOH)
 Total Flow : 100.0g/min
 Back Pressure : 100bar
 UV : 212nm
 Stack time : 1.8min
 Load/inj : 5.5mg
 LCMS (*m/z*) 313.3 [M+H]⁺.

¹H NMR (400 MHz, DMSO-d₆): δ 6.87 (d, J = 8.1 Hz, 1H), 6.49 (d, J = 4.9 Hz, 1H), 5.58 (d, J = 8.1 Hz, 1H), 3.60 (dd, J = 10.8, 8.4 Hz, 1H), 3.47 (s, 1H), 3.39 (dd, J = 10.6, 5.8 Hz, 2H), 3.30 (s, 2H), 3.14 - 2.98 (m, 2H), 2.90 (d, J = 4.5 Hz, 1H), 2.50 (qd, J = 3.2, 2.2, 1.6 Hz, 1H), 2.31 - 2.13 (m, 1H), 2.05 (d, J = 7.1 Hz, 1H), 1.72 (dt, J = 7.0, 3.0 Hz, 2H), 1.44 (s, 1H), 1.16 (d, J = 14.4 Hz, 1H).

Synthesis of (9S)-2-(3-(trifluoromethyl)pyrrolidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine



(1,3-Bis(2,6-di-isopropylphenyl)-4,5-dihydroimidazol-2-ylidene)chloro) (3-phenylallyl) palladium (0.062 g, 0.095 mmol) added to a degassed suspension of (9S)-2-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (0.5 g, 2.385 mmol), 3-(trifluoromethyl)pyrrolidine (0.663 g, 4.77 mmol) and KOtBu (0.535 g, 4.77 mmol) in 1,2-dimethoxyethane (20 mL) at RT. The reaction mixture was further degassed for 10 min and was stirred for 16 h at 80 °C. The reaction mixture was cooled to RT and was filtered through a pad of celite. The filtrate was evaporated to obtain brown residue. The residue was partitioned between water (15 mL) and EtOAc (2X 25 mL). The organic layer was washed with water followed by brine solution and dried over anhydrous sodium sulfate, filtered and filtrate was evaporated to get the crude (TLC eluent: 80% EtOAc/hexane, R_f value: 0.4, UV active). The crude was purified by column chromatography (100-200 mesh) using silica gel, and the product was eluted 50% ethyl acetate in pet ether to give (30:70) mixture of enantiomers which on further SFC purification afforded (9S)-2-(3-(trifluoromethyl)pyrrolidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (0.125 g, 0.393 mmol, 16.46 % yield) as a brown solid.

Analytical SFC Conditions: Peak I (major)

Chiralpak AD-H (250 X 4.6)mm, 5μ

% CO₂ : 70.0%

% Co solvent : 30.0% (0.5% DEA In MeOH)

Total Flow : 3.0g/min

Back Pressure : 100 bar

Temperature :26.8°C

UV :212nm

Preparative SFC Conditions

Column/dimensions : Chiralpak AD-H (250 X 30)mm

5 % CO₂ :75%

% Co solvent :25.0% (MeOH)

Total Flow :100.0g/min

Back Pressure :100bar

UV :212nm

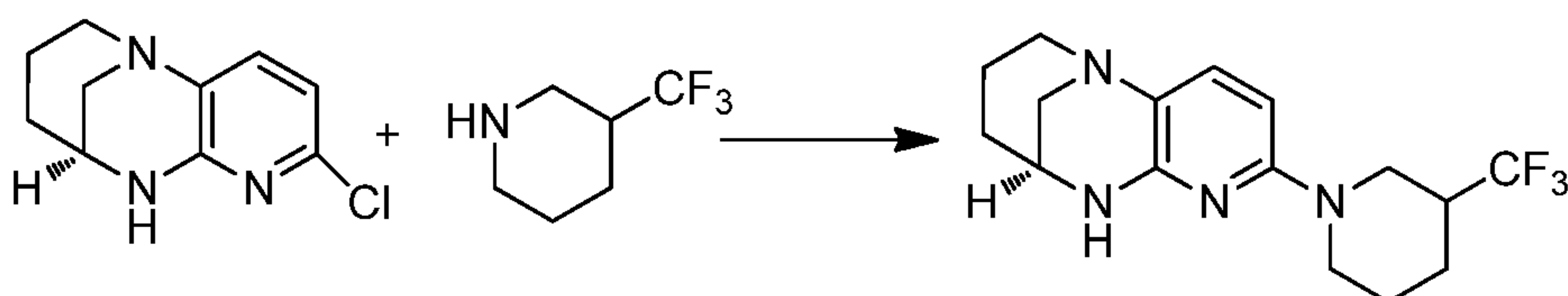
10 Stack time :1.8min

Load/inj :5.5mg

LCMS (*m/z*) 313.31[M+H]⁺.

¹H NMR (400 MHz, CDCl₃): δ 7.03 (dd, *J* = 8.2, 0.6 Hz, 1H), 5.67 (d, *J* = 8.2 Hz, 1H), 4.91 (s, 1H), 3.71 (dd, *J* = 10.8, 8.5 Hz, 1H), 3.62 (s, 1H), 3.52 (dd, *J* = 10.8, 7.2 Hz, 2H), 3.43 (dt, *J* = 9.6, 7.5 Hz, 1H), 3.24 - 3.08 (m, 3H), 2.99 (d, *J* = 8.2 Hz, 1H), 2.83 (d, *J* = 12.0 Hz, 1H), 2.27 - 2.08 (m, 2H), 1.91 - 1.75 (m, 2H), 1.65 (d, *J* = 5.0 Hz, 2H), 1.26 (t, *J* = 6.6 Hz, 1H).

20 Synthesis of (9*S*)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-*b*][1,4]diazocine.



A suspension of (9*S*)-2-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-*b*][1,4]diazocine (0.750g, 3.58 mmol), 3-(trifluoromethyl)piperidine (1.096 g, 7.15 mmol) and KOtBu (1.204 g, 10.73 mmol) in 1,2-Dimethoxyethane (DME) (30 mL) stirred and degassed with argon at room temp for 15 mins. and then (1,3-Bis(2,6-di-isopropylphenyl)-4,5-dihydroimidazol-2-ylidene)chloro) (3-phenylallyl)palladium(2) (0.093 g, 0.143 mmol) added to the reaction mixture. Then the reaction mixture was stirred for 16 h at 80 °C. The reaction mass filtered through celite and evaporated under reduced pressure completely. Reaction mixture was diluted with EtOAc (50 ml), washed with water followed by brine solution, dried with sodium sulfate, filtered and evaporated. The crude product was added

to a silica gel column and was eluted with Hex/EtOAc (1:1). Collected fractions were evaporated and the resulting residue was purified via chiral SFC separation to get pure (9S)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (0.450 g, 1.371 mmol, 38.3 % yield) as off white solid, (TLC: R_f value:0.4, 80% EtOAc/Hexane).

Analytical SFC Conditions: Peak-I

Column/dimensions : Chiralpak AD-H (250 X 4.6)mm,5u

% CO₂ : 60.0%

% Co solvent : 40.0% (0.5% DEA IN MeOH)

10 Total Flow : 4.0g/min

Back Pressure :100 bar

Temperature :26.8°C

UV :215nm

Preparative SFC Conditions

15 Column/dimensions : Chiralpak AD-H (250 X 30)mm

% CO₂ :65.0%

% Co solvent :35.0% (100% MeOH)

Total Flow :100.0g/min

Back Pressure :100bar

20 UV :215nm

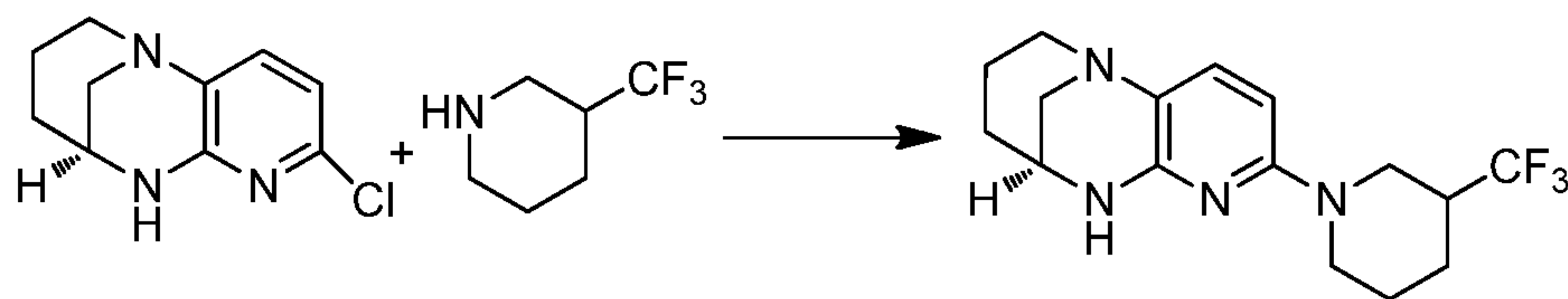
Stack time :2.2min

Load/inj :50.0mg

LCMS (*m/z*) 327.3 [M+H]⁺.

¹H NMR (400 MHz, DMSO-*d*₆): δ 6.89 (dd, *J* = 8.2, 0.7 Hz, 1H), 6.56 (d, *J* = 4.6 Hz, 1H), 5.89 (d, *J* = 8.3 Hz, 1H), 4.58 - 4.33 (m, 1H), 3.97 (d, *J* = 12.8 Hz, 1H), 3.48 (s, 1H), 3.17 - 2.98 (m, 2H), 2.92 (d, *J* = 4.4 Hz, 1H), 2.76 - 2.57 (m, 2H), 2.50 (p, *J* = 1.9 Hz, 2H), 1.96 (d, *J* = 10.6 Hz, 1H), 1.74 (dd, *J* = 9.4, 3.3 Hz, 3H), 1.61 - 1.32 (m, 3H), 1.25 - 1.08 (m, 1H).

Synthesis of (9S)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine



KOtBu (0.803 g, 7.15 mmol) was added to a stirred solution of (9S)-2-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (0.750 g, 3.58 mmol), and 3-(trifluoromethyl)piperidine (1.096 g, 7.15 mmol) in 1,2-Dimethoxyethane (DME) (20 mL). The reaction was stirred and degassed with argon at room temp for 15 mins. and then (1,3-Bis(2,6-di-isopropylphenyl)-4,5-dihydroimidazol-2-ylidene)chloro) (3-phenylallyl)palladium(2) (0.093 g, 0.143 mmol) added to the reaction mixture. The reaction mixture was stirred for 16 h at 80 °C. The reaction was cooled to room temperature, filtered through celite and evaporated completely, and was partitioned between water (20 mL) and EtOAc (50 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid (TLC eluent: 80% EtOAc : R_f-0.4; UV active). The crude was purified by column chromatography using (100-200 mesh) silica gel and was eluted with 50% EtOAc in Hexane to afford (69: 31) mixture and further purified by SFC to afford pure (9S)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (450 mg, 1.310 mmol, 36.6 % yield) as a pale yellow solid.

Analytical SFC Conditions: Peak-II

Column/dimensions : Chiralpak AD-H (250 X 4.6)mm,5u

% CO₂ : 60.0%

% Co solvent : 40.0% (0.5% DEA IN MeOH)

Total Flow : 4.0g/min

Back Pressure : 100 bar

Temperature : 26.8°C

UV : 215nm

Preparative SFC Conditions

Column/dimensions : Chiralpak AD-H (250 X 30)mm

% CO₂ : 65.0%

% Co solvent :35.0% (100% MeOH)

Total Flow :100.0g/min

Back Pressure :100bar

UV :215nm

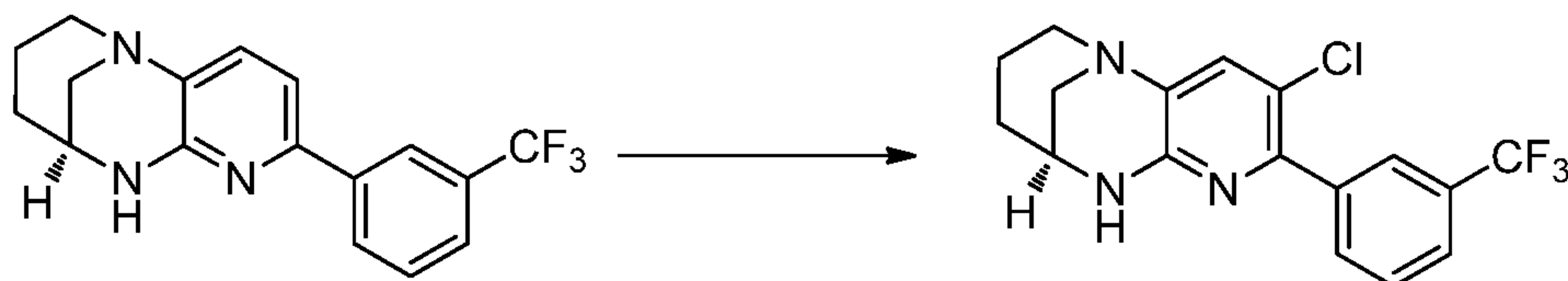
5 Stack time :2.2min

Load/inj :50.0mg

LCMS (*m/z*) 327.2 [M+H]⁺.

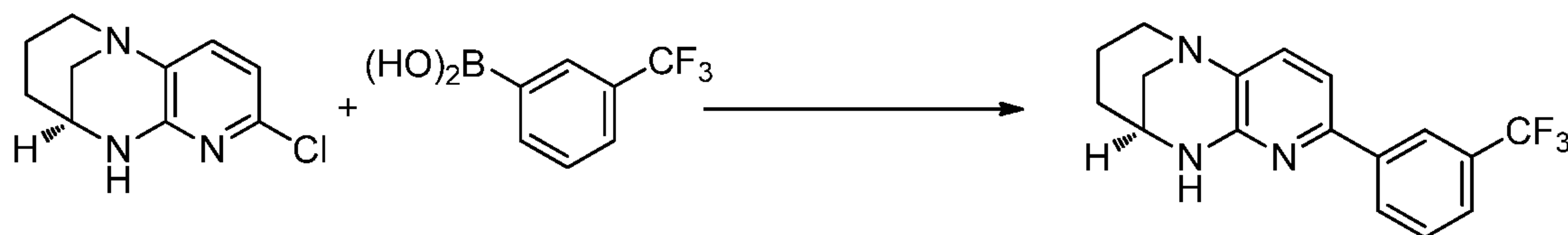
1H NMR (400 MHz, DMSO-d₆): δ ppm 6.89 (d, J=8.11 Hz, 1 H), 6.55 (br d, J=3.95 Hz, 1 H), 5.89 (d, J=8.33 Hz, 1 H), 4.49 (dt, J=12.44, 1.78 Hz, 1 H), 3.96 (d, J=13.15 Hz, 1 H),
 10 3.48 (br s, 1 H), 3.15 – 2.96 (m, 2 H), 2.94 - 2.80 (m, 1 H), 2.74 - 2.56 (m, 2 H), 2.55 -
 2.41 (m, 2 H), 2.12 – 1.84 (m, 1 H), 1.82 - 1.64 (m, 3 H), 1.58 - 1.32 (m, 3 H), 1.32 - 1.08
 (m, 1 H).

15 **Synthesis of (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine**



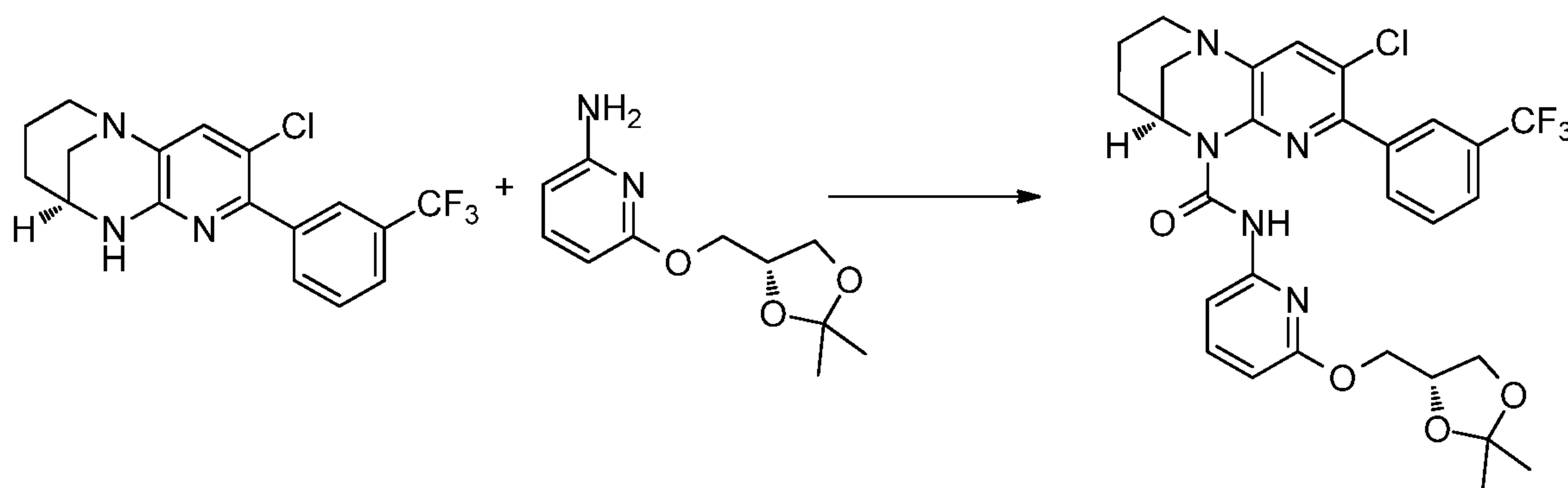
To a solution of (9S)-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (2 g, 6.26 mmol) in Chloroform (20 mL) stirred under nitrogen at 0°C was added NCS (1.004 g, 7.52 mmol). The reaction mixture was stirred at
 20 RT for 2 hr. Reaction mixture was quenched with water and extracted with 2x25 ml of DCM, organic layer was dried over Na₂SO₄ and concentrated under reduced pressure to afford crude compound. The crude product was purified by flash column chromatography with 100-200 silica gel and was eluted with 70% ethyl acetate in pet ether to afford pure compound (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (800 mg, 1.859 mmol, 29.7 % yield) as pale yellow
 25 semi solid, LCMS (*m/z*): 354.22 [M+H]⁺.

Synthesis of (9S)-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine



To solid (9S)-2-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (5 g, 23.85 mmol), (3-(trifluoromethyl)phenyl)boronic acid (6.79 g, 35.8 mmol) and potassium phosphate tri basic (15.19 g, 71.5 mmol) in 1,4-Dioxane (160 mL) and Water (40 mL) stirred and degas with argon for 10 mints then added solid x-phos (0.689 g, 4.77 mmol) and $\text{Pd}_2(\text{dba})_3$ (2.184 g, 2.385 mmol) again the reaction mixture degas for 5 mints and stirred at 110 °C for 16 hr. The organic phase was washed with water 50 mL, saturated brine 100 mL and dried over Na_2SO_4 and evaporated under vacuum to give the crude products as a brown solid. The crude products was washed with diethyl ether and pentane and filtered washed with diethyl ether to get pure compound (9S)-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (4.8 g, 13.81 mmol, 57.9 % yield), LCMS (m/z): 320.11 $[\text{M}+\text{H}]^+$.

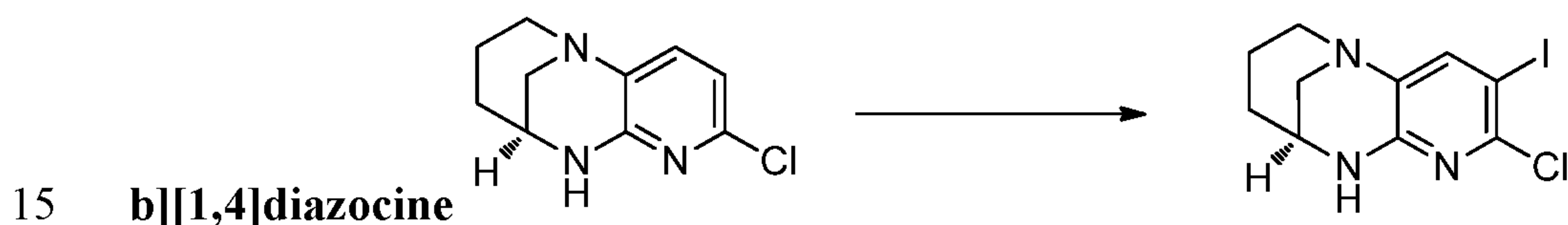
Synthesis of (9S)-3-chloro-N-(6-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



Triphosgene (377 mg, 1.272 mmol) was added to a stirred solution of (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (450 mg, 1.272 mmol), and TEA (0.886 mL, 6.36 mmol) in Tetrahydrofuran (THF) (50 mL) at 28 °C. The reaction mixture was stirred for 30 min and was added (R)-6-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-amine (571 mg, 2.54 mmol). The reaction

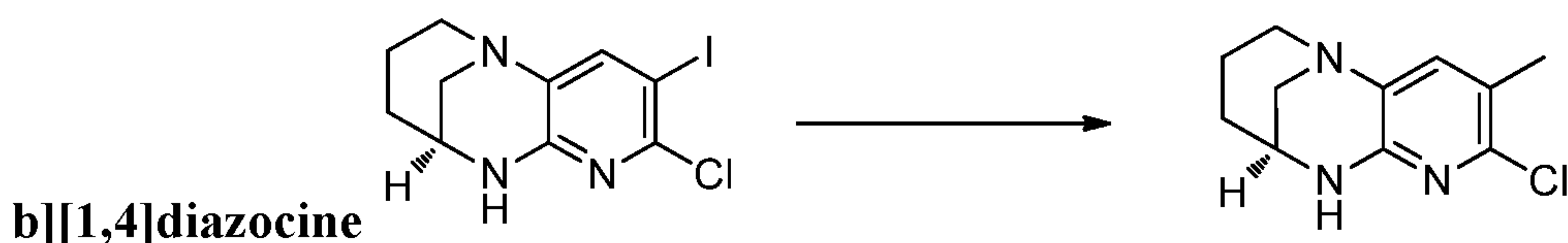
mixture was stirred for 10h at 70 °C. The reaction mixture was cooled to room temperature and was partitioned between water (5 mL) and EtOAc (15 mL). EtOAc layer was separated and was dried over anhydrous Na₂SO₄, filtered. The filtrate was evaporated to get crude. The crude was purified by GRACE using C-18 reserval column, Mobile phase A: 0.1% Formic Acid in water; B: MeOH, the product was eluted at 91% of MeOH in 0.1% Formic Acid in water. The solvent was evaporated and was basified with saturated NaHCO₃. The aqueous layer was extracted with DCM, DCM layer was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to afford (9S)-3-chloro-N-(6-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (250 mg, 0.391 mmol, 30.8 % yield) as yellow solid, LCMS (*m/z*): 604.14 [M+H]⁺.

Synthesis of (9S)-2-chloro-3-iodo-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine



NIS (2.58 g, 11.45 mmol) was added to a stirred solution of (9S)-2-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (2.0 g, 9.54 mmol) in Chloroform (40 mL) under nitrogen at 0°C and the reaction mixture was stirred 16 hr at 65 °C. The reaction mixture was cooled to room temp, solvent evaporated under reduced pressure completely to afford the crude product. The crude product was purified by column chromatography using neutral alumina and was eluted with 20% EtOAc in Hexane (gradient system) to afford the desired product (9S)-2-chloro-3-iodo-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (1.65 g, 4.53 mmol, 47.5 % yield) as a pale yellow solid, LCMS (*m/z*): 335.90 [M+H]⁺.

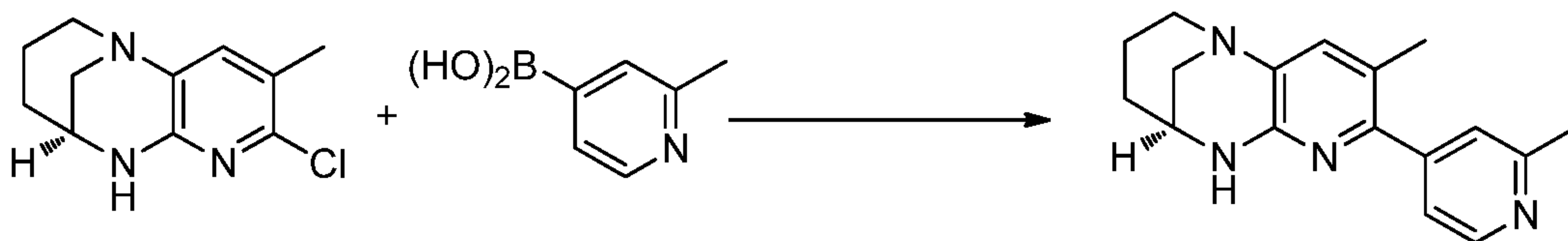
Synthesis of (9S)-2-chloro-3-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine



A suspension of (9S)-2-chloro-3-iodo-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (1.65 g, 4.92 mmol), trimethylboroxine (1.639 mL, 4.92 mmol) and

potassium carbonate (2.039 g, 14.75 mmol) in 1,4-Dioxane (15 mL) & Water (1.5 mL) stirred and degassed with argon at room temp for 15 mins, tetrakis(triphenylphosphine)palladium(0) (0.568 g, 0.492 mmol) was added to the reaction mixture. Then the reaction mixture was stirred 48 hr at 90 °C. The reaction mixture was cooled to room temp, and filtered through celite and washed with EtOAc (30 ml). Take filtrate and concentrated and dissolved with EtOAc (50 ml). EtOAc layer washed with water (15 ml) followed by brine solution (15 ml) and dried out with Na₂SO₄, filtered and concentrated to get crude product. The crude product was purified by column chromatography using neutral alumina and was eluted with 20% EtOAc in Hexane (gradient system) to afford the desired product (9S)-2-chloro-3-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (0.800 g, 3.55 mmol, 72.1 % yield) as a pale yellow solid (TLC eluent: 50% EtOAc in Hexane : R_f 0.3; UV active). LCMS (*m/z*): 224.90 [M+H]⁺.

15 Synthesis of (9S)-3-methyl-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine

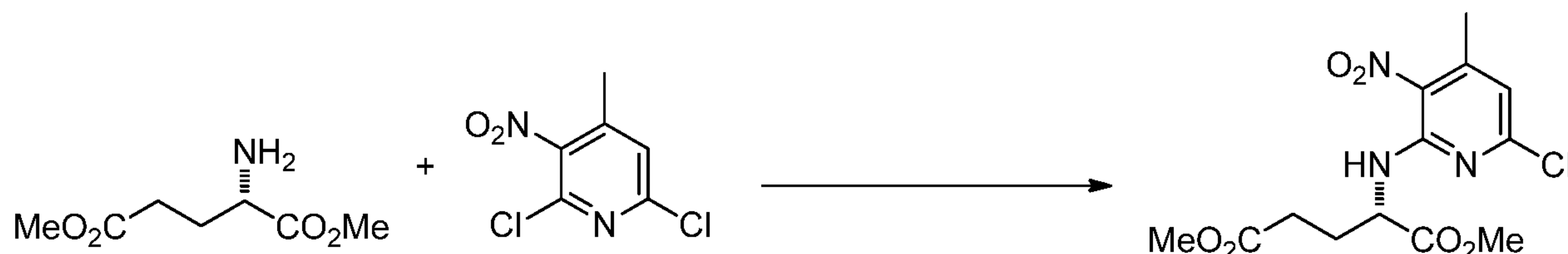


A suspension of (9S)-2-chloro-3-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (800 mg, 3.58 mmol), (2-methylpyridin-4-yl)boronic acid (612 mg, 4.47 mmol) and tripotassium phosphate (2277 mg, 10.73 mmol) in 1,4-Dioxane (20 mL) & Water (4 mL) stirred and degassed with argon at room temp for 15 mins. Pd₂(dba)₃ (164 mg, 0.179 mmol) and X-Phos (170 mg, 0.358 mmol) added to the reaction mixture. Then the reaction mixture was stirred 16 hr at 90 °C. The reaction was monitored by TLC. The reaction mixture was cooled to room temp and filtered through celite and washed with EtOAc. Take filtrate and concentrated and dissolved with EtOAc. EtOAc layer washed with water followed by brine solution and dried out with Na₂SO₄, filtered and concentrated to get crude product. The crude product was purified by ether (20 ml) washings to afford desired product (9S)-3-methyl-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (600 mg, 2.121 mmol, 59.3 % yield) as an off white solid, LCMS (*m/z*): 281.16 [M+H]⁺.

Synthesis of 2,6-dichloro-4-methyl-3-nitropyridine

- 5 To a suspension of 2,6-dichloro-4-methylpyridine (5g, 30.9 mmol) in trifluoroacetic anhydride (24.98 mL, 177 mmol) cooled to 0° C. was added dropwise nitric acid (2.90 mL, 64.8 mmol) into it. The resulting solution was stirred at RT for 18 hr. The reaction mixture was added slowly to a chilled solution of sodium metabisulfite (5.87 g, 30.9 mmol) in Water (40 mL) and stirred at RT for 2 hr. The reaction mixture was neutralized
- 10 to pH 7 using 8N NaOH (25 mL) solution and extracted twice with DCM (100 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure to afford 2,6-dichloro-4-methyl-3-nitropyridine (6.2g, 29.4 mmol, 95 % yield) as off white solid, LCMS (*m/z*): 206.98 [M+H]⁺.

15 **Synthesis of (S)-dimethyl 2-((6-chloro-4-methyl-3-nitropyridin-2-yl)amino)pentanedioate**



- Procedure: (S)-dimethyl 2-aminopentanedioate hydrochloride (147 g, 696 mmol) was
- 20 added to a suspension of 2,6-dichloro-4-methyl-3-nitropyridine (120 g, 580 mmol) and sodium bicarbonate (146 g, 1739 mmol) in Tetrahydrofuran (THF) (2.5 L) at 0 °C under nitrogen. The reaction mixture was stirred at 65 °C for 24 hr. The reaction was monitored by TLC. The reaction mixture was filtered and washed with EtOAc (2 x 30 mL). The filtrate was concentrated under vacuum to afford crude. The crude was purified by column
- 25 chromatography using silica gel (100-200 mesh), and the product was eluted with 10% EtOAc in Pet ether to afford (S)-dimethyl 2-((6-chloro-4-methyl-3-nitropyridin-2-yl)amino)pentanedioate (50g, 135 mmol, 23.31 % yield) as yellow solid, LCMS (*m/z*): 345.1 [M+H]⁺.

Synthesis of (S)-methyl 3-(6-chloro-8-methyl-2-oxo-1,2,3,4-tetrahydropyrido[2,3-b]pyrazin-3-yl)propanoate



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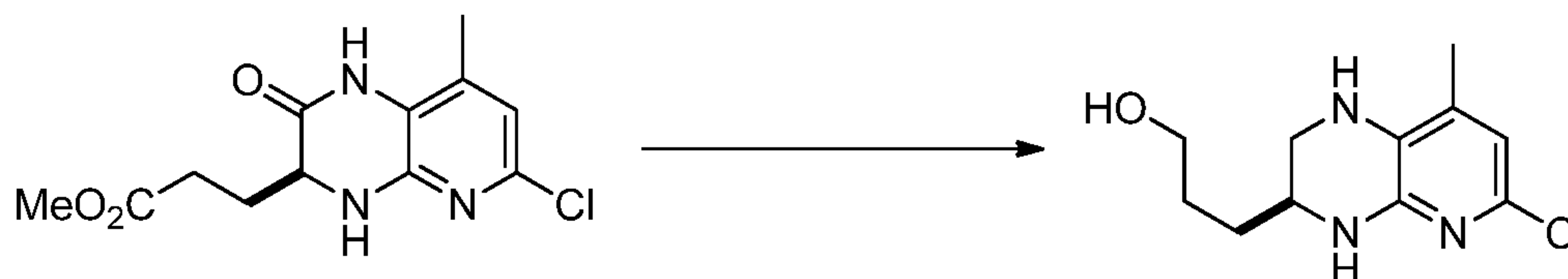
Iron (40.4 g, 723 mmol) was added to a stirred solution of (S)-dimethyl 2-((6-chloro-4-methyl-3-nitropyridin-2-yl)amino)pentanedioate (50 g, 145 mmol) in Isopropanol (450 mL) and Water (90 mL) at room temp. The reaction mixture was heated to 40°C and was added acetic acid (12.42 mL, 217 mmol). The reaction mixture was stirred at 80 °C for 2

10 hr. The reaction was monitored by TLC. The reaction mixture was cooled to RT, and basified with saturated NaHCO₃, filtered through a pad of celite and was washed with DCM (3 X 50mL). The filtrate was separated and was washed with brine solution, dried over anhydrous Na₂SO₄, filtered and filtrate was concentrated under reduced pressure to afford crude product. The crude product was purified by washed with ether (20 ml) to

15 afford desired product (S)-methyl 3-(6-chloro-8-methyl-2-oxo-1,2,3,4-tetrahydropyrido[2,3-b]pyrazin-3-yl)propanoate (29g, 101 mmol, 69.8 % yield) as an off-white solid, LCMS (*m/z*): 284.06 [M+H]⁺.

Synthesis of (S)-3-(6-chloro-8-methyl-1,2,3,4-tetrahydropyrido[2,3-b]pyrazin-3-yl)propan-1-ol

20

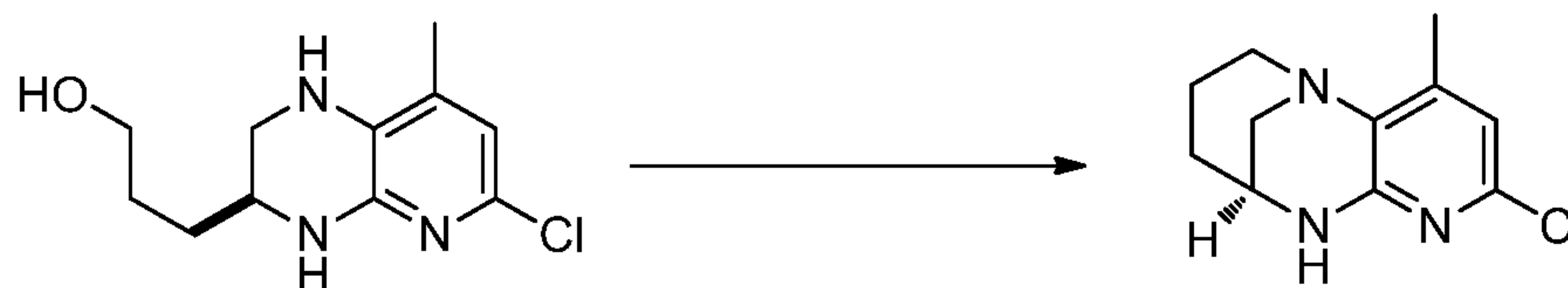


Procedure: 2M lithium aluminum hydride (256 mL, 511 mmol) was added dropwise to a stirred solution of aluminum chloride (19.08 g, 143 mmol), in Tetrahydrofuran (THF)

25 (290 mL) under nitrogen at a rate to control gas evolution. The reaction mixture was stirred for 30 min at rt. The reaction mixture (alane AlH₃) was added dropwise to a stirred solution of (S)-methyl 3-(6-chloro-8-methyl-2-oxo-1,2,3,4-tetrahydropyrido[2,3-b]pyrazin-

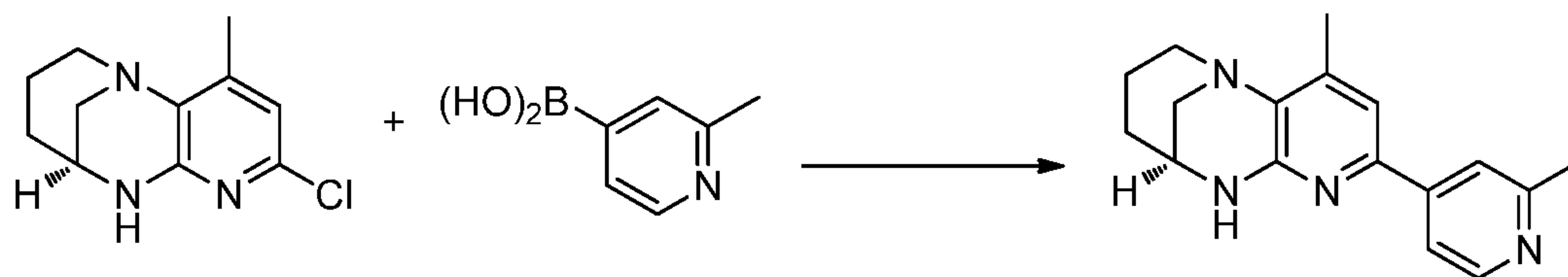
3-yl)propanoate (29 g, 102 mmol) in Tetrahydrofuran (THF) (450 mL) dropwise at -78 °C under nitrogen over 30 minutes. The reaction was allowed to warm to ambient temperature for 16 hr. The reaction was monitored by TLC. The reaction mixture was quenched with 10% NaOH solution at 0 °C and stirred 16 hr and filtered through a pad of celite and was washed with (300 ml) DCM. DCM layer was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to afford the crude product. The crude product was purified washing with ether (2 X 50 ml) to afford (S)-3-(6-chloro-8-methyl-1,2,3,4-tetrahydropyrido[2,3-b]pyrazin-3-yl)propan-1-ol (22 g, 69.8 mmol, 68.3 % yield) as an off-white yellow solid, LCMS (*m/z*): 242.0 [M+H]⁺.

Synthesis of (9S)-2-chloro-4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine



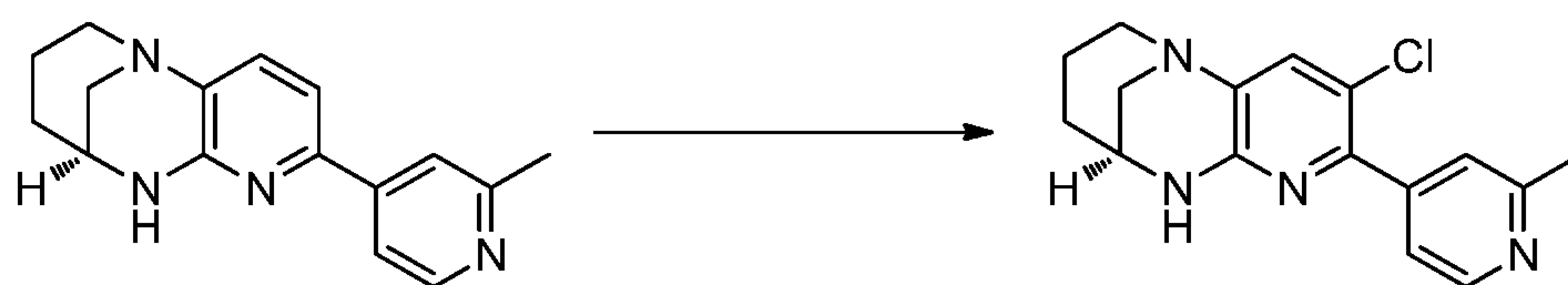
To a stirred solution of (S)-3-(6-chloro-8-methyl-1,2,3,4-tetrahydropyrido[2,3-b]pyrazin-3-yl)propan-1-ol (21 g, 87 mmol) was added HBr (11.79 ml, 217 mmol) at 28 °C and stirred for 12 hr at 100 °C. The reaction was monitored by TLC. The reaction mixture was cooled to 28 °C, and was poured in to ice water (50 mL). The aqueous layer was neutralized with saturated NaHCO₃ solution (120 ml). The aqueous layer was extracted with EtOAc (2X 50 ml). EtOAc layer was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to the crude. The crude was purified by column chromatography using neutral alumina and the product was eluted with 50% EtOAc in Pet ether to afford the compound. The compound was washed with n-pentane to afford pure (9S)-2-chloro-4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (13 g, 57.8 mmol, 66.5 % yield) as white solid, LCMS (*m/z*): 224.09 [M+H]⁺.

Synthesis of (9S)-4-methyl-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine



To a degassed solution of (9S)-2-chloro-4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (500 mg, 2.235 mmol), (2-methylpyridin-4-yl)boronic acid (459 mg, 3.35 mmol) and K_3PO_4 (1423 mg, 6.71 mmol) in 1,4-Dioxane (40 mL); Water (10 mL) and was added x-phos (213 mg, 0.447 mmol), $Pd_2(dba)_3$ (205 mg, 0.224 mmol). The reaction mixture was stirred at 110 °C for 12 hr. The reaction mixture was cooled to 28°C and was partitioned between water (15 mL) and EtOAc (2 X15 mL). EtOAc layer was separated and was dried over anhydrous Na_2SO_4 , filtered. The filtrate was evaporated to get crude. The crude was purified by washing with diethyl ether (15 mL) to afford pure (9S)-4-methyl-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (550 mg, 1.920 mmol, 86 % yield) as yellow solid, LCMS (m/z): 281.05 $[M+H]^+$.

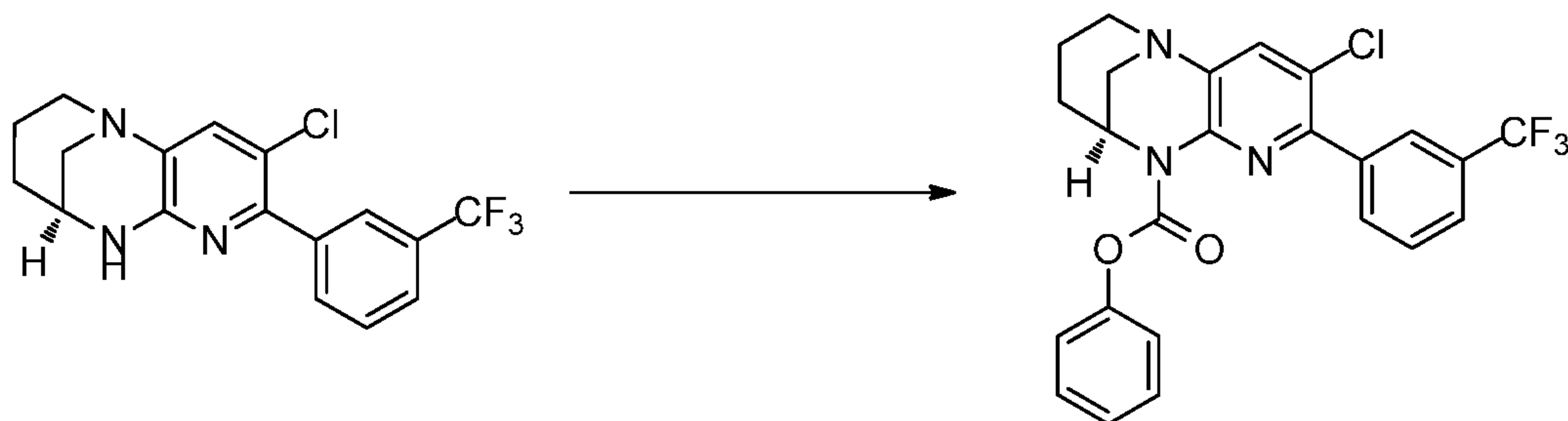
Synthesis of (9S)-3-chloro-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine



To a solution of (9S)-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (2.0 g, 7.51 mmol) in Chloroform (20 mL) stirred under nitrogen at 0°C was added NCS (1.504 g, 11.26 mmol) portionwise during 10 min. The reaction mixture was stirred at 0 °C for 4 hr .Reaction mass was quenched with the ice cold water (20 ml) and extracted with the DCM (3x30 ml).Organic phase was seperated and washed with the water (2x20 ml) and brine solution (2x20 ml),then organic phase was dried over anhydrous sodium sulphate,filtered it and filterate was evoporated under reduced pressure to get the crude product.The crude product (N35964-5-A1 & N35964-7-A1) was purified by column chromatography using neutral alumina and was eluted with 50% EtOAc in Hexane (gradient system) to afford the desired product (9S)-3-chloro-2-(2-

methanopyridin-4-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (1.2 g, 3.60 mmol, 47.9 % yield) as a pale yellow solid, LCMS (m/z): 300.17 $[M+H]^+$.

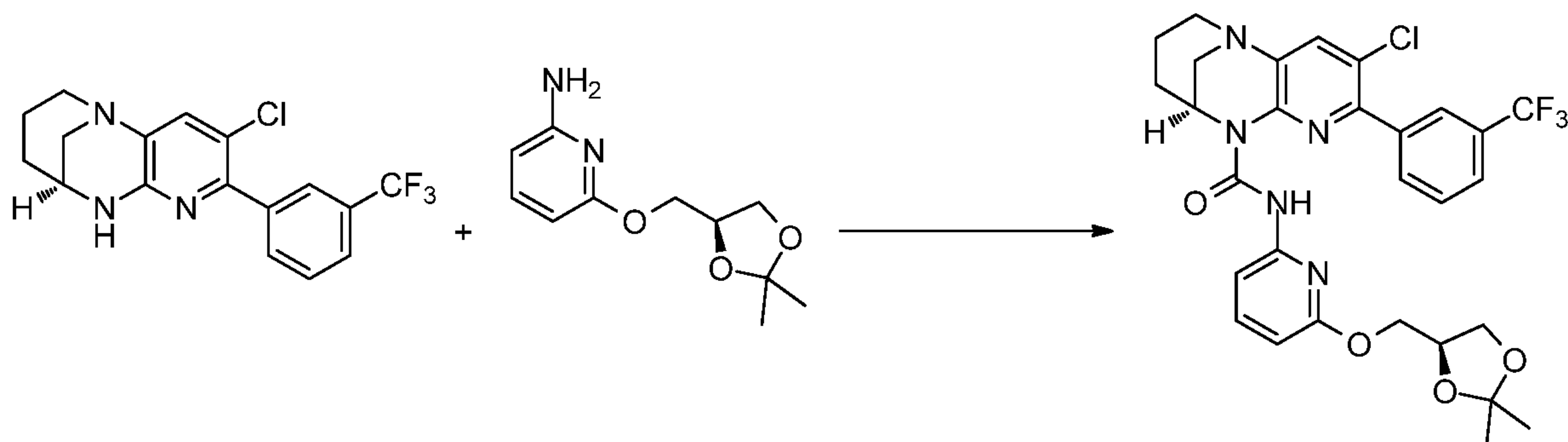
Synthesis of (9S)-phenyl 3-chloro-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxylate



To a solution of phenyl carbonochloridate (0.195 mL, 1.555 mmol) and pyridine (0.149 mL, 1.837 mmol) in Dichloromethane (DCM) (35 mL) stirred under nitrogen at 0°C was added (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-

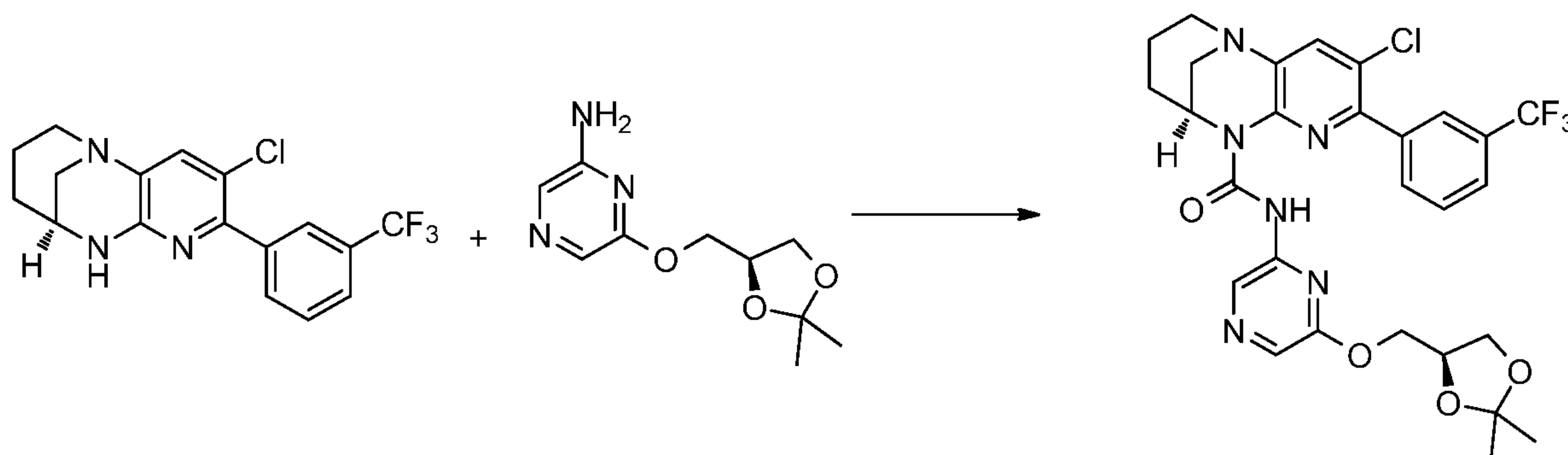
methanopyrido[2,3-b][1,4]diazocine (500 mg, 1.413 mmol). The reaction mixture was stirred at RT for 2 h. The Reaction was monitored by TLC. The reaction mixture was quenched with saturated sodium bicarbonate solution and extracted with DCM (200 mL) twice. Combined DCM layer washed with water (80mL) and dried out with Na₂SO₄, filtered and concentrated under high vacuum to get crude product. The crude product was added to a silica gel (100-200 mesh) column and was eluted with 30%Hex/EtOAc the collected fraction was distilled under reduced pressure to afford a compound. The compound was washed with pentane to get a pure compound of (9S)-phenyl 3-chloro-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxylate (500 mg, 1.044 mmol, 73.9 % yield) as a white solid, LCMS (m/z): 474.30 $[M+H]^+$.

Synthesis of (9S)-3-chloro-N-(6-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



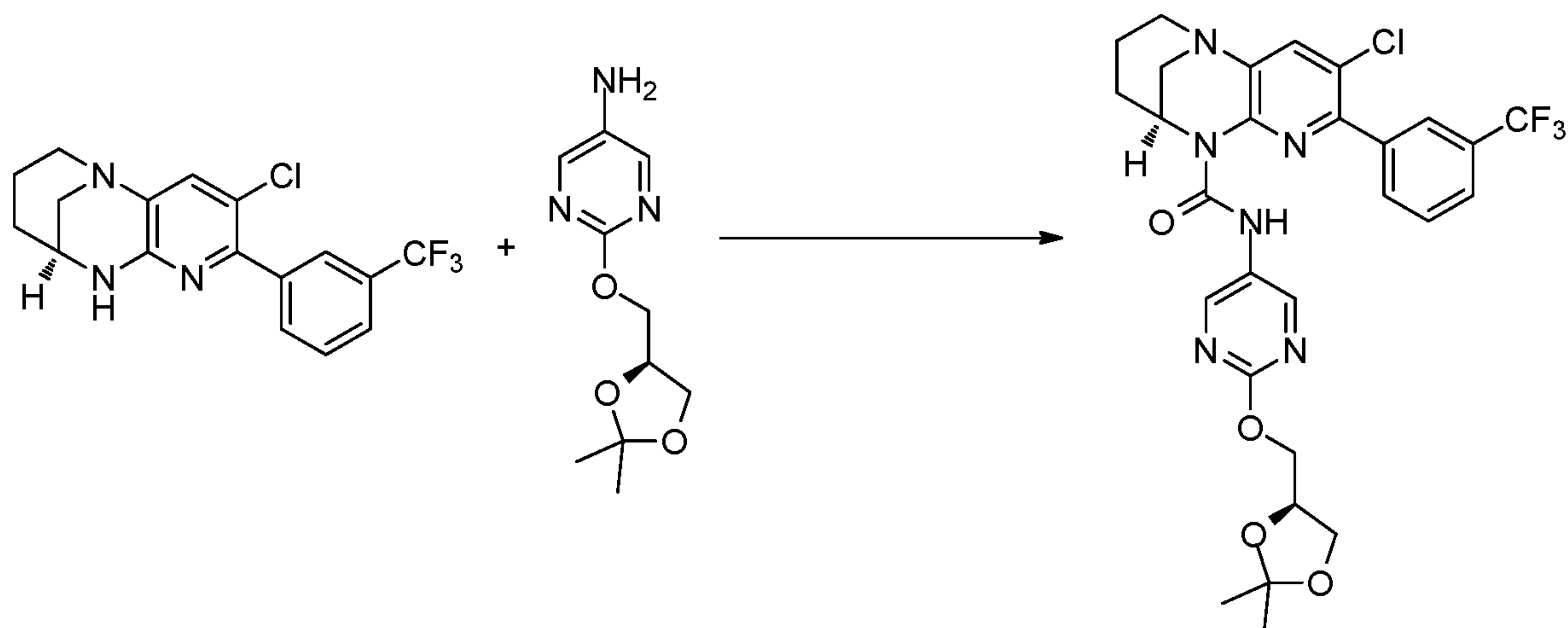
- 5 Triphosgene (377 mg, 1.272 mmol) was added to a stirred solution of (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (450 mg, 1.272 mmol), and TEA (0.886 mL, 6.36 mmol) in Tetrahydrofuran (THF) (50 mL) at 28°C. The reaction mixture was stirred for 30 min and was added (S)-6-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)pyridin-2-amine (571 mg, 2.54 mmol). The reaction
- 10 mixture was stirred for 10h at 70 °C. The reaction mixture was cooled to room temperature and was partitioned between water (15 mL) and EtOAc (25 mL). EtOAc layer was separated and was dried over anhydrous Na₂SO₄, filtered. The filtrate was evaporated to get crude. The crude was purified by GRACE using C-18 reserval column, Mobile phase A: 0.1% Formic Acid in water; B: MeOH, the product was eluted at 91% of MeOH
- 15 in 0.1% Formic Acid in water. The solvent was evaporated and was basified with saturated NaHCO₃. The aqueous layer was extracted with DCM, DCM layer was dried over anhydrous Na₂SO₄, filtered, and filtrate was evaporated to afford (9S)-3-chloro-N-(6-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-
- 20 carboxamide (270 mg, 0.430 mmol, 33.8 % yield) as yellow solid, LCMS (*m/z*): 606.11 [M+H]⁺.

Synthesis of (9S)-3-chloro-N-(6-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



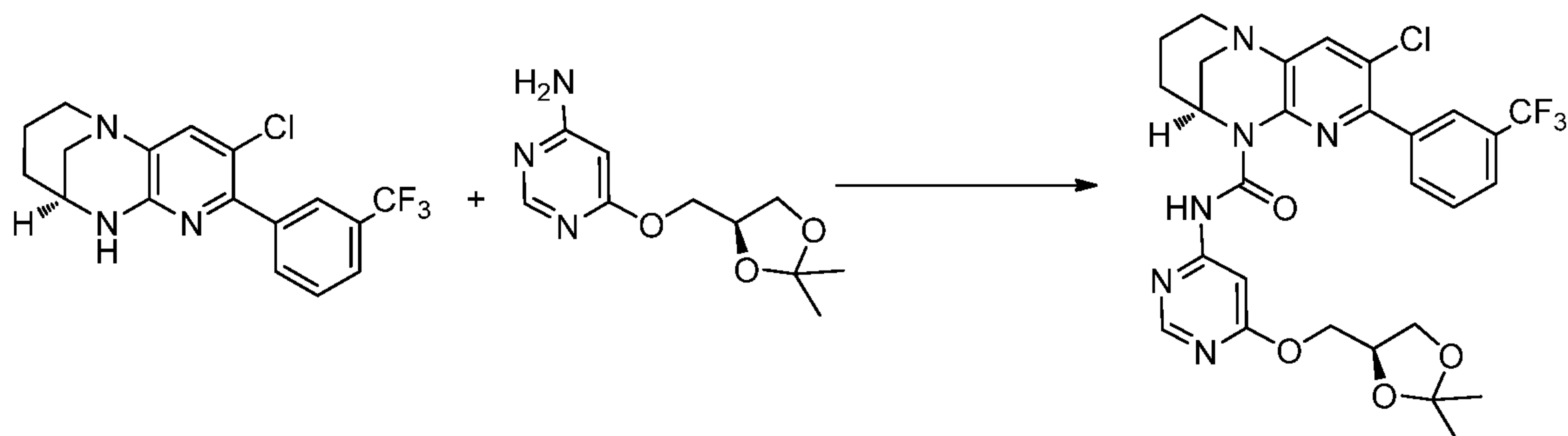
- 5 TEA (1.064 mL, 7.63 mmol) and triphosgene (377 mg, 1.272 mmol) was added to a stirred solution of (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (450 mg, 1.272 mmol) in Tetrahydrofuran (THF) (50 mL) under nitrogen at room temp. The reaction mixture was stirred at RT for 30 min. (S)-6-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-amine (573 mg, 2.54 mmol) was
- 10 added and the reaction mixture was stirred 16 hr at 65 °C. The reaction mixture was cooled to room temp, solvent evaporated under reduced pressure completely and was partitioned between water (10 mL) and EtOAc (50 mL). Organic layer was separated, dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to afford crude product. The crude product was purified by column chromatography using neutral alumina and was
- 15 eluted with 50% EtOAc in Hexane (gradient system) to afford the desired product (9S)-3-chloro-N-(6-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (200 mg, 0.271 mmol, 21.27 % yield) as an off white solid, LCMS (*m/z*): 605.2 [M+H]⁺.

Synthesis of (9S)-3-chloro-N-(2-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-5-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



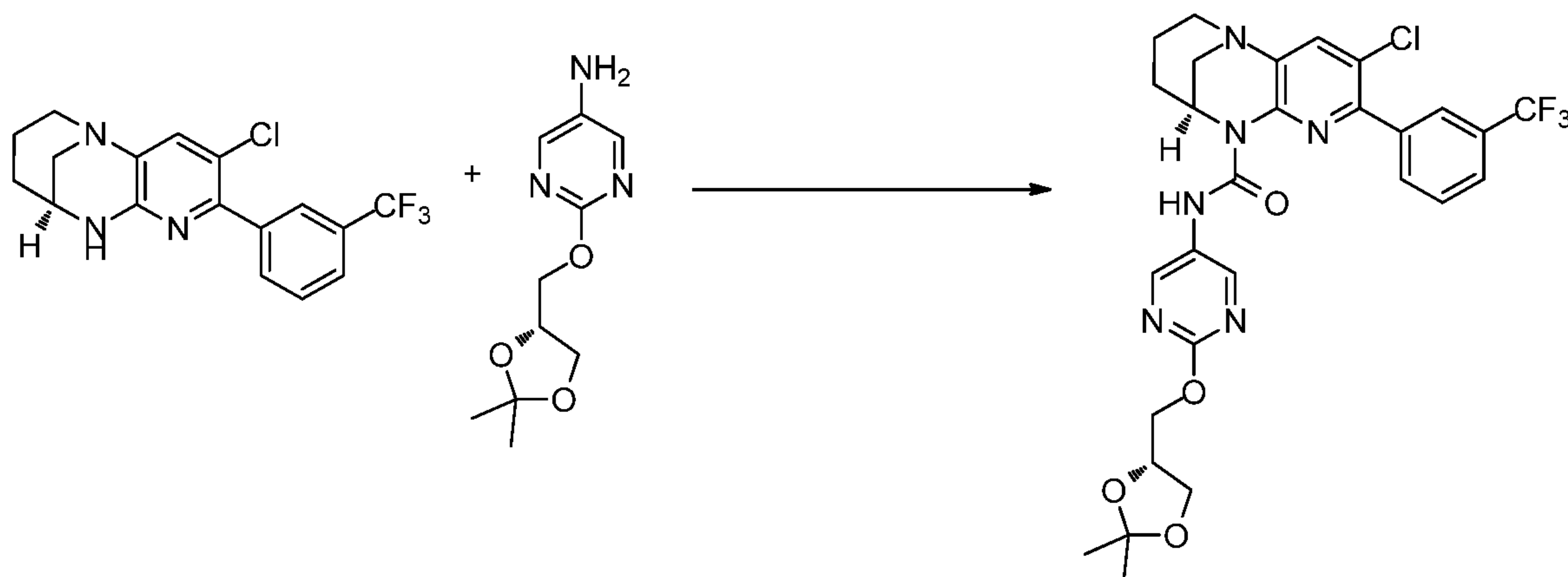
- 5 TEA (1.064 mL, 7.63 mmol) and triphosgene (377 mg, 1.272 mmol) was added to a stirred solution of (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (450 mg, 1.272 mmol) in Tetrahydrofuran (THF) (45 mL) under nitrogen at room temp. The reaction mixture was stirred at RT for 30 min. (S)-2-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-5-amine (860 mg, 3.82 mmol) was
- 10 added and the reaction mixture was stirred 16 hr at 65 °C. The reaction mixture was cooled to room temp, solvent evaporated under reduced pressure completely and was partitioned between water (30 mL) and EtOAc (100 mL). Organic layer was separated, dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to afford crude product. The crude product was purified by column chromatography using neutral alumina and was
- 15 eluted with 20% EtOAc in Hexane (gradient system) to afford the desired product (9S)-3-chloro-N-(2-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-5-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (200 mg, 0.317 mmol, 24.91 % yield) as a pale yellow solid, LCMS (*m/z*): 605.19 [M+H]⁺.

Synthesis of (9S)-3-chloro-N-(6-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



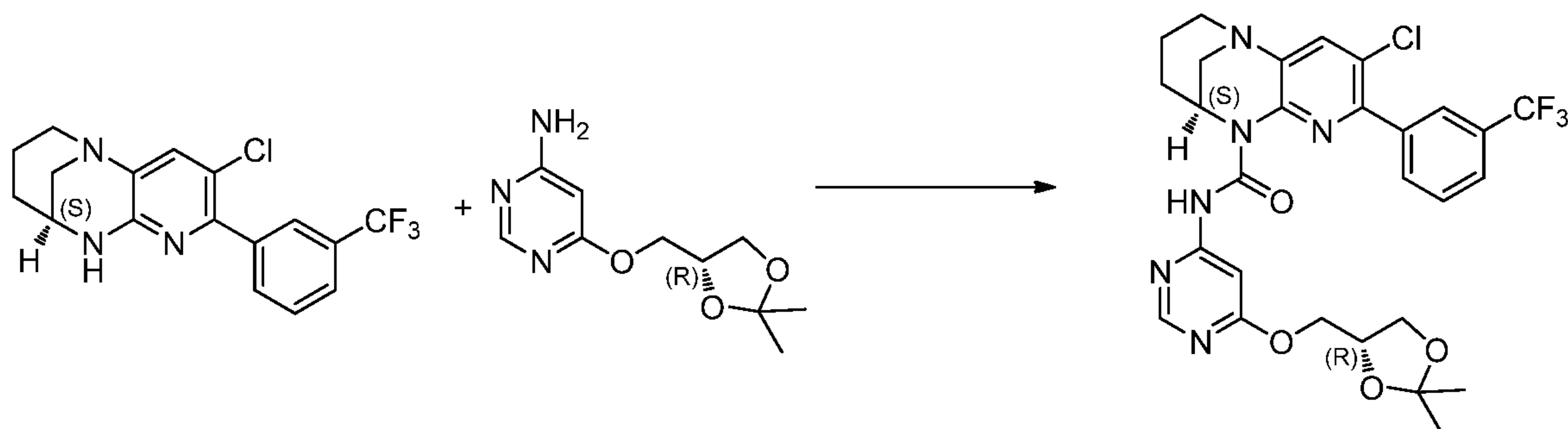
- 5 A solution of (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (0.4 g, 1.131 mmol) and TEA (0.788 mL, 5.65 mmol) in Tetrahydrofuran (THF) (50 mL) was added triphosgene (0.336 g, 1.131 mmol) and stirred under nitrogen at room temp for 1h. To this reaction mixture (S)-6-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-yl)pyrimidin-4-amine (0.382 g, 1.696 mmol) was added. The
- 10 reaction mixture was stirred at 65 °C for 16 h and progress of the reaction was monitored by TLC and LCMS. The reaction mixture was cooled to room temperature, poured in to water (50 mL) and extracted with EtOAc (2 X 50mL). The organic layer was washed with water and brine. The organic layer was filtered through Na₂SO₄ and concentrated to obtain crude compound. The crude compound was purified by neutral alumina and eluted in 50%
- 15 ethyl acetate in hexane to afford (9S)-3-chloro-N-(6-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (0.3 g, 0.486 mmol, 43.0 % yield) as off white solid, LCMS (*m/z*): 605.55 [M+H]⁺.

Synthesis of (9S)-3-chloro-N-(2-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-5-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



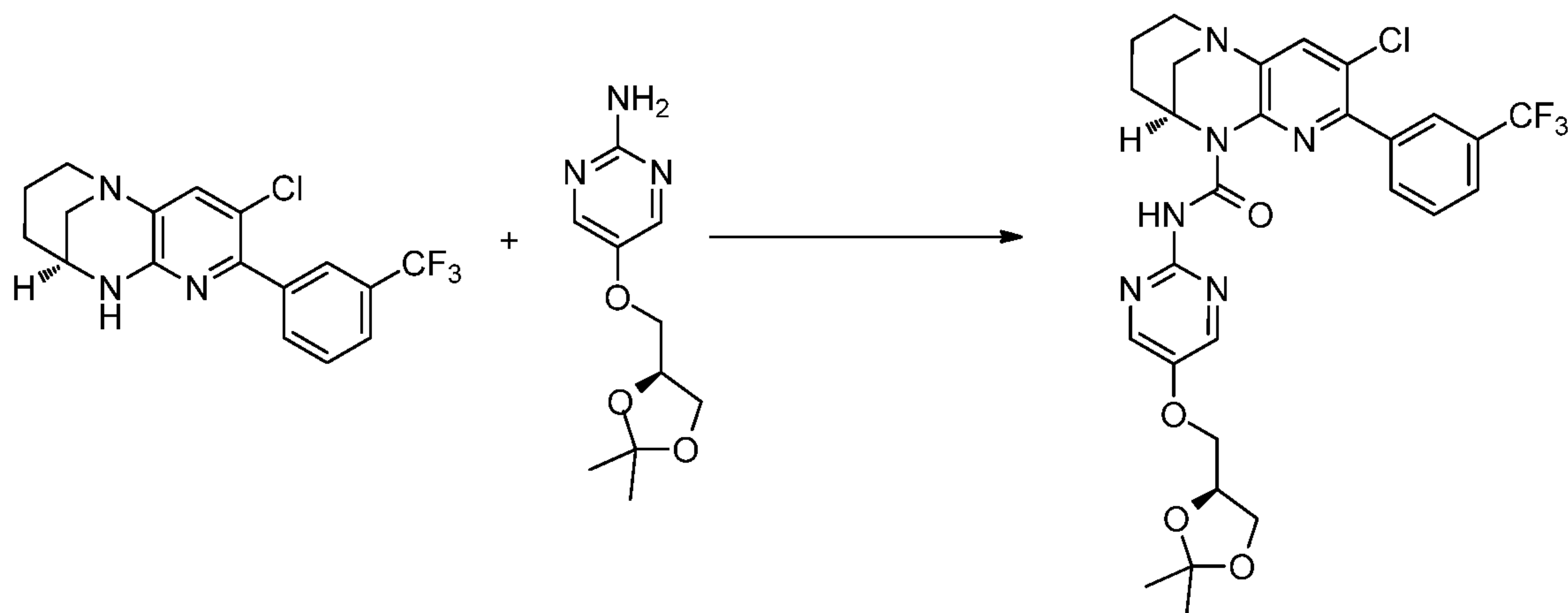
- 5 Triphosgene (417 mg, 1.405 mmol) was added to a stirred solution of (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-6,7,8,9,10,10a-hexahydro-4aH-5,9-methanopyrido[2,3-b][1,4]diazocine (500 mg, 1.405 mmol) and TEA (1.175 mL, 8.43 mmol) in Tetrahydrofuran (THF) (50 mL) at room temp. The reaction mixture was stirred for 1 h and (R)-2-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-5-amine (791 mg, 3.51
- 10 mmol) was added. The reaction mixture was stirred at 65 °C for 16 h. Reaction was monitored by TLC. The reaction mixture was evaporated under reduced pressure and diluted with water (50 mL) and extracted with Ethyl acetate (2 X 75 mL) and followed by brine solution (50 mL) and separated the layer, dried with anhydrous Na₂SO₄, filtered and concentrated to get crude product. The crude product was submitted to Neutral Alumina
- 15 column by eluting 50-60% Ethyl acetate in Hexane. The collected fraction was evaporated under reduced pressure to get the pure compound of (9S)-3-chloro-N-(2-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-5-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (350 mg, 0.544 mmol, 38.7 % yield) as a Off white solid, LCMS (*m/z*): 605.23 [M+H]⁺.

Synthesis of (9S)-3-chloro-N-(6-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



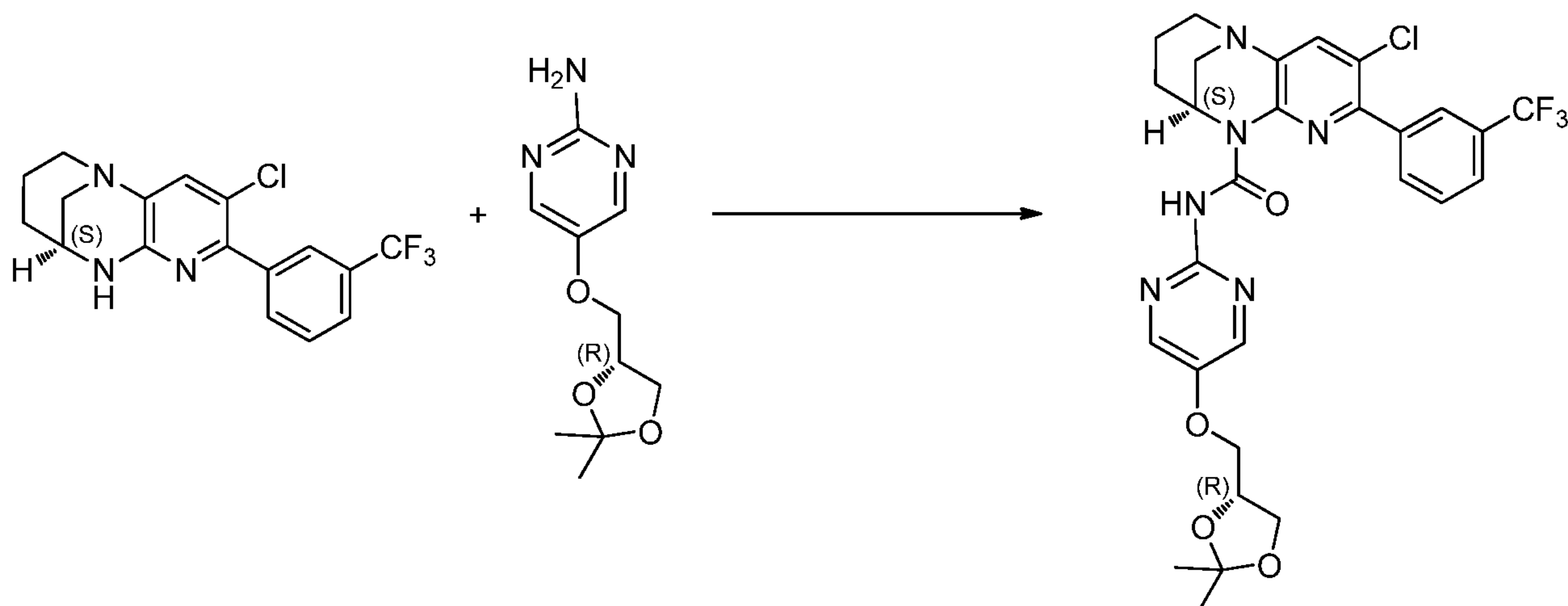
- 5 Triphosgene (252 mg, 0.848 mmol) followed by triethylamine (1.182 mL, 8.48 mmol) were added to a stirred solution of (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (500 mg, 1.413 mmol) in Tetrahydrofuran (THF) (15 mL) at RT and stirred for 30 min. Then (R)-6-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-yl)amine (637 mg, 2.83 mmol) were added to the
- 10 reaction mixture at RT and stirred at 80 °C for 15.5 h. Reaction mixture was cooled to RT, diluted with water (40mL), extracted with ethyl acetate (2X60 mL) and washed with brine solution (30 mL). Organic layer was separated, dried over Na₂SO₄, filtered and concentrated to get crude compound. The crude compound was purified by column chromatography using silica gel (100-200 mesh), 30 % ethyl acetate in pet ether as an
- 15 eluent to afford (9S)-3-chloro-N-(6-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (200 mg, 0.324 mmol, 22.92 % yield) as brown gummy solid, LCMS (*m/z*): 606.99 [M+H]⁺.

Synthesis of (9S)-3-chloro-N-(5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



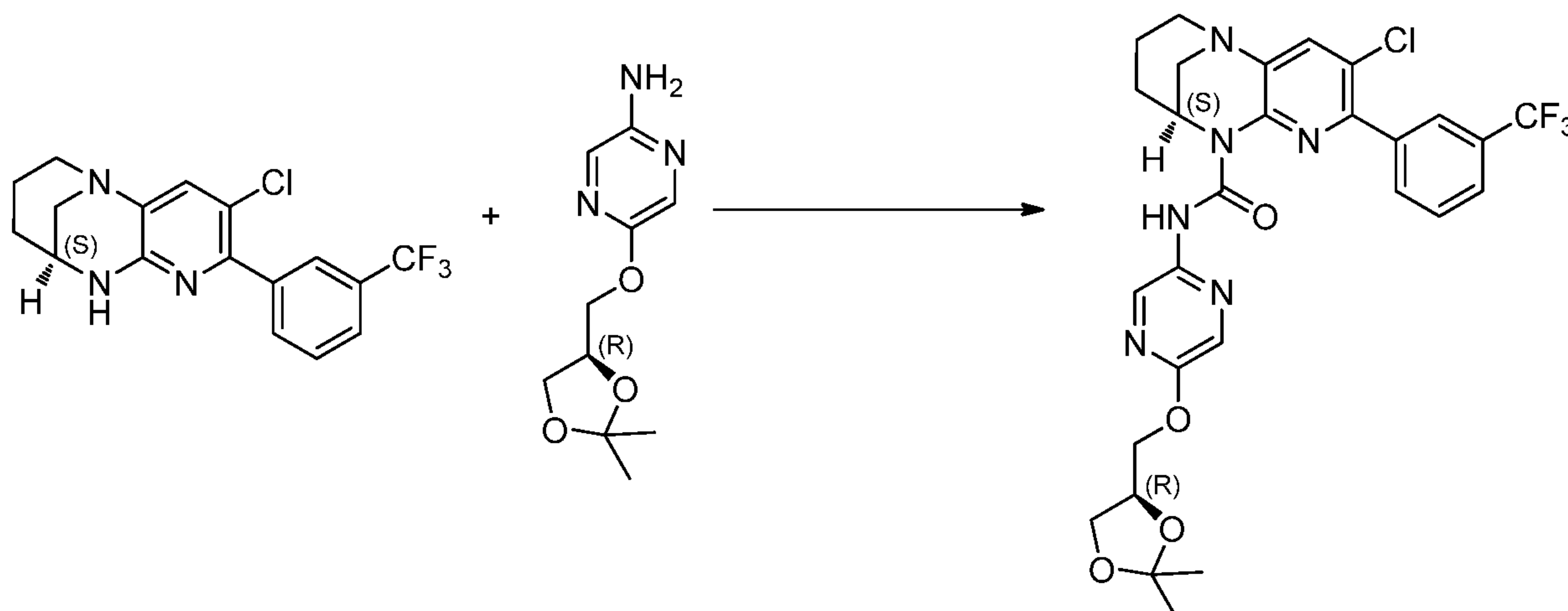
- 5 Triphosgene (419 mg, 1.413 mmol) was added to a stirred solution of (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (500 mg, 1.413 mmol), and TEA (1.182 mL, 8.48 mmol) in Tetrahydrofuran (THF) (50 mL) under nitrogen at 28 °C. The reaction mixture was stirred at RT for 30 min. and was added (S)-5-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-amine (955 mg, 4.24 mmol). The reaction mixture was stirred 16 hr at 65 °C. The reaction mixture was cooled to 28 °C, the reaction mixture was partitioned between water (20 mL) and EtOAc (2x 25 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude. The crude was purified by GRACE using C-18 reserval column, Mobile phase A: 0.1% Formic Acid in water; B: ACN, the product was eluted at 50% of ACN in 0.1% Formic Acid in water. The solvent was evaporated and was basified with saturated NaHCO₃. The precipitated solid was filtered, and was dried to afford (9S)-3-chloro-N-(5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (120 mg, 0.191 mmol, 13.48 % yield) as off white solid, LCMS (*m/z*): 605.23 [M+H]⁺.

Synthesis of (9S)-3-chloro-N-(5-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



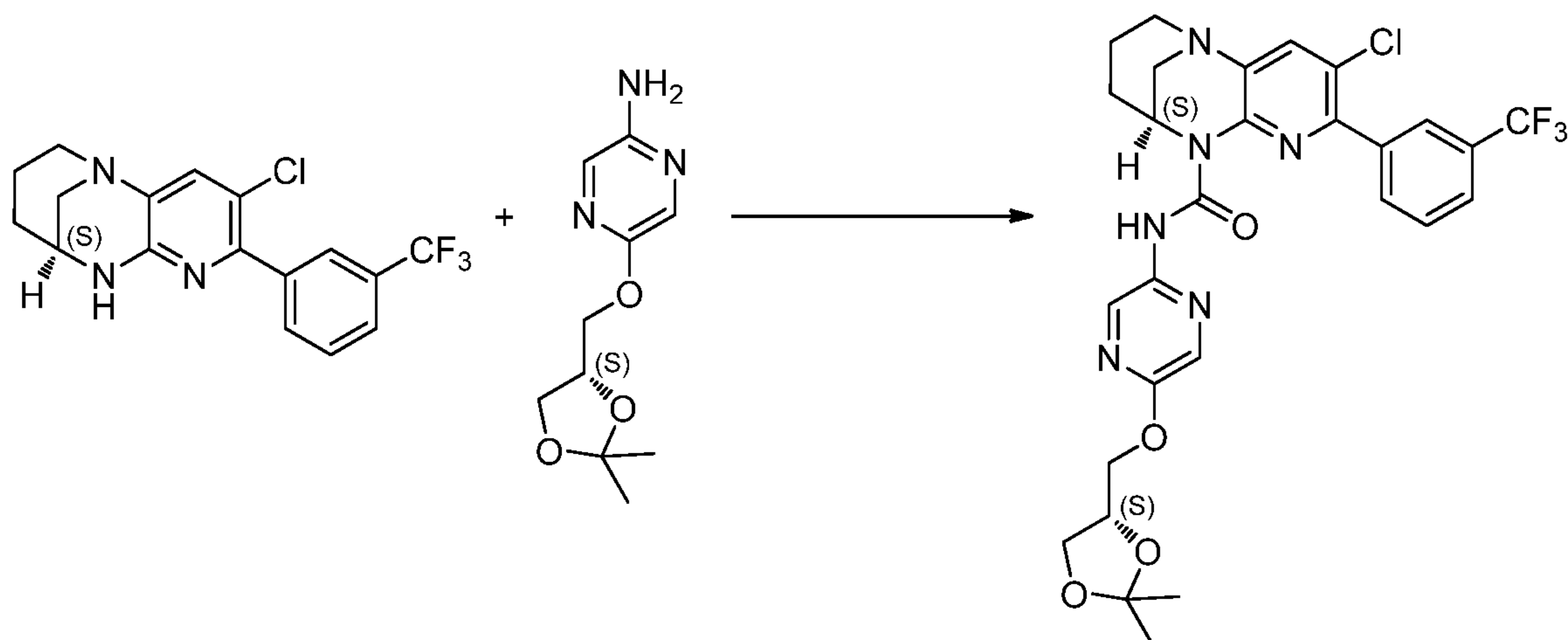
- 5 Triphosgene (0.419 g, 1.413 mmol) was added to a solution of (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (0.5 g, 1.413 mmol), TEA (0.985 mL, 7.07 mmol) in Tetrahydrofuran (THF) (15 mL) was stirred under nitrogen at room temp for 1h. To this reaction mixture (R)-5-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-amine (0.637 g, 2.83 mmol) was added. The
- 10 reaction mixture was stirred at 65 °C for 16 h and progress of the reaction was monitored by TLC and LCMS. The reaction mixture was cooled to room temperature, poured in to ice water (50 mL) and extracted with EtOAc (2 X 100 mL). The combined organic layer was washed with water (50 mL), brine solution (50 mL), dried over Na₂SO₄, filtered and evaporated to obtain crude compound. The crude compound was purified by column
- 15 chromatography using neutral alumina and eluted in 50% EtOAc in hexane to afford (9S)-3-chloro-N-(5-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (0.52g, 0.387 mmol, 27.4 % yield) as pale yellow solid, LCMS (*m/z*): 605.20 [M+H]⁺.

Synthesis of (9S)-3-chloro-N-(5-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



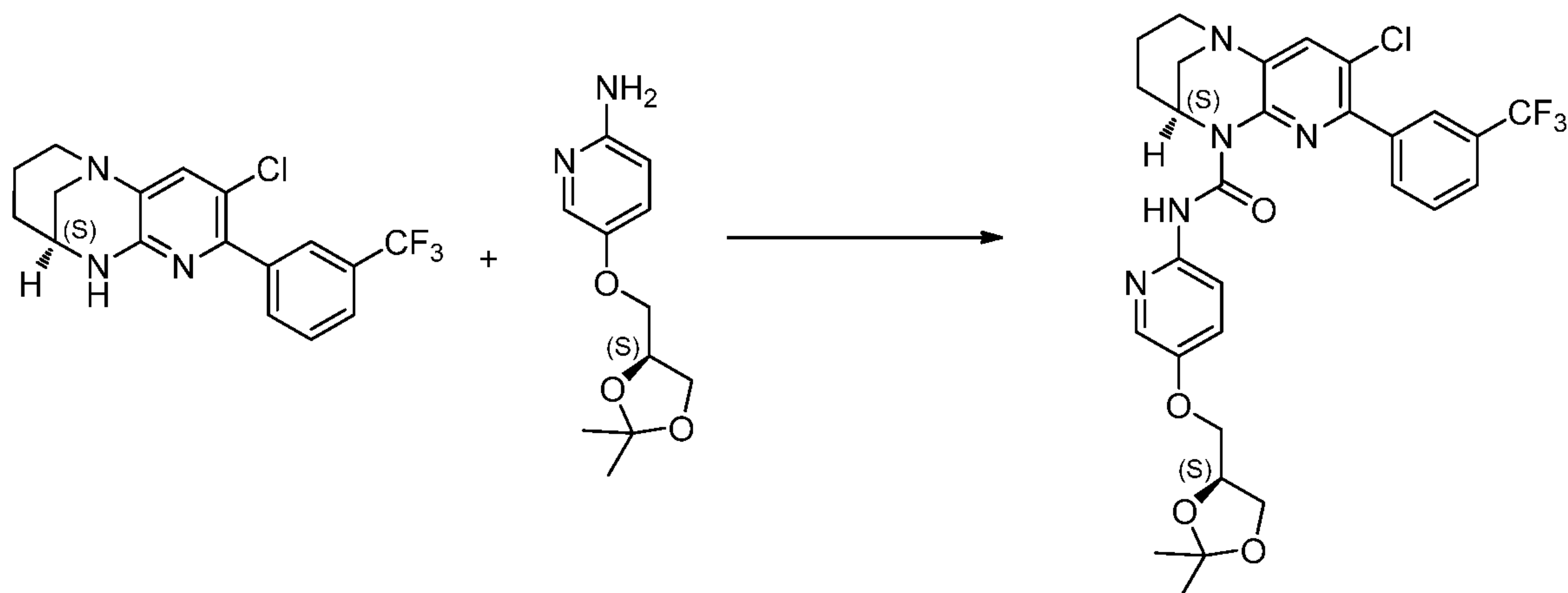
- 5 (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (600 mg, 1.696 mmol) followed by triphosgene (302 mg, 1.018 mmol) were added to a solution of (R)-5-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-amine (458 mg, 2.035 mmol) and TEA (1.182 mL, 8.48 mmol) in Tetrahydrofuran (THF) (20 mL) at 25 °C, stirred for 16h at 70 °C. Progress of the reaction
- 10 was monitored by LCMS and TLC. The reaction mixture was cooled to 28 °C and was partitioned between water (20 mL) and EtOAc (2X50 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to get crude. The sample was loaded in dichloromethane and purified on silica 5g using a 0-15% methanol-dichloromethane over 80 min. The appropriate fractions were combined and
- 15 evaporated in vacuum to give the required product 220 mg as an off-white solid, LCMS (*m/z*): 605.17 [M+H]⁺.

Synthesis of (9S)-3-chloro-N-(5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



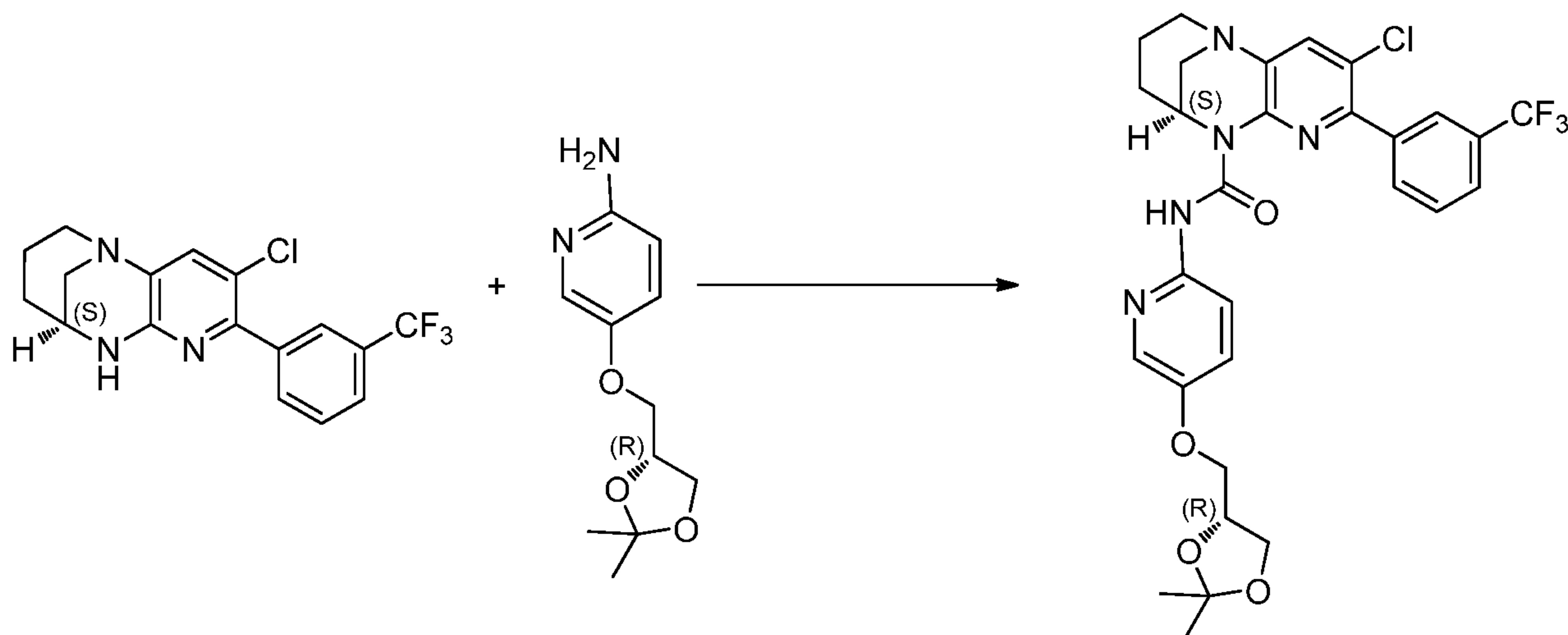
- 5 To a solution of (S)-5-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-amine (637 mg, 2.83 mmol) in Tetrahydrofuran (THF) (20 mL) stirred under nitrogen at room temp was added triphosgene (419 mg, 1.413 mmol) and triethylamine (1.182 mL, 8.48 mmol), To this (S)-5-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-amine (637 mg, 2.83 mmol) was added and the reaction mixture was stirred at 65 °C for 16 hr. Reaction
- 10 mixture was quenched with ice water and extracted with 2x25 ml of ethyl acetate, combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure to afford crude compound. The crude product was purified by flash column chromatography (100-200 silica gel) eluting at 2% methanol in DCM to afford pure compound (9S)-3-chloro-N-(5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-
- 15 yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (200 mg, 0.308 mmol, 21.82 % yield) as pale brown solid, LCMS (*m/z*): 605.1 [M+H]⁺.

Synthesis of (9S)-3-chloro-N-(5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



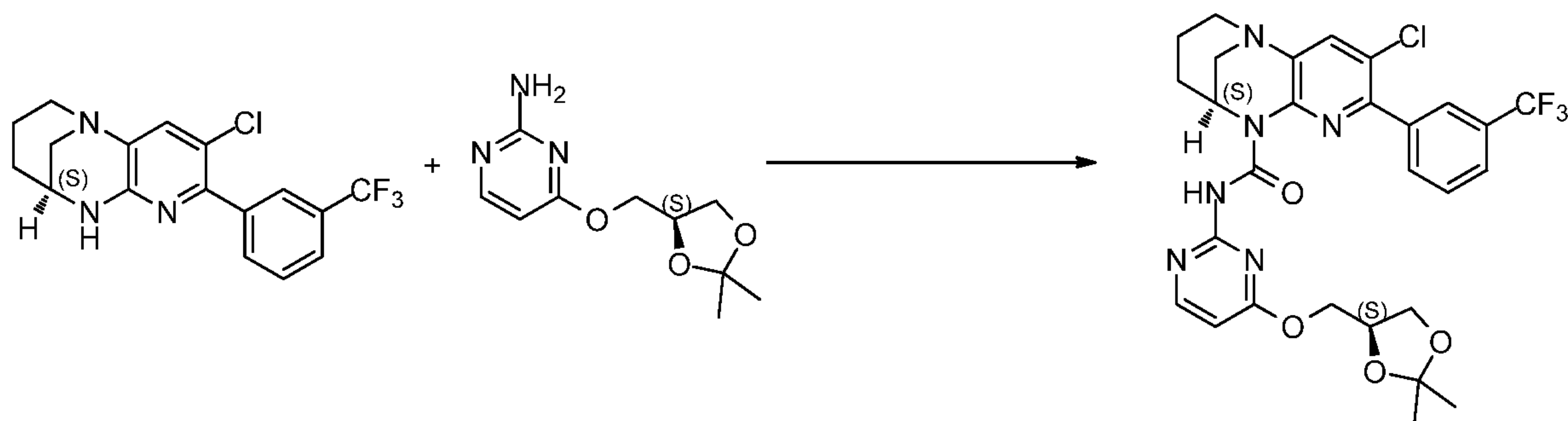
- 5 (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (1 g, 2.83 mmol) was dissolved in Tetrahydrofuran (THF) (30 mL) stirred under nitrogen at 0 °C were added triphosgene (0.839 g, 2.83 mmol), DIPEA (2.468 mL, 14.13 mmol). The reaction mixture was stirred for 16 at RT. To this (S)-5-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-amine (1.268 g, 5.65
- 10 mmol) was added and stirred for 16 h at 80 °C in sealed tube. The reaction mixture allowed to room temperature and quenched with 200 ml of water and extracted with 3x200 ml of ethyl acetate, the combined organic layer was dried over Na₂SO₄ and concentrated under reduced pressure to obtain crude compound. The crude product was purified by flash column chromatography to afford (9S)-3-chloro-N-(5-(((S)-2,2-dimethyl-1,3-
- 15 dioxolan-4-yl)methoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (300 mg, 0.486 mmol, 17.18 % yield) as an off white solid. LCMS (*m/z*): 604.20 [M+H]⁺.

Synthesis of (9S)-3-chloro-N-(5-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



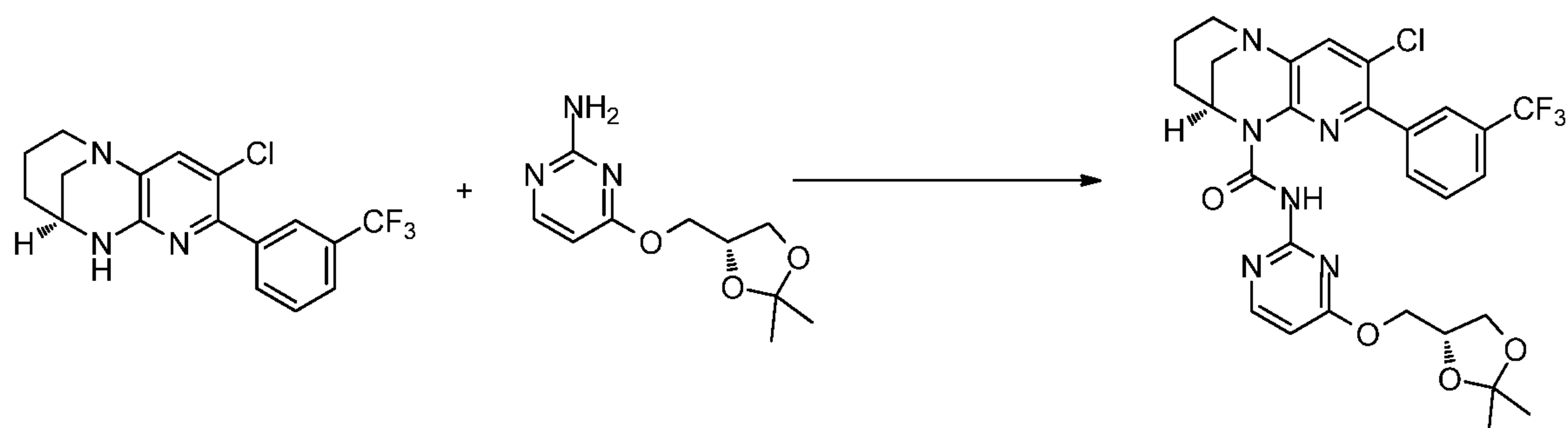
- 5 TEA (1.970 mL, 14.13 mmol) followed by triphosgene (0.839 g, 2.83 mmol) were added to a solution of (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (1 g, 2.83 mmol) in Tetrahydrofuran (THF) (20 mL) at RT and stirred for 6 h at RT and (R)-5-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-amine (0.697 g, 3.11 mmol) was added and heated at 80 °C for 10h.
- 10 The reaction mixture was cooled to 28 °C and was partitioned between water (25 mL) and EtOAc (40 mL x 2). Organic layers were separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to get crude, then it was purified by column chromatography (using 100-200 silica gel, column eluted at 80% ethyl acetate in hexane) to afford the (9S)-3-chloro-N-(5-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-
- 15 yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (350 mg, 0.562 mmol, 19.88 % yield) as an off white solid, LCMS (*m/z*): 604.24 [M+H]⁺.

Synthesis of (9S)-3-chloro-N-(4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



- 5 DIPEA (2.52 mL, 14.13 mmol) followed by triphosgene (0.503 g, 1.696 mmol) were added to a solution of (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (1.0 g, 2.83 mmol) and (S)-4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-amine (0.828 g, 3.67 mmol) in Tetrahydrofuran (THF) (50 mL) at 25 °C, stirred for 16h at 70 °C. Progress of the reaction was monitored
- 10 by LCMS and TLC. The reaction mixture was cooled to 28 °C and was partitioned between water (100 mL) and EtOAc (2X100 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to get crude. The sample was loaded in dichloromethane and purified on silica (Si) 5g using a 0-15% methanol-dichloromethane over 80 min. The appropriate fractions were combined and
- 15 evaporated in vacuum to give the required product, 300 mg as a off-white solid, LCMS (*m/z*): 605.21 [M+H]⁺.

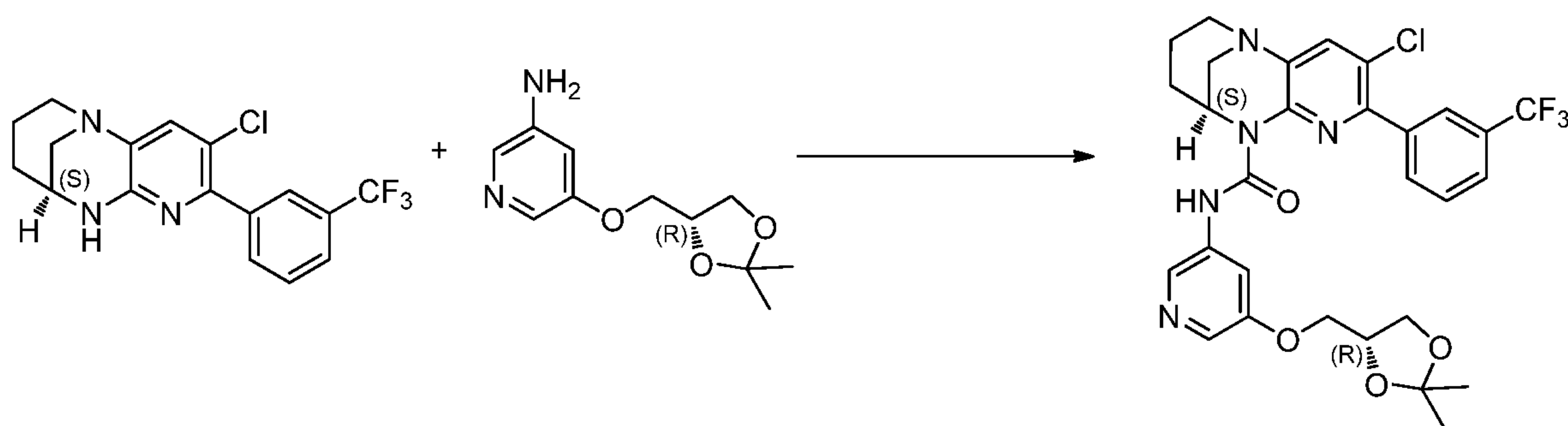
Synthesis of (9S)-3-chloro-N-(4-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



TEA (1.970 mL, 14.13 mmol) followed by triphosgene (0.839 g, 2.83 mmol) were added to a solution of (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-

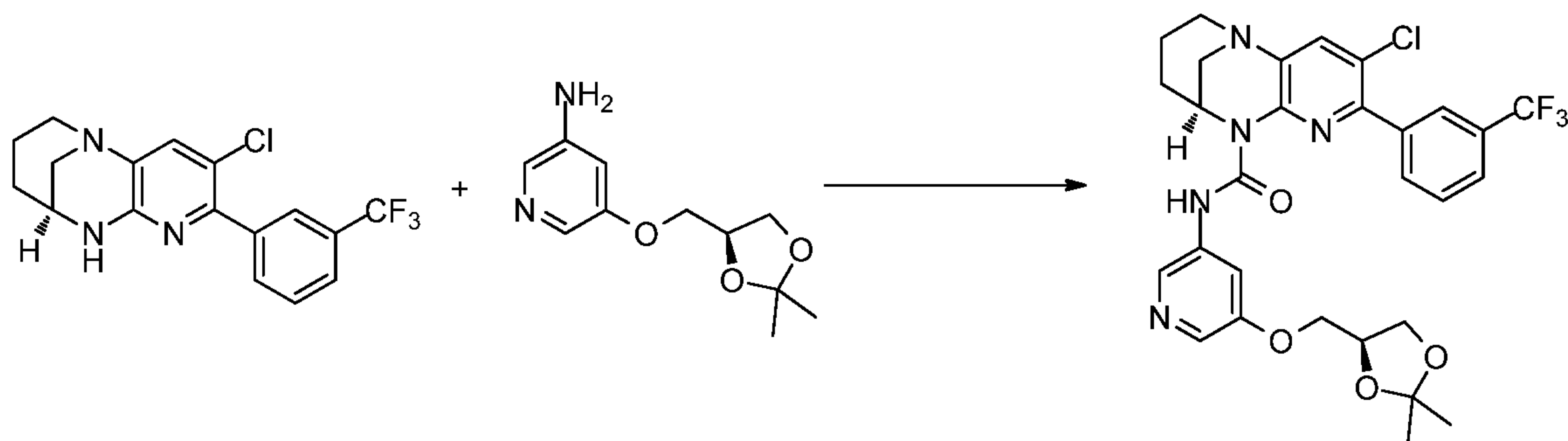
methanopyrido[2,3-b][1,4]diazocine (1 g, 2.83 mmol) in Tetrahydrofuran (THF) (30 mL) at RT and stirred for 6 h at RT and (R)-4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-amine (0.700 g, 3.11 mmol) was added and heated at 80 °C for 10h. The reaction mixture was cooled to 28 °C and was partitioned between water (25 mL) and EtOAc (40 mL x 2). Organic layers were separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to get (9S)-3-chloro-N-(4-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (300 mg, 0.411 mmol, 14.52 % yield) as a gum, LCMS (*m/z*): 605.26 [M+H]⁺.

Synthesis of (9S)-3-chloro-N-(5-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



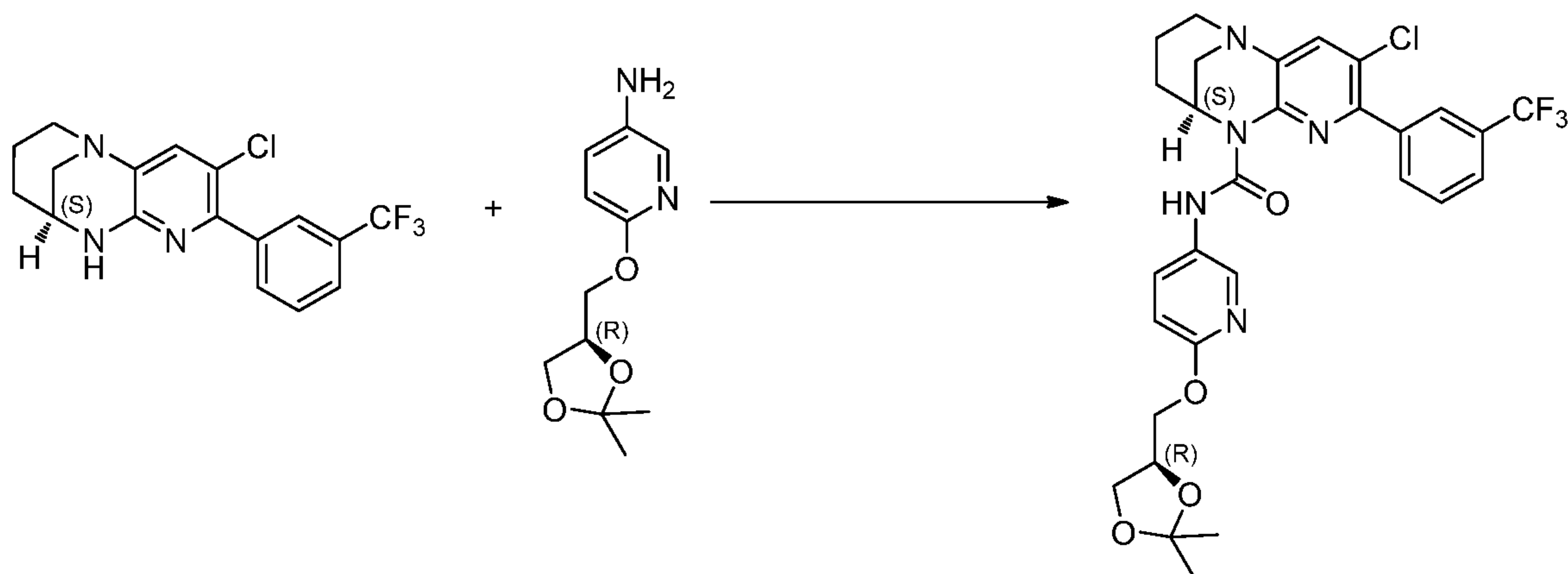
DIPEA (329 mg, 2.54 mmol) followed by triphosgene (252 mg, 0.848 mmol) were added to a solution of (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (300 mg, 0.848 mmol) in Tetrahydrofuran (THF) (50 mL) at 25 °C, stirred for 3h and (R)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-amine (380 mg, 1.696 mmol) was added and stirred at 70 °C for 16h. The reaction mixture was cooled to 28 °C and was partitioned between water (20 mL) and EtOAc (50 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to get crude. The crude mass was purified by column chromatography (100-200) mesh eluted with 30% EtOAc in pet ether to obtained (9S)-3-chloro-N-(5-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (250 mg, 0.414 mmol, 48.8 % yield), as a pale yellow solid, LCMS (*m/z*): 604.00[M+H]⁺.

Synthesis of (9S)-3-chloro-N-(5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



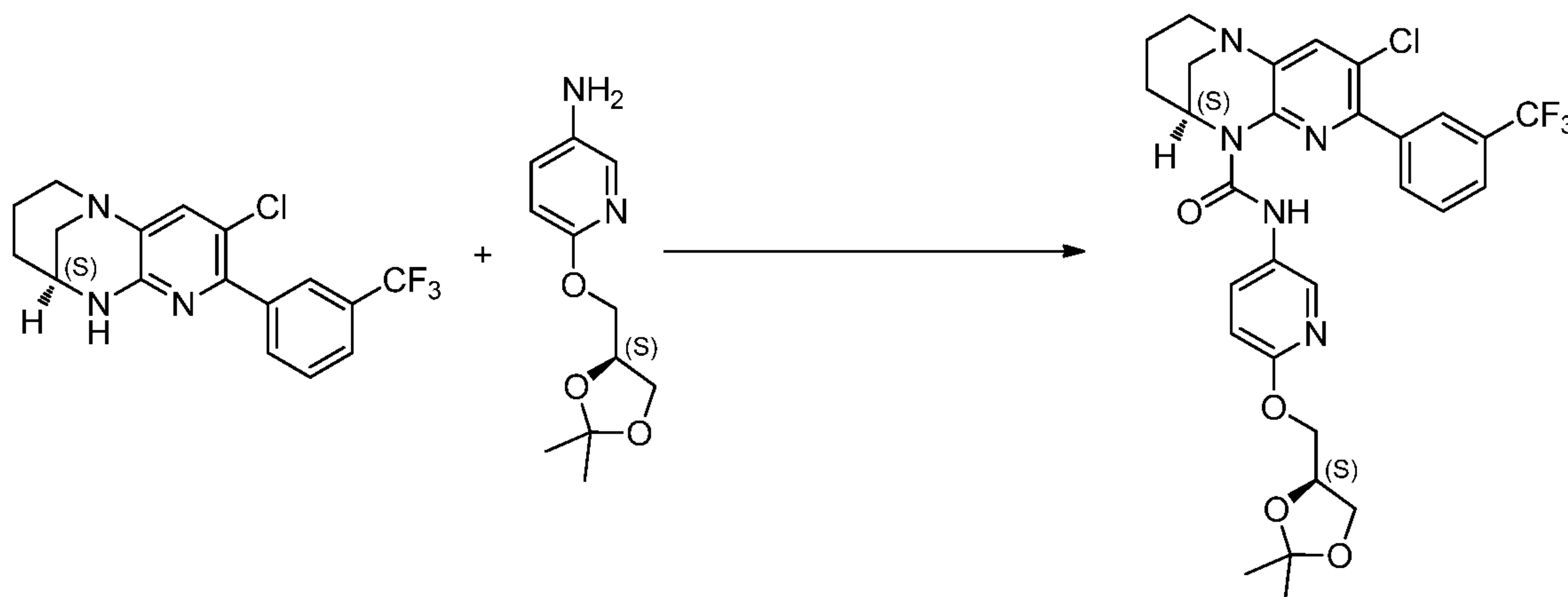
- 5 triphosgene (419 mg, 1.413 mmol) was added to a stirred solution of (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (500 mg, 1.413 mmol), and TEA (0.985 mL, 7.07 mmol) in Tetrahydrofuran (THF) (20 mL) at 28°C. The reaction mixture was stirred for 30 min and was added (S)-5-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-amine (634 mg, 2.83 mmol). The reaction
- 10 mixture was stirred for 15.5 h at 70 °C. The reaction mixture was cooled to room temperature and was partitioned between water (30 mL) and EtOAc (100 mL). EtOAc layer was separated and was dried over anhydrous Na₂SO₄, filtered. The filtrate was evaporated to get crude. The sample was loaded in dichloromethane and purified on silica (Si) 5g using a 0-50% of Ethyl acetate/Pet ether over 80 mins. The appropriate fractions
- 15 were combined and evaporated in vacuum to give the required product 250 mg as an off-white solid. LCMS (*m/z*): 604.22 [M+H]⁺.

Synthesis of (9S)-3-chloro-N-(6-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



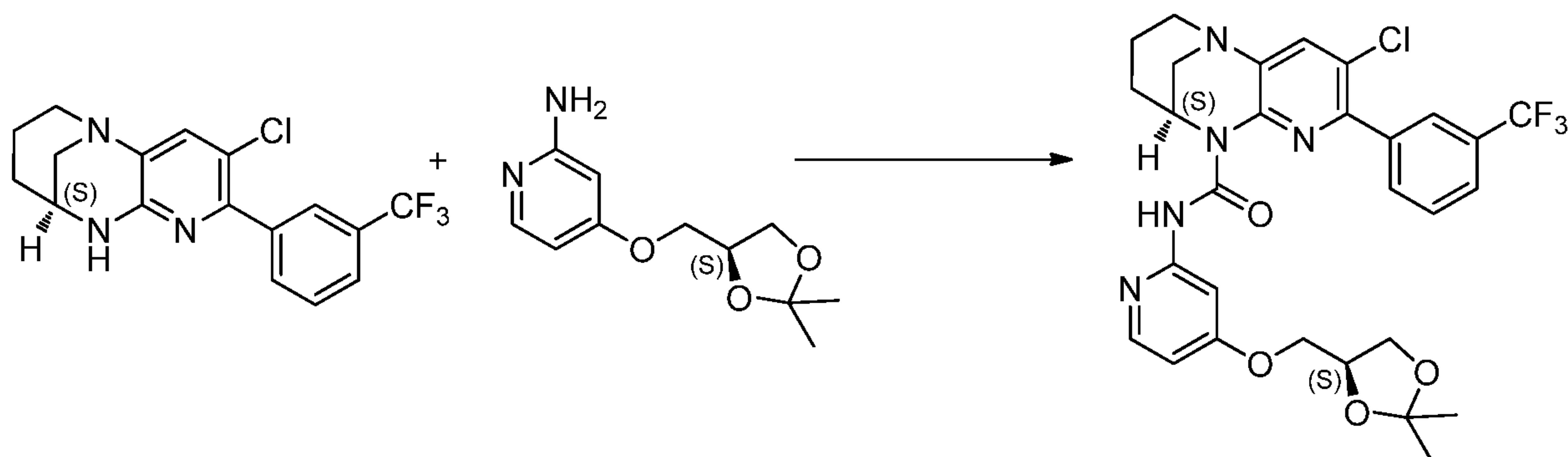
- 5 To solid (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (600 mg, 1.696 mmol) in Tetrahydrofuran (THF) (20 mL) stirred under nitrogen at room temp was added TEA (1.182 mL, 8.48 mmol) and solid triphosgene (302 mg, 1.018 mmol) stirred for 6hrs and was added (R)-6-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-amine (571 mg, 2.54 mmol). The reaction mixture
- 10 was stirred at 80 °C for 16 hr. The reaction mixture was monitored by TLC. The organic phase was evaporated, added water 50 mL and Extracted with Ethyl acetate and washed with saturated brine 100 mL dried over Na₂SO₄ and evaporated in vacuum to give the crude products. The residue was purified via combiflash (100% ACN reverse phase column). Collected fractions and evaporated to get pure compound (9S)-3-chloro-N-(6-
- 15 (((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (180 mg, 0.273 mmol, 16.12 % yield), LCMS (*m/z*): 604.09 [M+H]⁺.

Synthesis of (9S)-3-chloro-N-(6-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



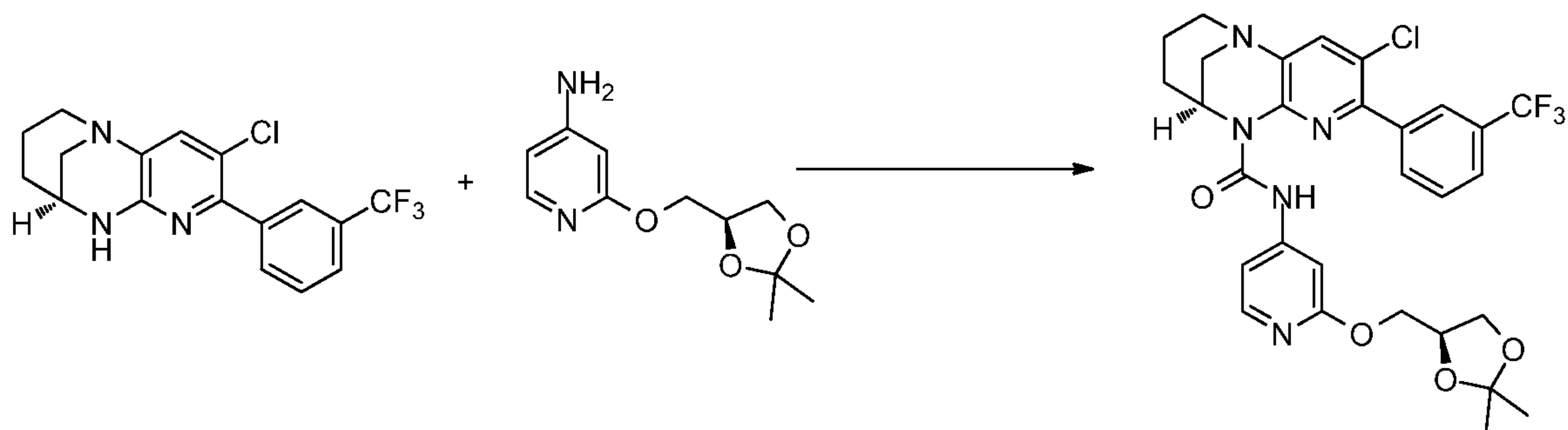
- 5 DIPEA (877 mg, 6.78 mmol) followed by triphosgene (671 mg, 2.261 mmol) were added to a solution of (S)-6-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-amine (1014 mg, 4.52 mmol) in Tetrahydrofuran (THF) (25 mL) at 25 °C, stirred for 1h and (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (800 mg, 2.261 mmol) was added and heated at 70 °C for 18 hr. The
- 10 reaction mixture was cooled to 28 °C and was partitioned between water (20 mL) and EtOAc (50 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to get crude (TLC eluent: 5% methanol in DCM R_f: 0.3; UV active). The crude compound was purified by column chromatography (C-18: eluting with 80% ACN in 1% aq formic acid) to get 460 mg with LCMS: 73%. Further
- 15 purified by flash column chromatography (silica-gel: 100-200 mesh) eluted with 50% EtOAc in hexane to afford (9S)-3-chloro-N-(6-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (300 mg, 0.490 mmol, 21.65 % yield), as a brownish sticky, LCMS (*m/z*): 604.0 (M+H)⁺.

Synthesis of (9S)-3-chloro-N-(4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



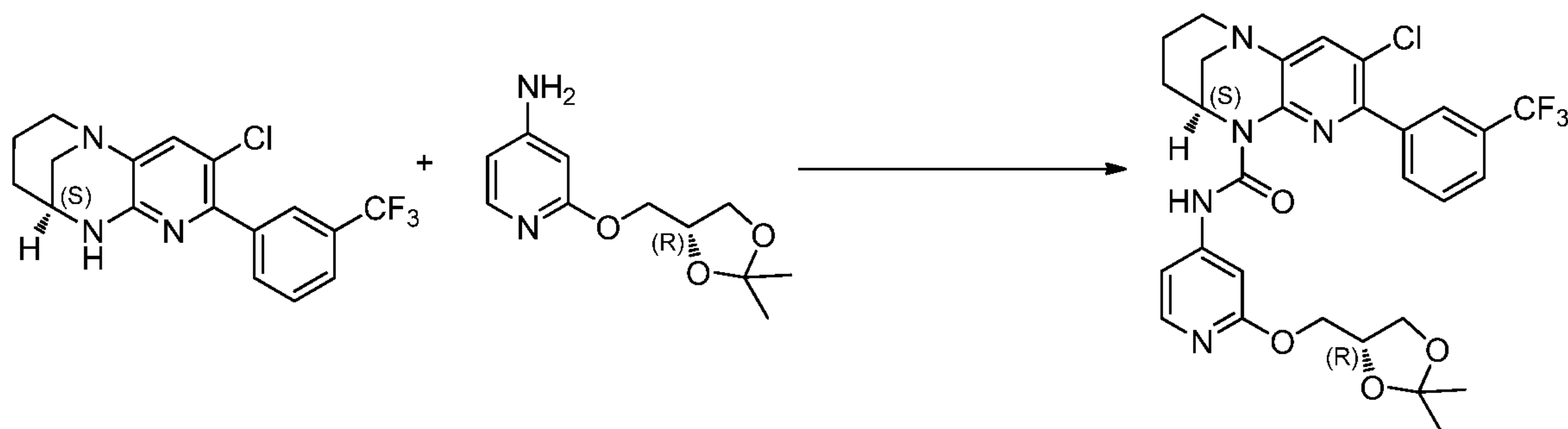
- 5 To solid (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (600 mg, 1.696 mmol) in Tetrahydrofuran (THF) (20 mL) stirred under nitrogen at room temp was added TEA (1.182 mL, 8.48 mmol) and solid triphosgene (302 mg, 1.018 mmol) stirred for 16hrs and was added (S)-4-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)pyridin-2-amine (571 mg, 2.54 mmol). The reaction mixture
- 10 was stirred at 65 °C for 16 hr. The reaction mixture was monitored by TLC. The organic phase was evaporated added water 50 mL and Extracted with Ethyl acetate and washed with saturated brine 100 mL dried over Na₂SO₄ and evaporated in vacuum to give the crude products. The crude product purified by flash chromatography, collected fractions to get compound, was washed with diethyl ether and pentane and filtered and washed
- 15 pentane to get pure compound (9S)-3-chloro-N-(4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (216 mg, 0.319 mmol, 18.79 % yield), LCMS (*m/z*): 604.11 [M+H]⁺.

Synthesis of (9S)-3-chloro-N-(6-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



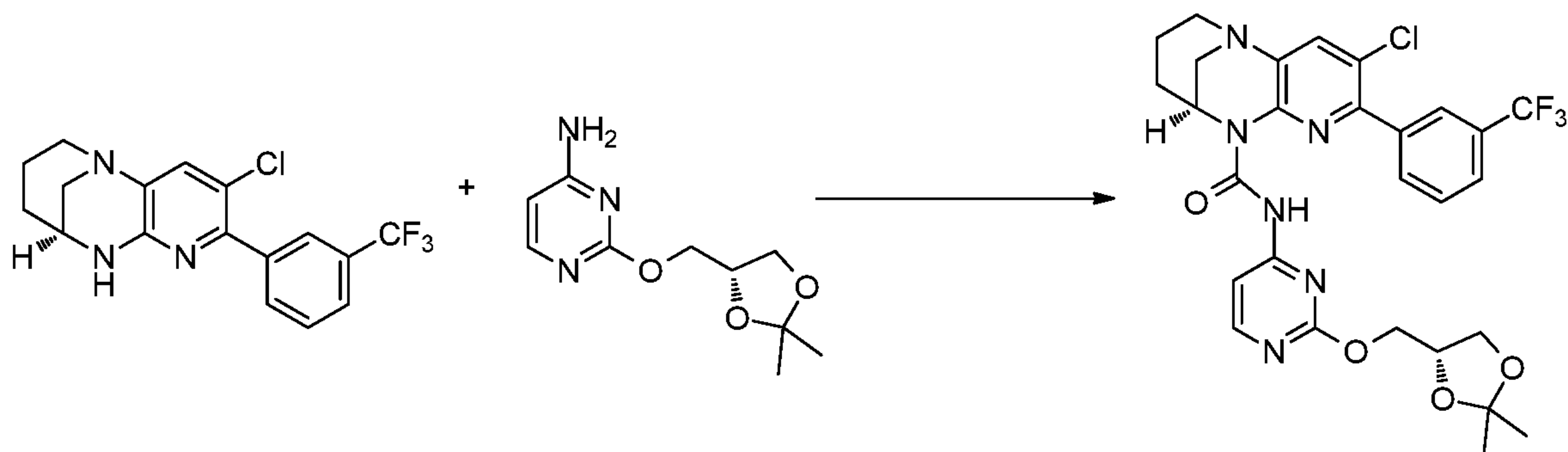
- 5 Procedure: triphosgene (377 mg, 1.272 mmol) was added to a stirred solution of (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (450 mg, 1.272 mmol), and TEA (0.886 mL, 6.36 mmol) in Tetrahydrofuran (THF) (10.0 mL) at 28°C. The reaction mixture was stirred for 30 min and was added (S)-2-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-4-yl)amine (571 mg, 2.54 mmol). The reaction mixture was stirred for 10h at 70 °C. The reaction mixture was cooled to room temperature and was partitioned between water (5 mL) and EtOAc (15 mL). EtOAc layer was separated and was dried over anhydrous Na₂SO₄, filtered. The filtrate was evaporated to get crude. The crude was purified by chromatography (GRACE using C-18 reserval column, Mobile phase A: 0.1% Formic Acid in water; B: MeOH, eluent 91% B in A). Combined fractions were concentrated basified with saturated NaHCO₃. The aqueous layer was extracted with DCM, DCM layer was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to afford (9S)-3-chloro-N-(6-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (300.0 mg, 0.489 mmol, 38.5 % yield) as off-white solid, LCMS (*m/z*): 604.13[M+H]⁺.

Synthesis of (9S)-3-chloro-N-(2-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



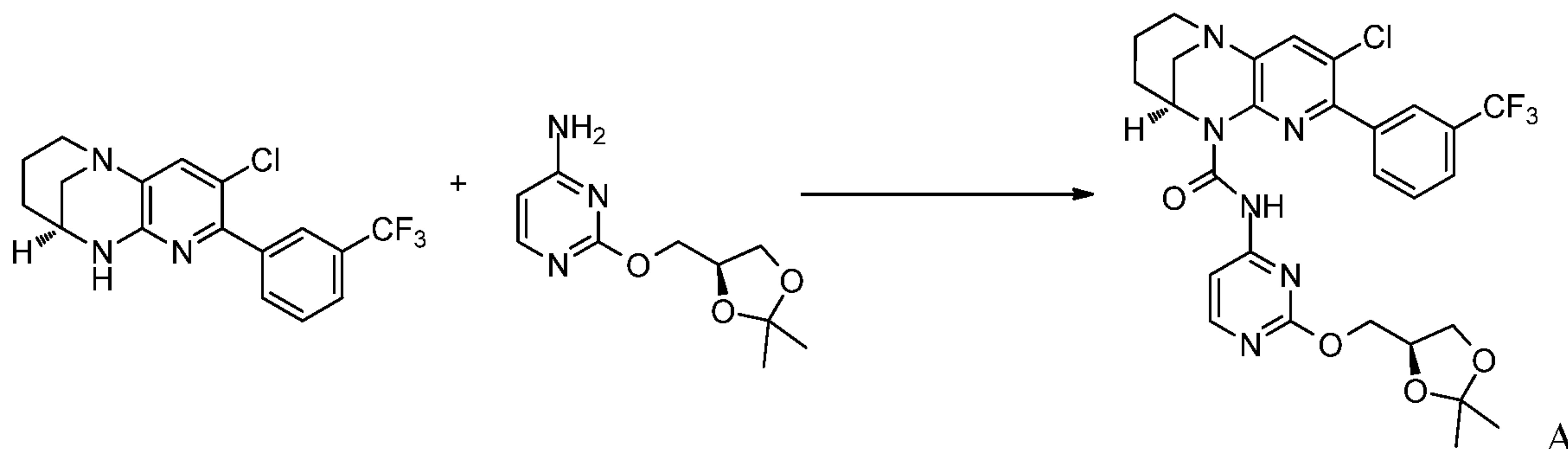
- 5 To solid (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (600 mg, 1.696 mmol) in Tetrahydrofuran (THF) (50 mL) stirred under nitrogen at room temp was added TEA (0.236 mL, 1.696 mmol) and solid triphosgene (503 mg, 1.696 mmol) stirred for 30 min and was added (R)-2-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-4-amine (380 mg, 1.696 mmol). The
- 10 reaction mixture was stirred at 65 °C for 16 hr. The reaction mixture was monitored by TLC. The organic phase was evaporated added water 50 mL and Extracted with Ethyl acetate and washed with saturated brine 100 mL dried over Na₂SO₄ and evaporated in vacuum to give the crude products. The crude product was added to a silica gel column and was eluted with DCM/MeOH. Collected fractions to get some pure compound again
- 15 purified by via combiflash (100% ACN; 120g reverse phase column). Collected fractions to get pure compound (9S)-3-chloro-N-(2-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (450 mg, 0.732 mmol, 43.2 % yield), LCMS (*m/z*): 604.02 [M+H]⁺.

Synthesis of (9S)-3-chloro-N-(2-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



- 5 Triphosgene (377 mg, 1.272 mmol) was added to a stirred solution of (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (450 mg, 1.272 mmol) and TEA (1.064 mL, 7.63 mmol) in Tetrahydrofuran (THF) (30 mL) at 28°C. The reaction mixture was stirred for 2 h and was added (R)-2-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-yl)pyrimidin-4-amine (573 mg, 2.54 mmol). The
- 10 reaction mixture was stirred for 16 hr at 65 °C. TLC eluent: 100 % Ethyl acetate R_f :0.3, UV active. The reaction mixture was cooled to room temp, solvent evaporated under reduced pressure completely and was partitioned between water (10 mL) and EtOAc (2 X 50 mL). Organic layer was separated, dried over anhydrous Na_2SO_4 , filtered and filtrate was evaporated to give crude as brown solid. Crude was diluted with DCM and absorbed
- 15 with neutral alumina and eluted with 35-40% EtOAc in pet ether fractions were collected and concentrated to get (9S)-3-chloro-N-(2-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (220 mg, 0.244 mmol, 19.18 % yield) as a brown solid, LCMS (m/z): 605.00 $[\text{M}+\text{H}]^+$.

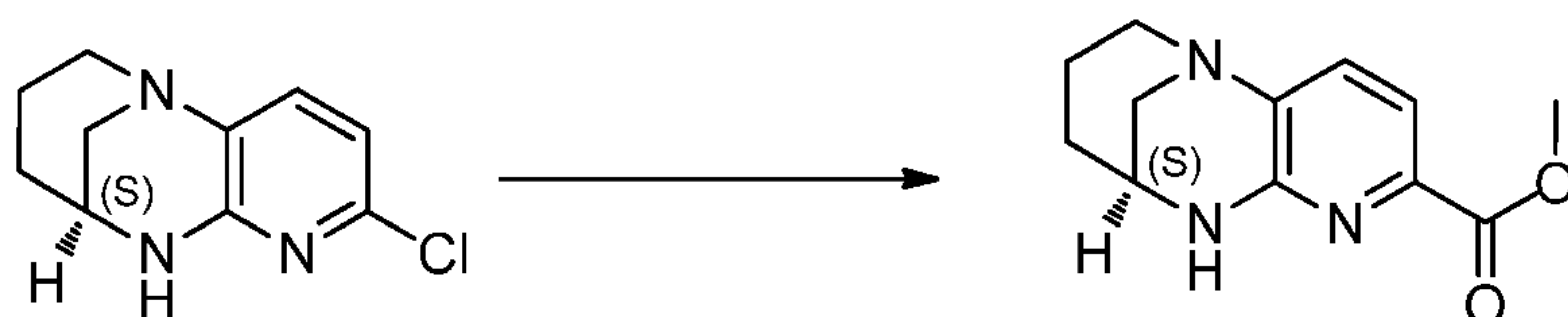
Synthesis of (9S)-3-chloro-N-(2-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



- 5 solution of (9S)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (400 mg, 1.131 mmol), triphosgene (336 mg, 1.131 mmol) and triethylamine (0.788 mL, 5.65 mmol) in Tetrahydrofuran (THF) (20 mL) was stirred under nitrogen at room temp for 30 min. To this reaction mixture (S)-2-(((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-amine (509 mg, 2.261 mmol) was
- 10 added. The reaction mixture was stirred at 70 °C for 16 h and progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature, poured in to water (10 mL) and extracted with EtOAc (3 X 20 mL). The combined organic layer was washed with water (20 mL), brine solution (20 mL), dried over Na₂SO₄, filtered and evaporated to get crude compound. TLC eluent: 100%EtOAc/Hexane, R_f: 0.3, UV active.
- 15 The crude compound was purified by column chromatography using Neutral Alumina and eluted at 20% EtOAc in Petether to afford pure (9S)-3-chloro-N-(2-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (300 mg, 0.395 mmol, 34.9 % yield) as off white solid, LCMS (*m/z*): 605.23 [M+H

20

Synthesis of (9S)-methyl 7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate



- 25 To a suspension of (9S)-2-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-

b][1,4]diazocine (50 g, 238 mmol), in Methanol (500 mL) at room temp was added TEA (100 mL, 715 mmol). and PdCl₂(dppf)-CH₂Cl₂ adduct (9.74 g, 11.92 mmol). in autoclave was filled with CO(100psi) gas. This was degassed and again filled with CO(300-350 psi) gas. The reaction mixture was heated to 130 °C for 16 hr. Progress of the reaction was monitored by TLC. TLC indicated starting material was consumed. Cooled the reaction mass to room temperature, filtered through celite, washed the celite bed with Methanol(500mL). The filtrate was concentrated to get black sticky compound. To this added Methanol(200mL) and stirred for 15 minutes and filtered the solid and dried to afford (9S)-methyl 7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (30 g, 108 mmol, 45.1 % yield))(N36489-30-A2) as Off-white solid, LCMS (*m/z*): 234.07 (M+H)⁺.

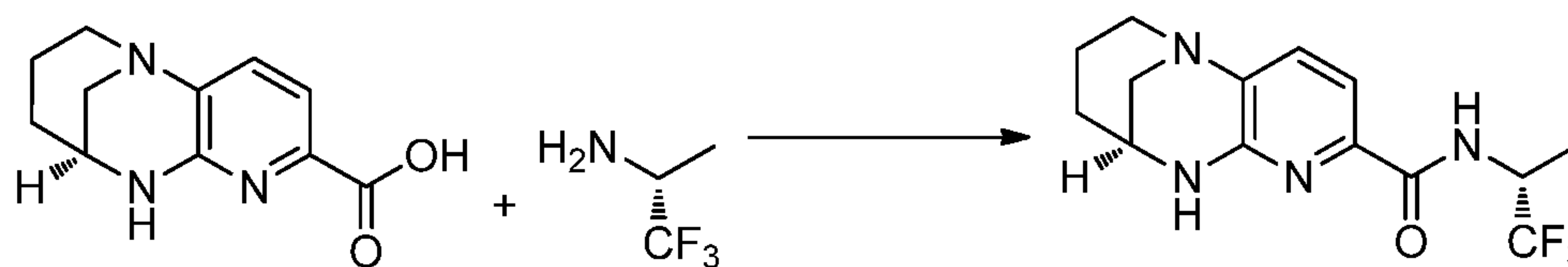
Synthesis of (9S)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid



carboxylic acid

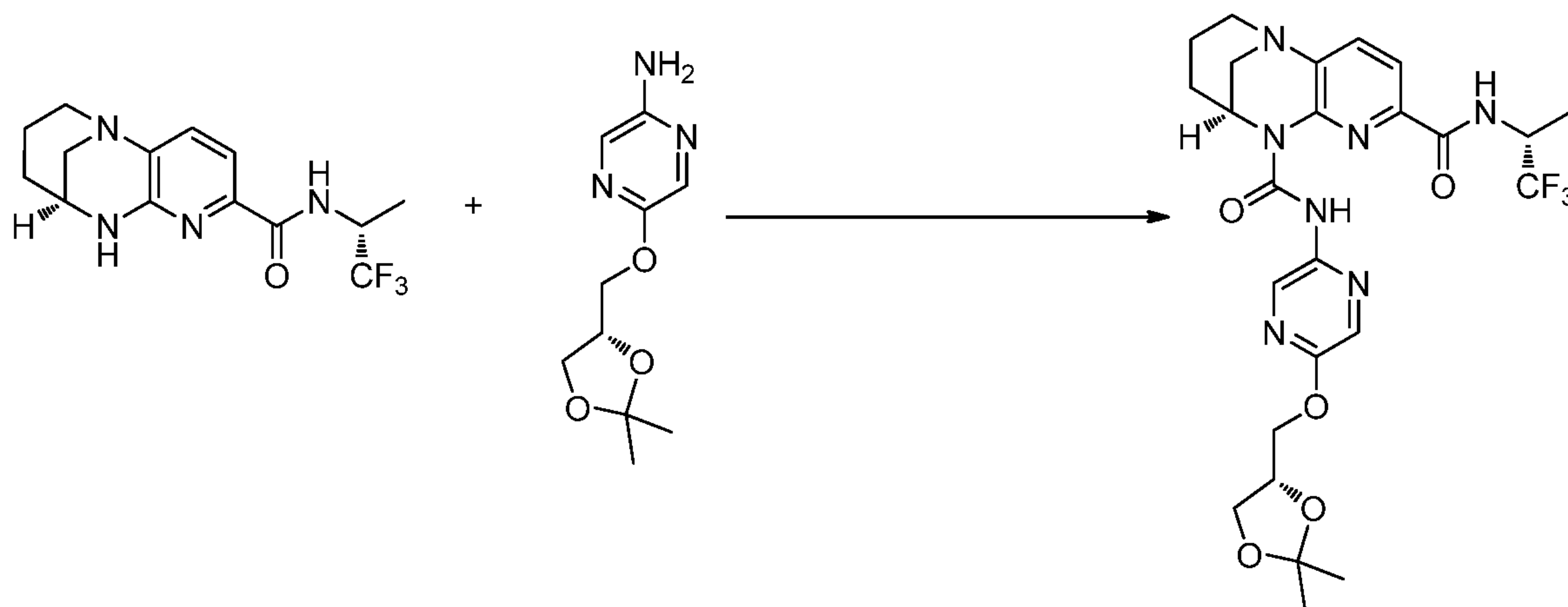
To a solution of (9S)-methyl 7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (3 g, 12.86 mmol) in Tetrahydrofuran (THF) (20 mL), Water (20 mL). The reaction mixture was cooled to 0 °C and added a solution of LiOH (0.462 g, 19.29 mmol) in Water (10 mL). The reaction was stirred at 28 °C for 2h and progress of the reaction was monitored by TLC. The solvent was evaporated under reduced pressure, diluted with water and acidified with 1N HCl (pH;4-5) at 0 °C. The solid was filtered, washed with water and dried under vacuum to afford (9S)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (2.0 g, 8.54 mmol, 66.4 % yield) as an off white solid, LCMS (*m/z*): 220.00 [M+H]⁺.

Synthesis of (9S)-N-((R)-1,1,1-trifluoropropan-2-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxamide



To a stirred solution of (9S)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (2.0 g, 9.12 mmol), HATU (5.20 g, 13.68 mmol) in N,N-Dimethylformamide (DMF) (20 mL) was added DIPEA (6.37 mL, 36.5 mmol). (R)-1,1,1-trifluoropropan-2-amine (1.032 g, 9.12 mmol) was added to the reaction mixture at 0 °C. The reaction mixture was stirred for 16 hr at 28 °C and progress of the reaction was monitored by TLC. The reaction mixture was poured in to water (40 mL) and extracted with EtOAc(3X30 mL). The combined organic layer was washed with brine solution dried over anhydrous Na₂SO₄, filtered and evaporated to get crude compound. The crude compound was purified by column chromatography using neutral alumina and eluent at 30% EtOAc in Pet ether to afford (9S)-N-((R)-1,1,1-trifluoropropan-2-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxamide (1.8 g, 5.59 mmol, 61.3 % yield) as an off white solid, LCMS (*m/z*): 314.85 [M+H]⁺.

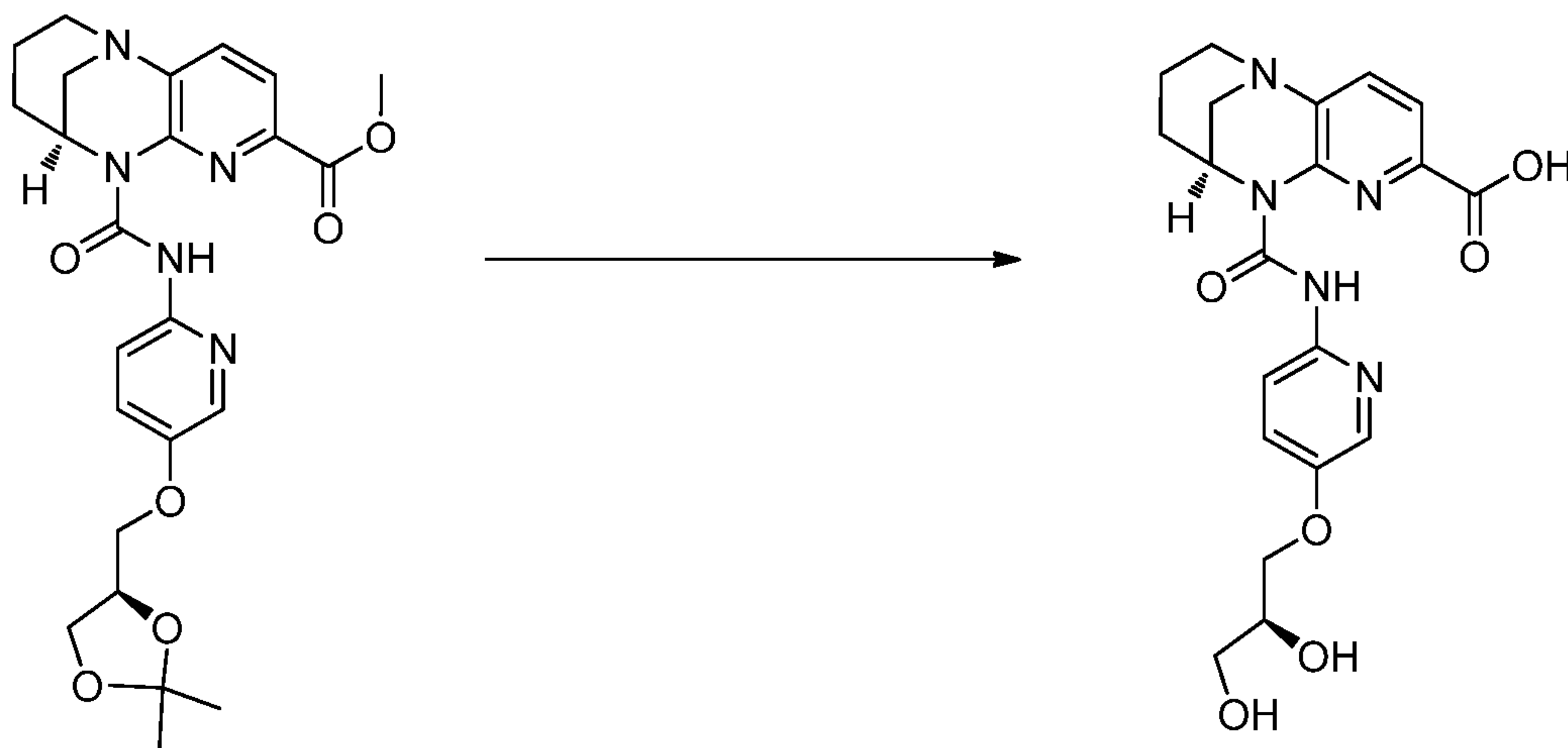
Synthesis of (9S)-N10-(5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-yl)-N2-((R)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide



To a stirred solution of (9S)-N-((R)-1,1,1-trifluoropropan-2-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxamide (300 mg, 0.954 mmol), triphosgene (283 mg, 0.954 mmol) and triethylamine (0.665 mL, 4.77 mmol) in Tetrahydrofuran (THF) (20 mL) under nitrogen at 28 °C and the reaction mixture was stirred at rt for 30 min. (S)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-amine (322 mg, 1.432 mmol) was added to the reaction mixture. The reaction mixture was stirred for 16 hr at 70 °C and progress of the reaction was monitored by TLC. The reaction mixture was cooled to rt, partitioned between water (30 mL) and EtOAc (3X30 mL).

Organic layer was separated and was dried over anhydrous Na_2SO_4 , filtered and filtrate was evaporated to give crude. The crude was purified by GRACE using C-18 reserval column, Mobile phase A: 0.1% Formic Acid in water; B: MeOH, the product was eluted at 65% of MeOH and 0.1% Formic Acid in water. The solvent was evaporated and basified with saturated NaHCO_3 . The precipitated solid was filtered, and was dried to afford (9S)-N10-(5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-yl)-N2-((R)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide (200 mg, 0.349 mmol, 36.6 % yield) as an off-white solid, LCMS (m/z): 566.35 $[\text{M}+\text{H}]^+$.

Synthesis of (9S)-10-((5-((S)-2,3-dihydroxypropoxy)pyridin-2-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid

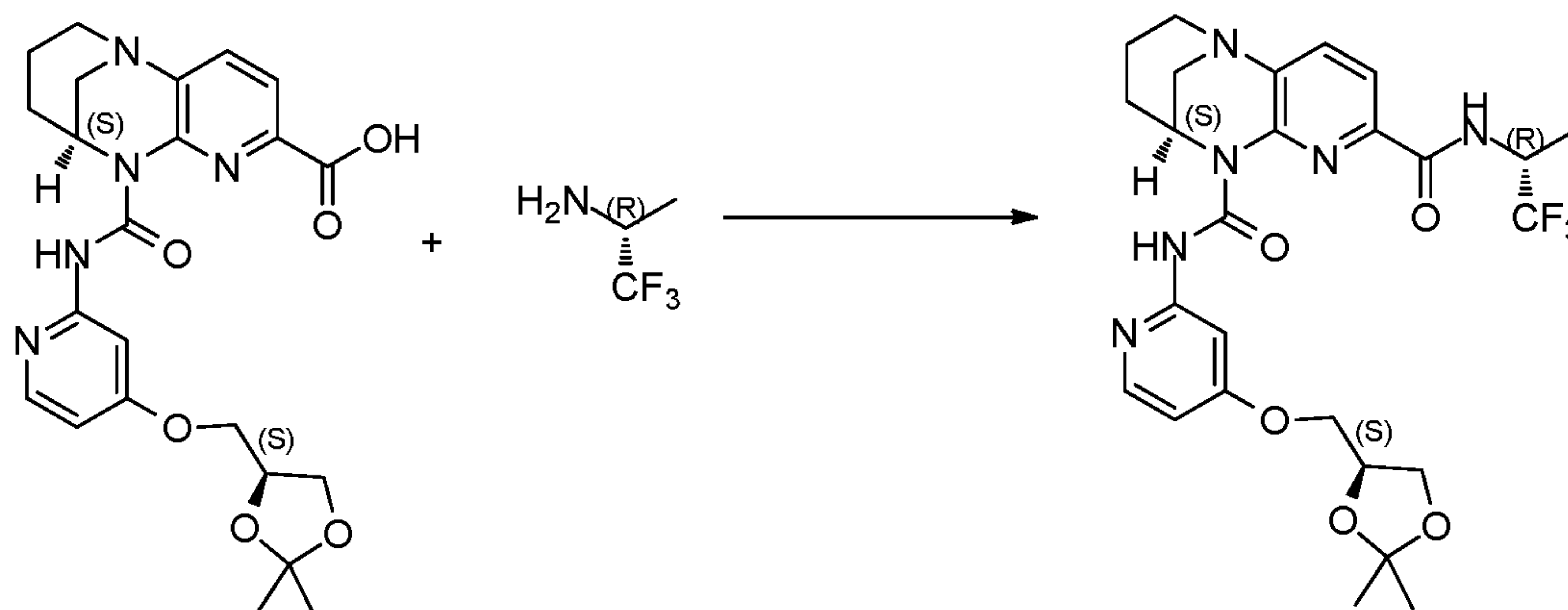


To a stirred solution of (9S)-methyl 10-((5-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (400 mg, 0.827 mmol) in Tetrahydrofuran (THF) (10 mL), Water (10 mL) was added a solution of LiOH (29.7 mg, 1.241 mmol) in water (1 mL) at 0 °C. The reaction mixture was stirred for 2 h at 28 °C and progress of the reaction was monitored by TLC. The reaction solvent was evaporated under reduced pressure, diluted with water and acidified with 1N HCl (pH;2-3) at 0 °C and extracted with DCM (3x40 mL). The combined organic layer was washed with water, brine solution dried over Na_2SO_4 Filtered and evaporated to get crude compound. The crude was purified by GRACE using C-18 reserval column, Mobile phase A: 0.1% Formic Acid in water; B: MeOH, the product was eluted at 50% of MeOH and 0.1% Formic Acid in water. The

solvent was evaporated and basified with saturated NaHCO₃. The precipitated solid was filtered and was dried to afford (9S)-10-((5-((S)-2,3-dihydroxypropoxy)pyridin-2-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (350 mg, 0.802 mmol, 97 % yield) as an off-white solid, LCMS (*m/z*):

5 430.46 [M+H]⁺.

Synthesis of (9S)-N10-(4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-N2-((R)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide



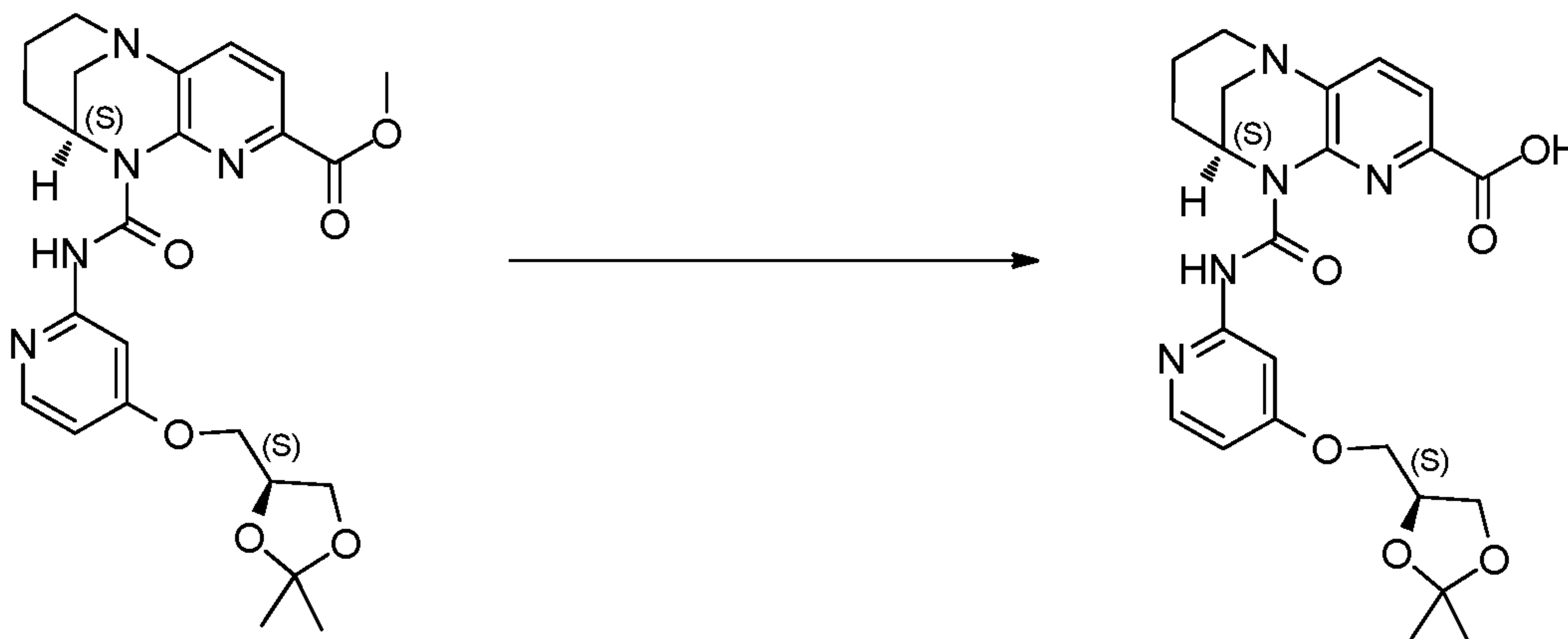
To a solution of (9S)-10-((4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (500 mg, 1.065 mmol) and (R)-1,1,1-trifluoropropan-2-amine (241 mg, 2.130 mmol) in Tetrahydrofuran (THF) (10 mL) stirred under nitrogen at 28 °C was added

15 HATU (486 mg, 1.278 mmol) and DIPEA (0.372 mL, 2.130 mmol) and the reaction mixture was stirred at 28 °C for 16 hr. Reaction mixture was quenched with ice water and extracted with 3x50 ml of ethyl acetate, combined organic layers were washed with 100 ml of brine solution and dried over Na₂SO₄ and concentrated under reduced pressure to afford crude compound. The crude compound was purified by column chromatography (100-200

20 silica gel) using gradient mixture of 80% EtOAc in Petether as eluent, to afford the (9S)-N10-(4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-N2-((R)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide (200 mg, 0.341 mmol, 32.0 % yield) as an off white solid, LCMS (*m/z*):

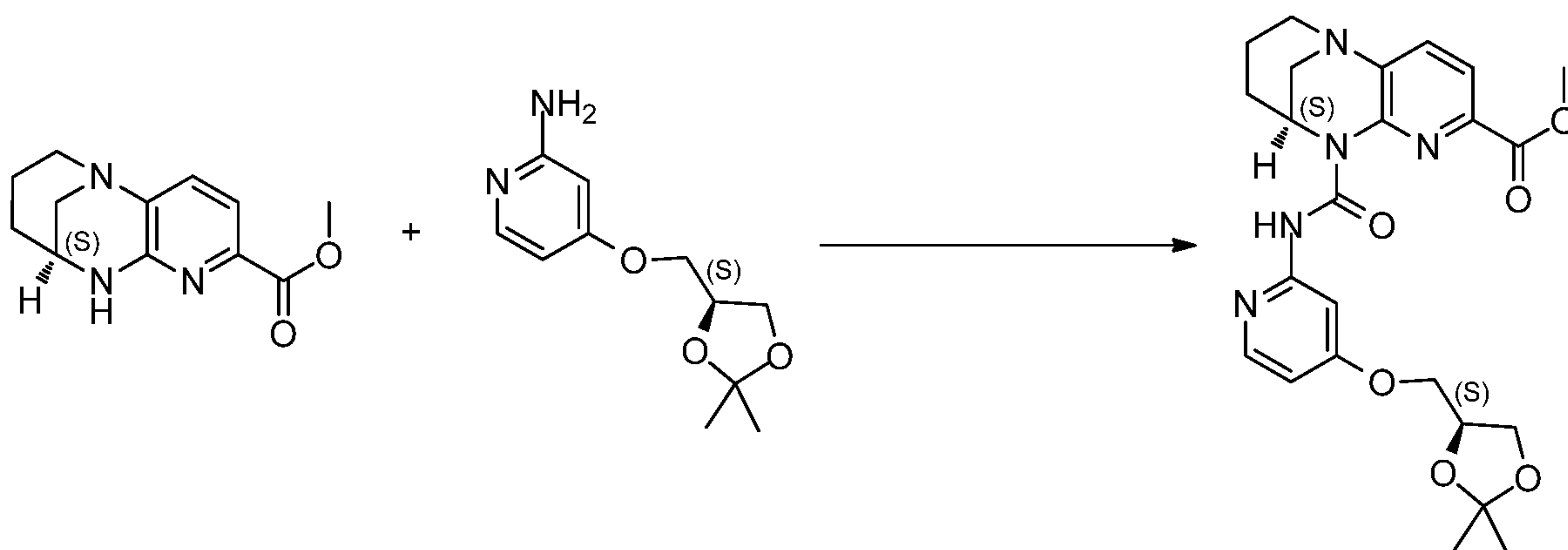
565.15 [M+H]⁺.

Synthesis of (9S)-10-((4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid



- 5 To a solution of (9S)-methyl 10-((4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (1.0 g, 2.068 mmol) in Tetrahydrofuran (THF) (35 mL) and Water (35.0 mL) was added LiOH (0.074 g, 3.10 mmol). The reaction mixture was stirred at RT for 1 hr. Reaction mixture was concentrated under reduced pressure to afford
- 10 compound (9S)-10-((4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (900 mg, 1.876 mmol, 91 % yield) as Off white solid, LCMS (m/z): 470.16 ($M+H$)⁺.

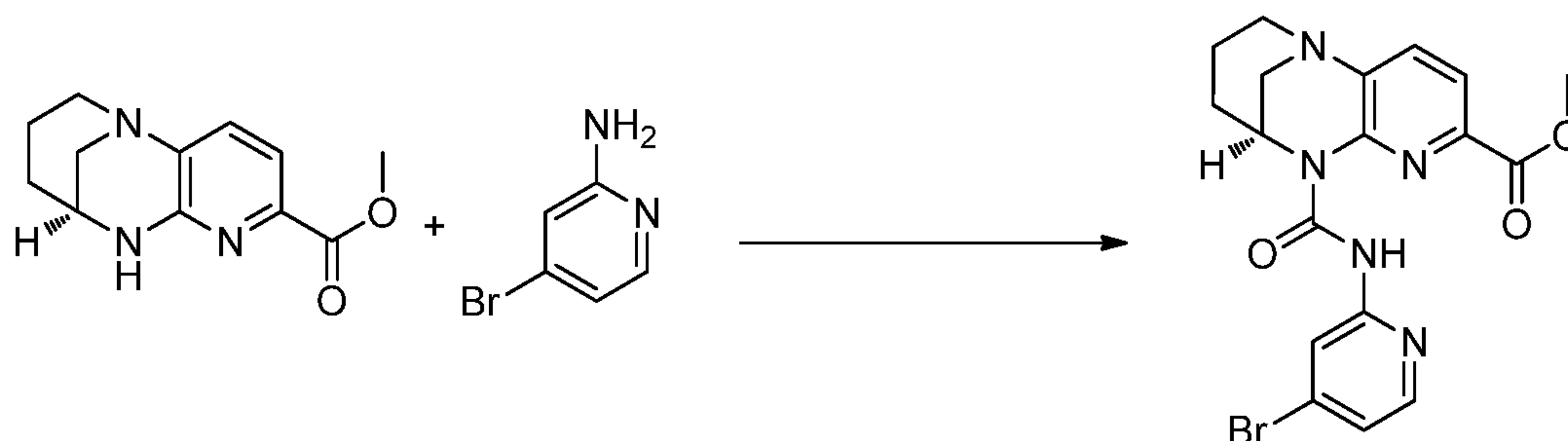
- 15 **Synthesis of (9S)-methyl 10-((4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate**



To a solution of (9S)-methyl 7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-

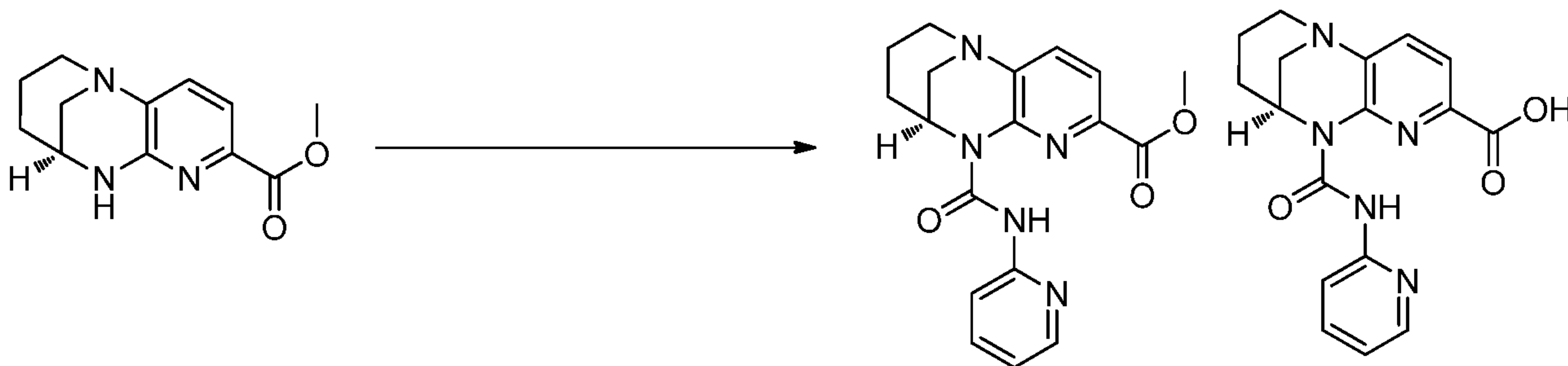
b][1,4]diazocine-2-carboxylate (1.0 g, 4.29 mmol), triphosgene (0.763 g, 2.57 mmol) in Tetrahydrofuran (THF) (30 mL) stirred under nitrogen at 0°C and added DIPEA (3.74 mL, 21.43 mmol). Then the reaction mixture was stirred at 28 °C for 30 min and added (S)-4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-amine (1.442 g, 6.43 mmol), then the
 5 reaction mixture was stirred at 80 °C for 15.5hr. The reaction was monitored by LCMS and TLC. The reaction mixture was poured in to the cold water (50 mL) and extracted with ethyl acetate (2x 100mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated under vacuum to give crude product, LCMS (*m/z*): 484.14 (M+H)⁺.

10 **Synthesis of (9S)-methyl 10-((4-bromopyridin-2-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate**



To a solution of (9S)-methyl 7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (1.0 g, 4.29 mmol), TEA (3.59 mL, 25.7 mmol) in
 15 Tetrahydrofuran (THF) (30 mL) stirred under nitrogen at 0°C and added triphosgene (1.272 g, 4.29 mmol). Then the reaction mixture was stirred at 30 °C for 30 min and added 4-bromopyridin-2-amine (2.225 g, 12.86 mmol), then the reaction mixture was stirred at 80 °C for 15.5hr. The reaction was monitored by LCMS and TLC. The reaction mixture was poured in to the cold water (20 mL) and extracted with ethyl acetate (2x50mL). The
 20 organic layer was dried over anhydrous Na₂SO₄ and concentrated under vacuum to give crude product, LCMS (*m/z*): 431.9 (M+H)⁺.

Synthesis of (9S)-methyl 10-(pyridin-2-ylcarbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate; and (9S)-10-(pyridin-2-ylcarbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid



5

To a suspension of NaH (8.57 g, 214 mmol) in Tetrahydrofuran (THF) (100 mL), stirred under nitrogen at room temperature, was added solid (9S)-methyl 7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (10 g, 42.9 mmol), after 15 min at room temperature was added 3-(pyridin-2-yl)-2H-pyrido[1,2-a][1,3,5]triazine-2,4(3H)-dione (15.45 g, 64.3 mmol). The resulting reaction mixture was stirred at 80 °C for 4 hr.

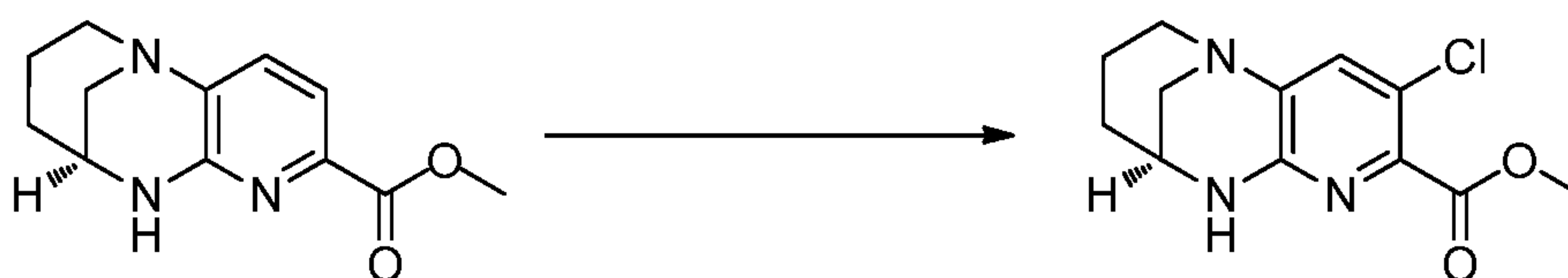
10

Progress of the reaction was monitored by TLC. TLC indicated starting material was consumed. The reaction mass was cooled to rt, diluted with Ethyl acetate(100mL), quenched in ice-cold water(250mL). Separated the organic layer and was dried over Na₂SO₄, filtered and concentrated to get (9S)-methyl 10-(pyridin-2-ylcarbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (10 g, 11.35 mmol, 26.5 % yield) as brown solid compound. LCMS (*m/z*): 354.16 (M+H)⁺. The aqueous layer was acidified with 2N HCl to pH 5 to 6. The aqueous layer was concentrated under vacuum to get the off white solid compound. This was dissolved in 10%Methanol in DCM (1.0L) and filtered the inorganic. The filtrate was concentrated and dried to afford (9S)-10-(pyridin-2-ylcarbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (3 g, 5.46 mmol, 12.75 % yield) as light brown solid. LCMS (*m/z*): 340.0 (M+H)⁺.

15

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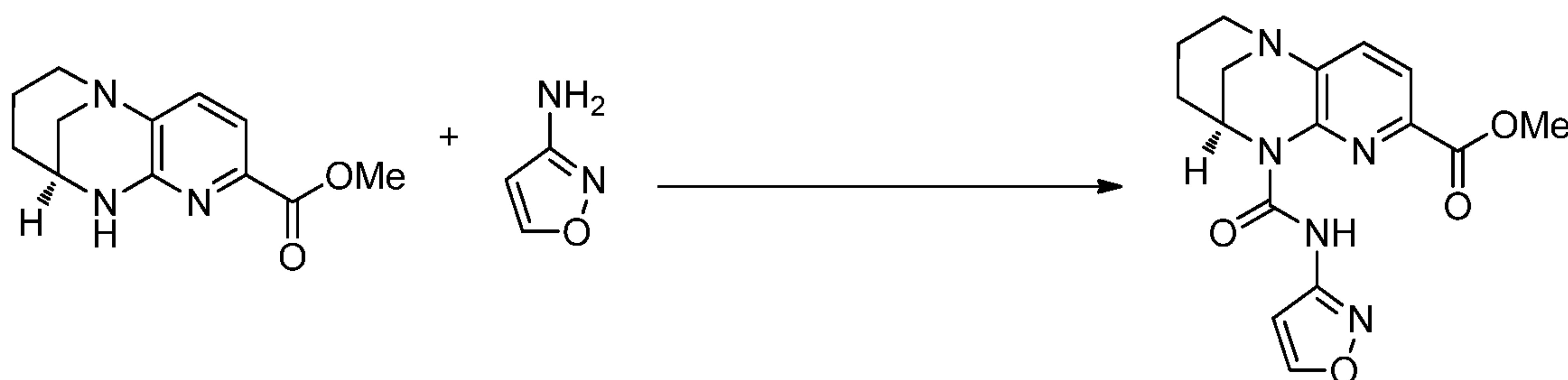
Synthesis of (9S)-methyl 3-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate



25

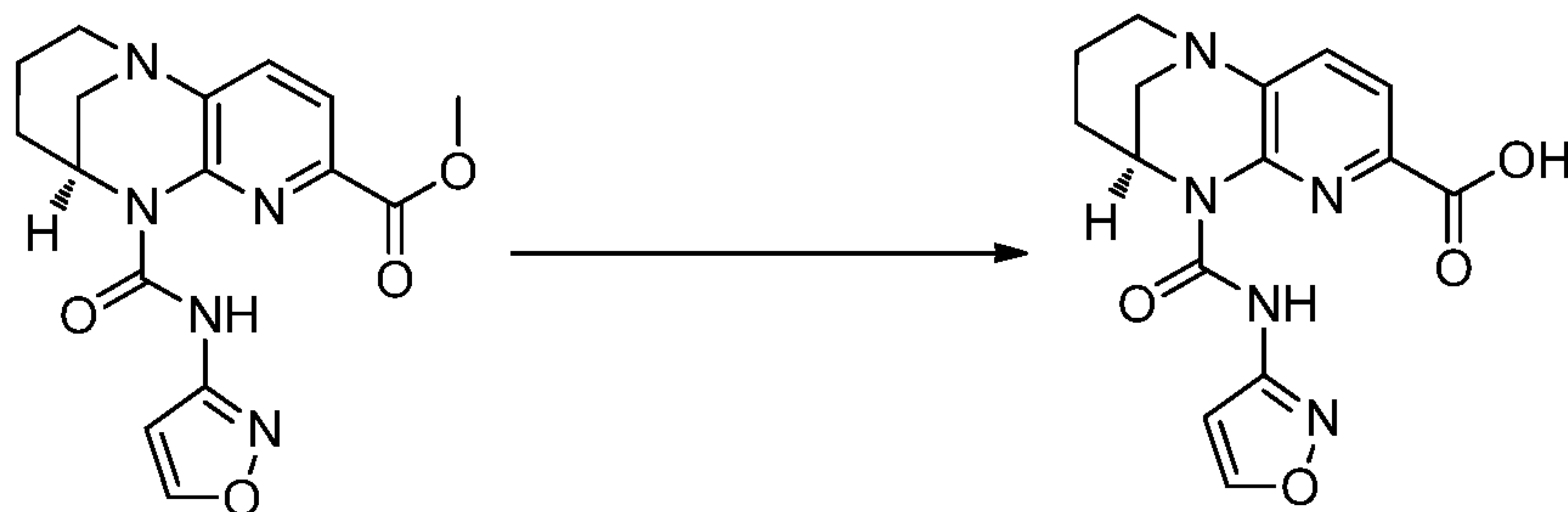
To a suspension of (9S)-methyl 7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (2.2 g, 9.43 mmol), in Chloroform (25 mL) stirred under nitrogen at 0 °C, was added 1-chloropyrrolidine-2,5-dione (1.637 g, 12.26 mmol) in lot wise over 2 min at 0 °C. After 30 min at 0 °C reaction mixture stirred at room temperature for 3 hr. Progress of the reaction was monitored by TLC. TLC indicated two non polar spots and small amount of un-reacted SM. Reaction mass was diluted with 50 ml of water, extracted with (2X100 ml) of DCM. Combined organic layers were dried over Na₂SO₄, filtered and concentrated to get crude. Crude material was purified by combiflash using silica gel column (40 g, 50% EtOAc in Hexane). Fractions containing pure compound were combined and concentrated to afford the desired compound (9S)-methyl 3-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (1g, 3.29 mmol, 34.9 % yield) as pale brown color viscous liquid, LCMS (*m/z*): 267.96 (M+H)⁺.

Synthesis of (9S)-methyl 10-(isoxazol-3-ylcarbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate



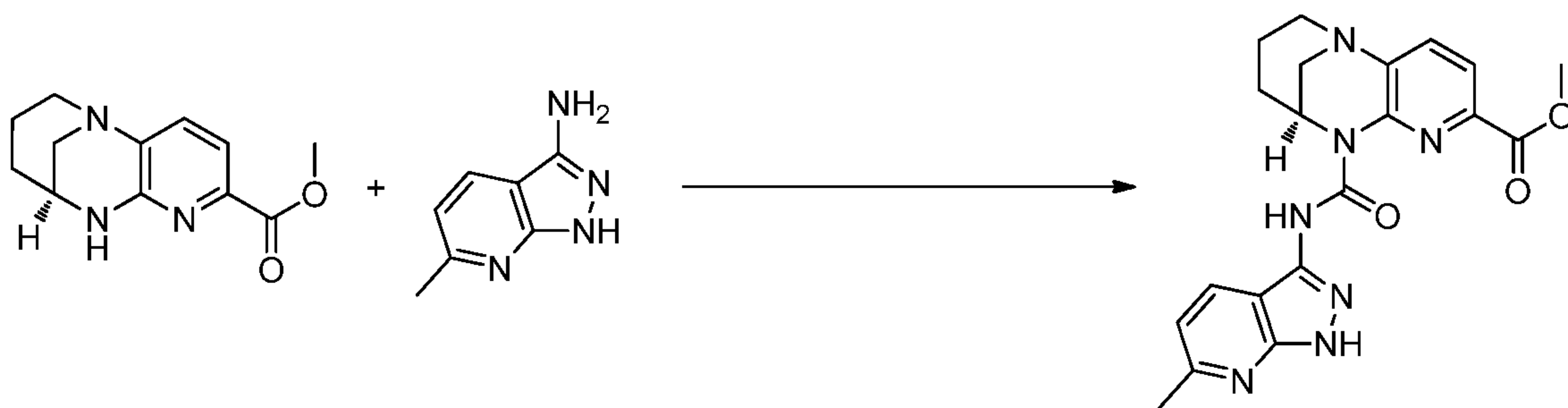
To a solution of (9S)-methyl 7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (600 mg, 2.57 mmol), triphosgene (458 mg, 1.543 mmol) in Tetrahydrofuran (THF) (10 mL) stirred under nitrogen at 0 °C and added DIPEA (2.246 mL, 12.86 mmol). Then the reaction mixture was stirred at 30 °C for 30 min and added isoxazol-3-amine (324 mg, 3.86 mmol), then the reaction mixture was stirred at 80 °C for 15.5hr. The reaction was monitored by LCMS and TLC. The reaction mixture was poured in to the cold water (50 mL) and extracted with ethyl acetate (2x 50mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated under vacuum to give crude product, LCMS (*m/z*): 344.17 (M+H)⁺.

Synthesis of (9S)-10-(isoxazol-3-ylcarbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid



- 5 To a solution of (9S)-methyl 10-(isoxazol-3-ylcarbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (0.5 g, 1.456 mmol) in Tetrahydrofuran (THF) (25 mL) and Water (25.00 mL) was added LiOH (0.052 g, 2.184 mmol). The reaction mixture was stirred at RT for 1 hr. Reaction mixture was concentrated under reduced pressure to remove all the THF and then acidified with 2N.HCl up to pH=4. then
- 10 the precipitated solid filtered and dried to afford compound (9S)-10-(isoxazol-3-ylcarbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (450 mg, 1.346 mmol, 92 % yield) as an off white solid, LCMS (*m/z*): 330.05 (M+H)⁺.

15 **Synthesis of (9S)-methyl 10-((6-methyl-1H-pyrazolo[3,4-b]pyridin-3-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate**

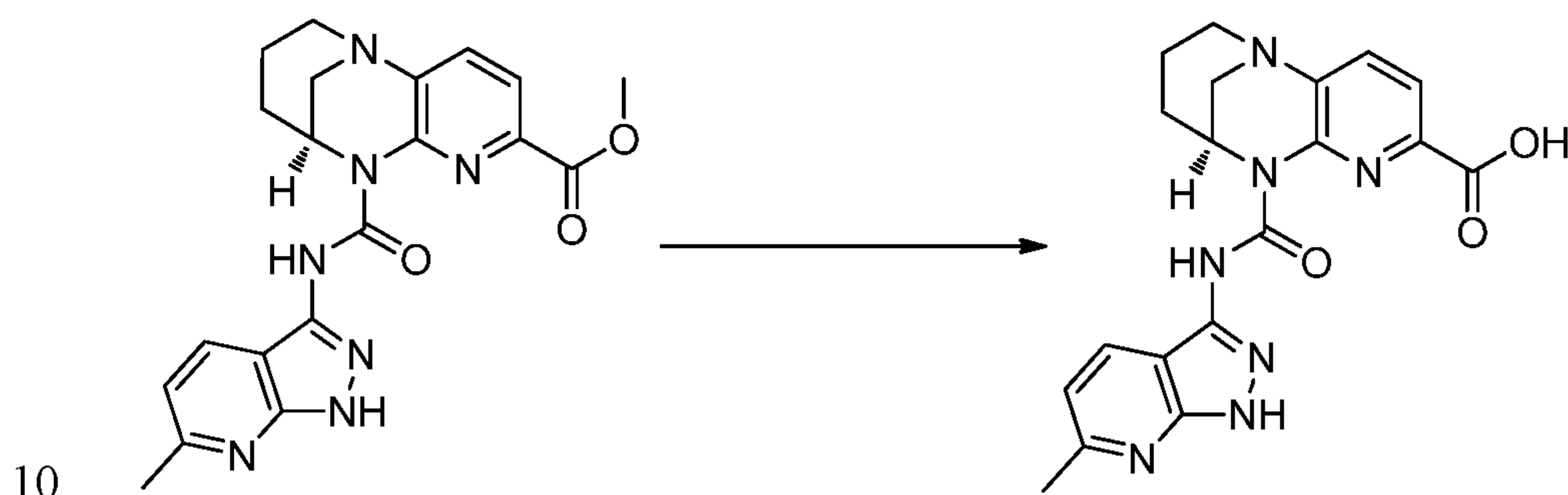


To

- a solution of (9S)-methyl 7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (2 g, 8.57 mmol) in stirred under nitrogen at 0°C triphosgene (2.54 g, 8.57
- 20 mmol) and TEA (7.17 mL, 51.4 mmol) was added. Then the reaction mixture was stirred at 30 °C for 30 min and added 6-methyl-1H-pyrazolo[3,4-b]pyridin-3-amine (507 mg, 3.42 mmol), then the reaction mixture was stirred at 90 °C for 16 hr. The reaction was monitored by LCMS and TLC. The reaction mixture was poured in to the cold water (30 mL) and extracted with ethyl acetate (2x 50 mL). The organic layer was dried over

anhydrous Na_2SO_4 and concentrated under vacuum to obtained crude compound. The crude compound was purified by column chromatography (100-200 silica gel) using gradient mixture of 10% methanol in DCM as eluent to afford the compound (9S)-methyl 10-((6-methyl-1H-pyrazolo[3,4-b]pyridin-3-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (1.5 g, 2.338 mmol, 27.3 % yield) as pale brown solid, LCMS (m/z): 408.00 ($\text{M}+\text{H}$)⁺.

Synthesis of (9S)-10-((6-methyl-1H-pyrazolo[3,4-b]pyridin-3-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid



To a solution of (9S)-methyl 10-((6-methyl-1H-pyrazolo[3,4-b]pyridin-3-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (1.5 g, 3.68 mmol) in Tetrahydrofuran (THF) (10 mL) and Water (10.00 mL) was added LiOH (0.132 g, 5.52 mmol). The reaction mixture was stirred at RT for 2 hr. Reaction mixture was concentrated under reduced pressure to afford compound as Li salt (9S)-10-((6-methyl-1H-pyrazolo[3,4-b]pyridin-3-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (1.2 g, 3.03 mmol, 82 % yield) as Pale brown solid, LCMS (m/z): 394.1 ($\text{M}+\text{H}$)⁺.

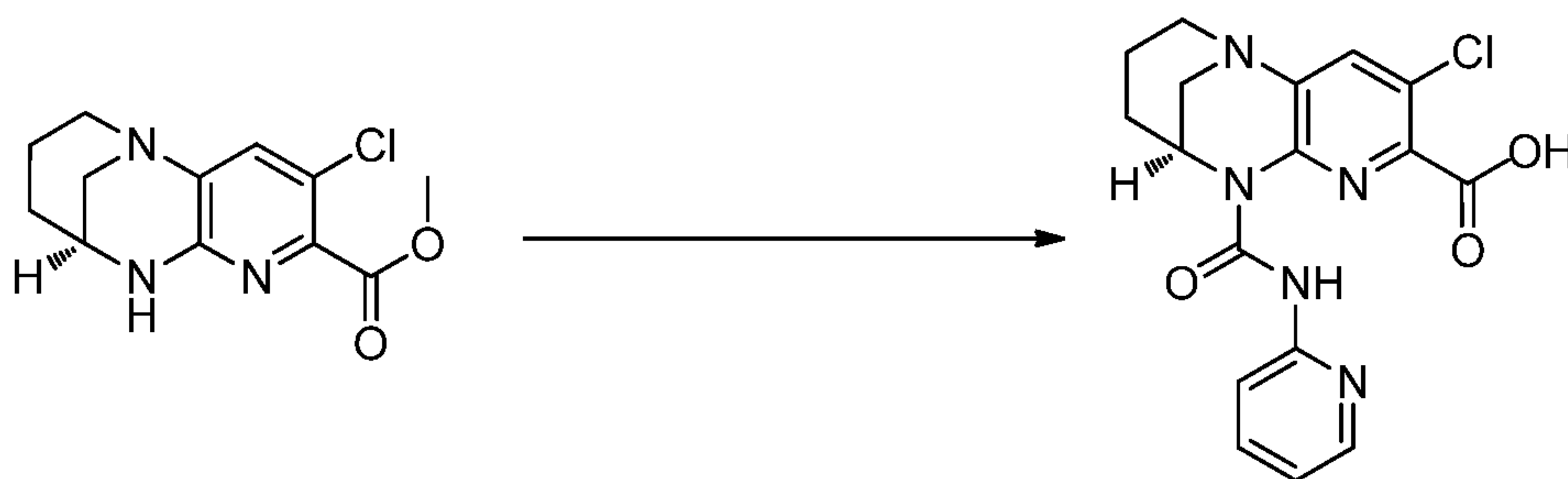
20 **Synthesis of (9S)-3-chloro-10-(pyridin-2-ylcarbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid**



To a stirred solution of NaH (0.448 g, 18.68 mmol) in Tetrahydrofuran (THF) (80 mL) at

room temperature, was added (9S)-methyl 3-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (1 g, 3.74 mmol) in portion wise during 1 min, After 15 min at room temperature was added 3-(pyridin-2-yl)-2H-pyrido[1,2-a][1,3,5]triazine-2,4(3H)-dione (1.077 g, 4.48 mmol). The resulting reaction mixture was stirred at room temperature for 1 hr. And then at 70 °C for 3hr. Progress of the reaction was monitored by TLC. TLC indicated formation of a non polar spot, a polar spot and complete consumption of SM. Reaction mass was diluted with 100 ml of ice cold water, aqueous layer was washed with (100 ml) of EtOAc, pH of aqueous layer adjusted to 4 with 1N HCl, concentrated aqueous layer under reduced pressure to get crude, resulting crude solid was extracted with 10% MeOH in DCM and concentrated the organic layer to get (9S)-3-chloro-10-(pyridin-2-ylcarbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (500 mg, 0.744 mmol, 19.91 % yield), LCMS (m/z): 374.20 ($M+H$)⁺.

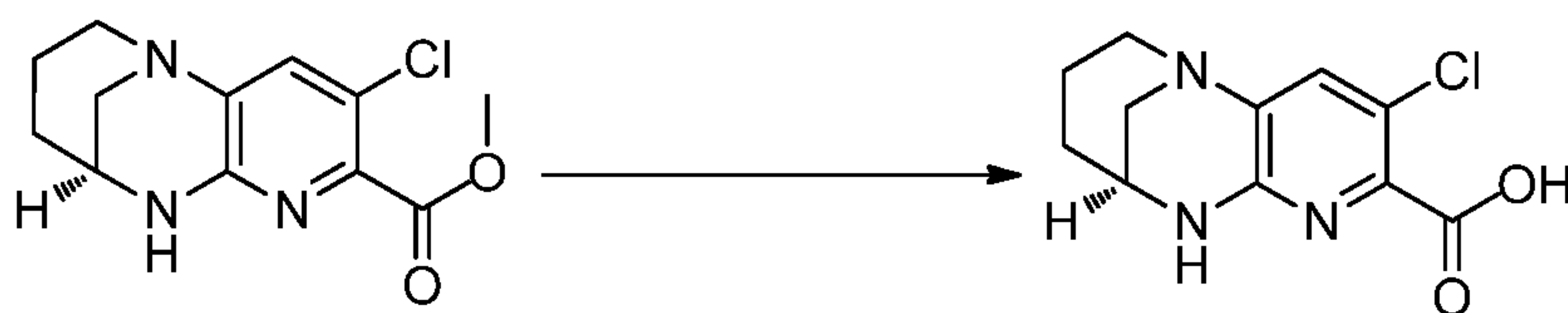
Synthesis of (9S)-3-chloro-10-(pyridin-2-ylcarbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid



To a stirred solution of NaH (224 mg, 5.60 mmol) in Tetrahydrofuran (THF) (30 mL) at room temperature, was added (9S)-methyl 3-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (500mg, 1.868 mmol) in portion wise during 1 min, After 15 min at room temperature was added 3-(pyridin-2-yl)-2H-pyrido[1,2-a][1,3,5]triazine-2,4(3H)-dione (538 mg, 2.241 mmol). The resulting reaction mixture was stirred at room temperature for 1 hr. And then at 70 °C for 3hr. Progress of the reaction was monitored by TLC. TLC indicated formation of a non polar spot, a polar spot and complete consumption of SM. Reaction mass was diluted with 100 ml of ice cold water, aqueous layer was washed with (100 ml) of EtOAc, pH of aqueous layer adjusted to 4 with 1N HCl, concentrated aqueous layer under reduced pressure to get crude, resulting crude solid was extracted with 10% MeOH in DCM and concentrated the organic layer to

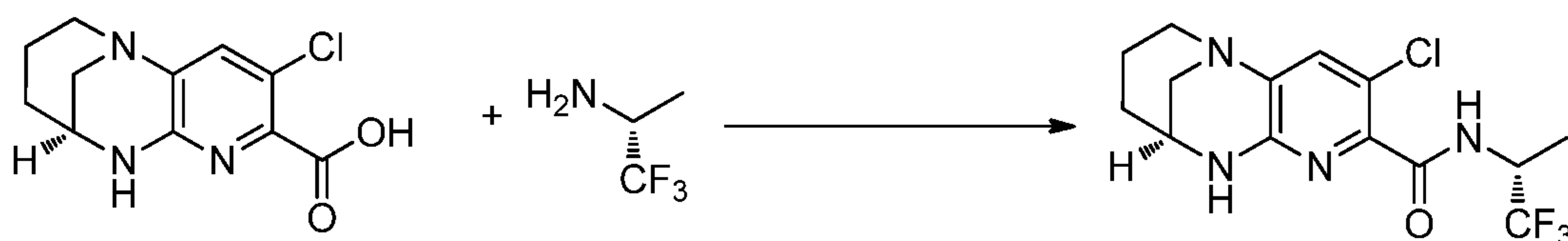
get (9S)-3-chloro-10-(pyridin-2-ylcarbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (300 mg, 0.525 mmol, 28.1 % yield) N35384-85-A1 as Brown color, LCMS (m/z): 374.08 ($M+H$)⁺.

5 **Synthesis of (9S)-3-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid**



To a solution of (9S)-methyl 3-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (2.0 g, 7.47 mmol) in Tetrahydrofuran (THF) (35 mL) and
 10 Water (35.0 mL), LiOH (0.268 g, 11.21 mmol) was added at RT and stirred for 16 hrs. (TLC eluent: 10% MeOH in ethyl acetate R_f : 0.2; UV active). Reaction mixture was diluted with water (100 mL), extracted with ethyl acetate (2X 100 mL) to remove all the impurities. The aqueous layer acidified with aq.HCl (5 mL) and concentrated under reduced pressure to afford (9S)-3-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (1.02 g, 3.32 mmol, 44.4 % yield) as a light brown
 15 colored solid. LCMS (m/z): 253.91 [$M+H$]⁺.

Synthesis of (9S)-3-chloro-N-((R)-1,1,1-trifluoropropan-2-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxamide



To a solution of (9S)-3-chloro-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (1.02 g, 4.02 mmol) in N,N-Dimethylformamide (DMF) (5 mL) under nitrogen at room temp, HATU (1.835 g, 4.82 mmol), DIPEA (1.404 mL, 8.04 mmol) and (R)-1,1,1-trifluoropropan-2-amine (0.682 g, 6.03 mmol) was added
 25 and stirred at RT for 16 h. Reaction mixture was diluted with ice water and extracted with 2x100 mL of ethyl acetate. The combined organic layers were washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure to afford crude compound. The crude product was purified by flash column chromatography (100-200 silica gel eluted

with 5% of CH₂Cl₂/MeOH) to afford (9S)-3-chloro-N-((R)-1,1,1-trifluoropropan-2-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxamide (800 mg, 1.991 mmol, 49.5 % yield) as an off white solid, LCMS (*m/z*): 348.96 [M+H]⁺.

5 **Synthesis of (9S)-10-(pyridin-2-ylcarbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid**

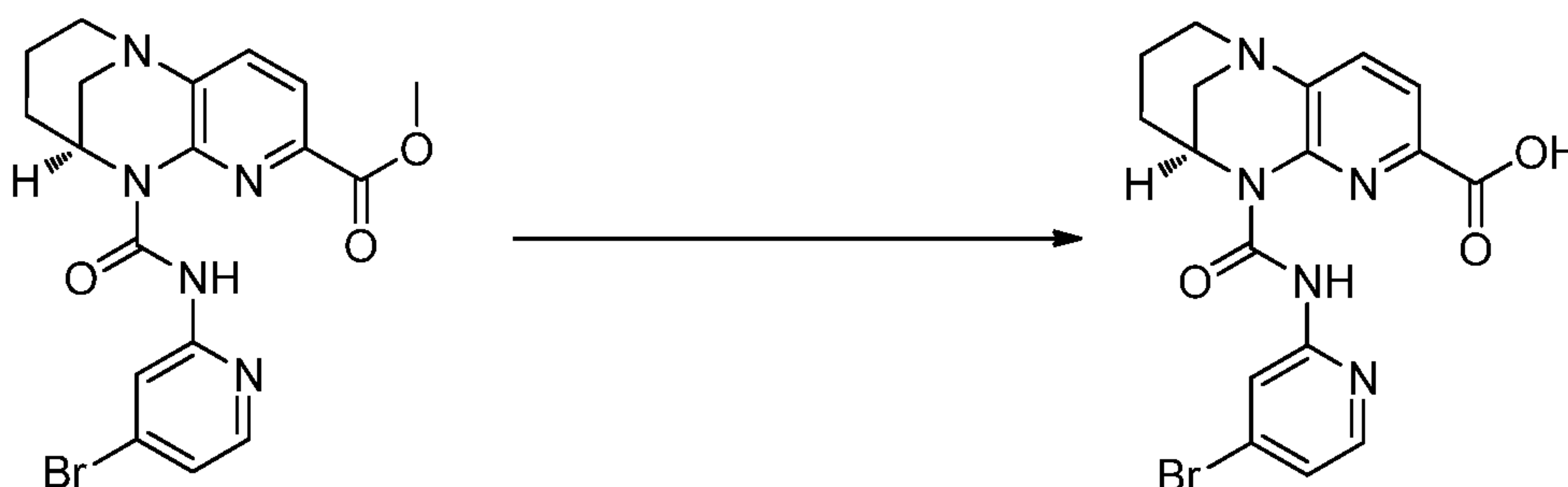


To a solution of (9S)-methyl 10-(pyridin-2-ylcarbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (10 g, 28.3 mmol), and in

10 Tetrahydrofuran (THF) (100 mL), Water (100 mL) at room temp was added lithium hydroxide (1.355 g, 56.6 mmol). The reaction mixture was stirred at rt for 3 hr. Progress of the reaction was monitored by TLC. TLC indicated starting material was consumed. The reaction mass was diluted with Ethyl acetate(100mL), separated the organic layer. The aqueous layer was acidified with 1N HCl to PH- 5-6. The aqueous layer was concentrated
15 to get light brown solid. To this added 10%Methanol in DCM(500mL) and stirred for 15 minutes and filtered the inorganic. The filtrate was concentrated to afford (9S)-10-(pyridin-2-ylcarbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (6 g, 17.47 mmol, 61.7 % yield) as light brown solid. LCMS (*m/z*): 340.15 (M+H)⁺.

20

Synthesis of (9S)-10-((4-bromopyridin-2-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid

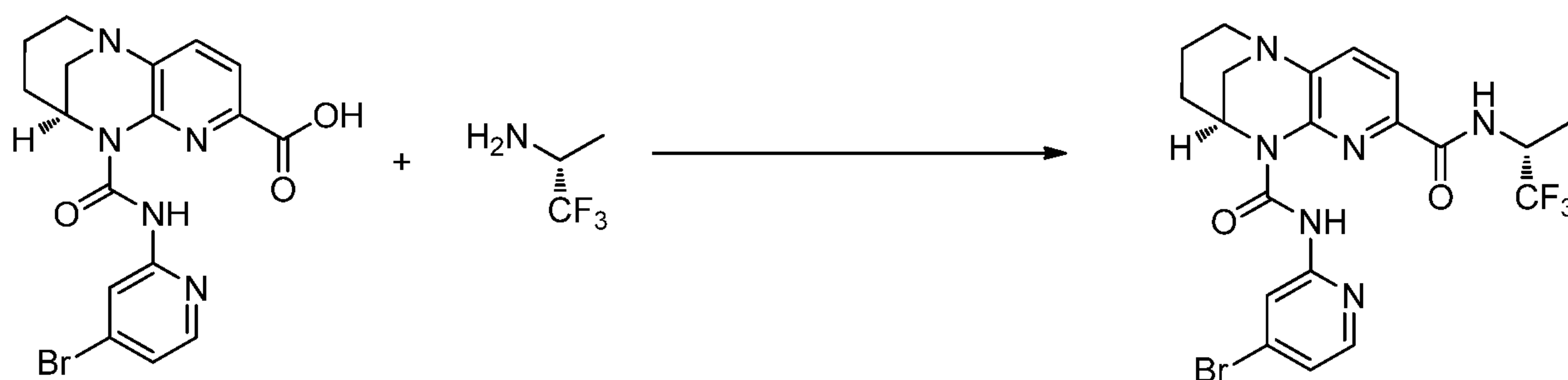


To a solution of (9S)-methyl 10-((4-bromopyridin-2-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (0.800 g, 1.851 mmol) in Tetrahydrofuran (THF) (35 mL) and Water (35.0 mL) was added LiOH (0.066 g, 2.78 mmol). The reaction mixture was stirred at RT for 1 hr. Reaction mixture was

5 concentrated under reduced pressure to afford Li salt of the product. Then the salt was diluted with water (20 mL) washed thoroughly with Ethyl acetate (2X 50 mL) then the aqueous layer acidified with aq. HCl to afford (9S)-10-((4-bromopyridin-2-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (600 mg, 1.357 mmol, 73.3 % yield) as an Off white solid, LCMS (m/z): 419.90 $[M+H]^+$.

10

Synthesis of (9S)-N10-(4-bromopyridin-2-yl)-N2-((R)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide

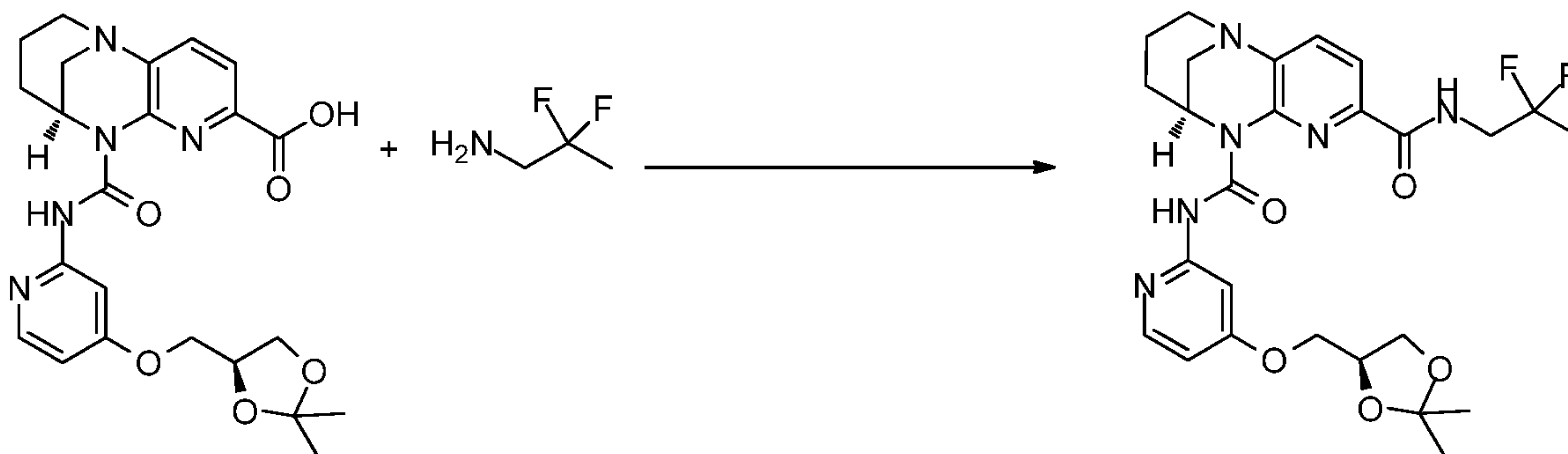


15 To a solution of (9S)-10-((4-bromopyridin-2-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (480 mg, 1.148 mmol) and (R)-1,1,1-trifluoropropan-2-amine (195 mg, 1.721 mmol) in N,N-Dimethylformamide (DMF) (15 mL) stirred under nitrogen at 28 °C was added HATU (524 mg, 1.377 mmol) and DIPEA (0.401 mL, 2.295 mmol) and the reaction mixture was stirred at 28 °C for 16 hr.

20 Reaction mixture was quenched with ice water and extracted with 3x20 ml of ethyl acetate, combined organic layers were washed with 20 ml of brine solution and dried over Na₂SO₄ and concentrated under reduced pressure to afford crude compound. Crude compound was purified by column chromatography using 100-200 silica gel and with an eluent 80-100 % of ethyl acetate/Hexane. Collected fractions were distilled off to afford (9S)-N10-(4-

25 bromopyridin-2-yl)-N2-((R)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide (500 mg, 0.960 mmol, 84 % yield) as an off white solid, LCMS (m/z): 565.9 $[M+H]^+$.

Synthesis of (9S)-N2-(2,2-difluoropropyl)-N10-(4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide

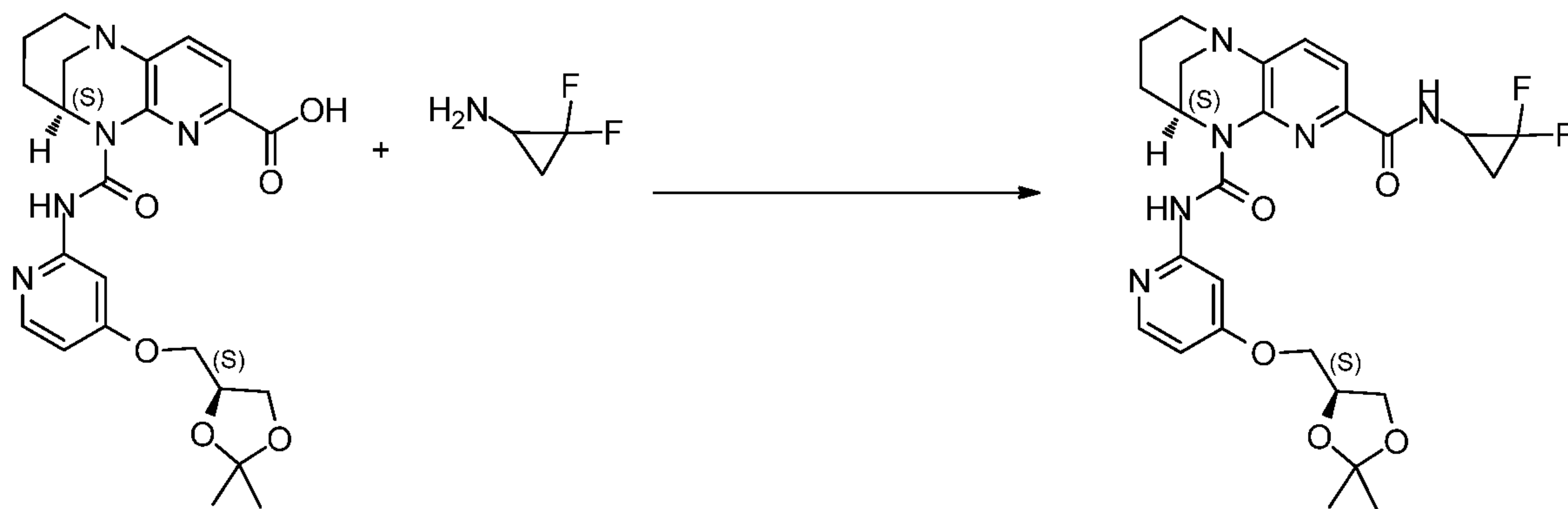


- 5 To a solution of (9S)-10-((4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (500 mg, 1.065 mmol) in Tetrahydrofuran (THF) (10 mL) stirred under nitrogen at 20 °C was added HATU (486 mg, 1.278 mmol) and DIPEA (0.372 mL, 2.130 mmol) and the reaction mixture was stirred at 28 °C for 16 hr. Reaction mixture was
- 10 diluted with ice water and extracted with 3x50 ml of ethyl acetate, combined organic layers were washed with 100 ml of brine solution and dried over Na₂SO₄ and concentrated under reduced pressure to afford crude compound. Crude compound was purified by column chromatography using 100-200 mesh silicagel and was eluted with 80% to Pure ethyl acetate, LCMS (*m/z*): 547.35 [M+H]⁺.

Synthesis of:

(9S)-N2-(2,2-difluorocyclopropyl)-N10-(4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide(Peak-1); and

5 **(9S)-N2-(2,2-difluorocyclopropyl)-N10-(4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide (Peak-2)**



To a stirred solution of (9S)-10-((4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (2.5 g, 5.32 mmol) in ACN (10 mL) was added HATU (4.05 g, 10.65 mmol) and DMAP (1.301 g, 10.65 mmol). Reaction mixture was stirred at RT for 15min, then added 2,2-difluorocyclopropanamine, Hydrochloride (1.380 g, 10.65 mmol) in sealed tube, reaction mixture was stirred at 90 °C for 16 hr. Progress of the reaction was monitored by TLC. Reaction mixture was cooled to RT, Water(50 mL) and extracted with ethyl acetate(2x50 mL), separated organic layer, dried over Na₂SO₄, concentrated under reduced pressure to obtain crude. Obtained crude was purified by column using silica (100-200 mesh/ 1-80% EtOH in Pet-ether as a eluent), collected fractions were concentrated pressure to get pure compound 700mg. The obtained pure compound was purified by SFC.

After SFC purification to get (9S)-N2-(2,2-difluorocyclopropyl)-N10-(4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide (Peak-1) (230 mg, 0.414 mmol, 7.77 % yield) and (9S)-N2-(2,2-difluorocyclopropyl)-N10-(4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide (Peak-2) (250 mg, 0.454 mmol, 8.53 % yield).

Column/dimensions : Chiralpak IC(250 X30)mm,5 μ
% CO₂ : 50.0%
% Co solvent : 50.0% (100% Methanol)
Total Flow : 100.0 g/min
5 Back Pressure : 100.0 bar
UV : 210 nm
Stack time : 6.5 min
Load/Inj : 9.5 mg

10 **Solubility : Methanol**

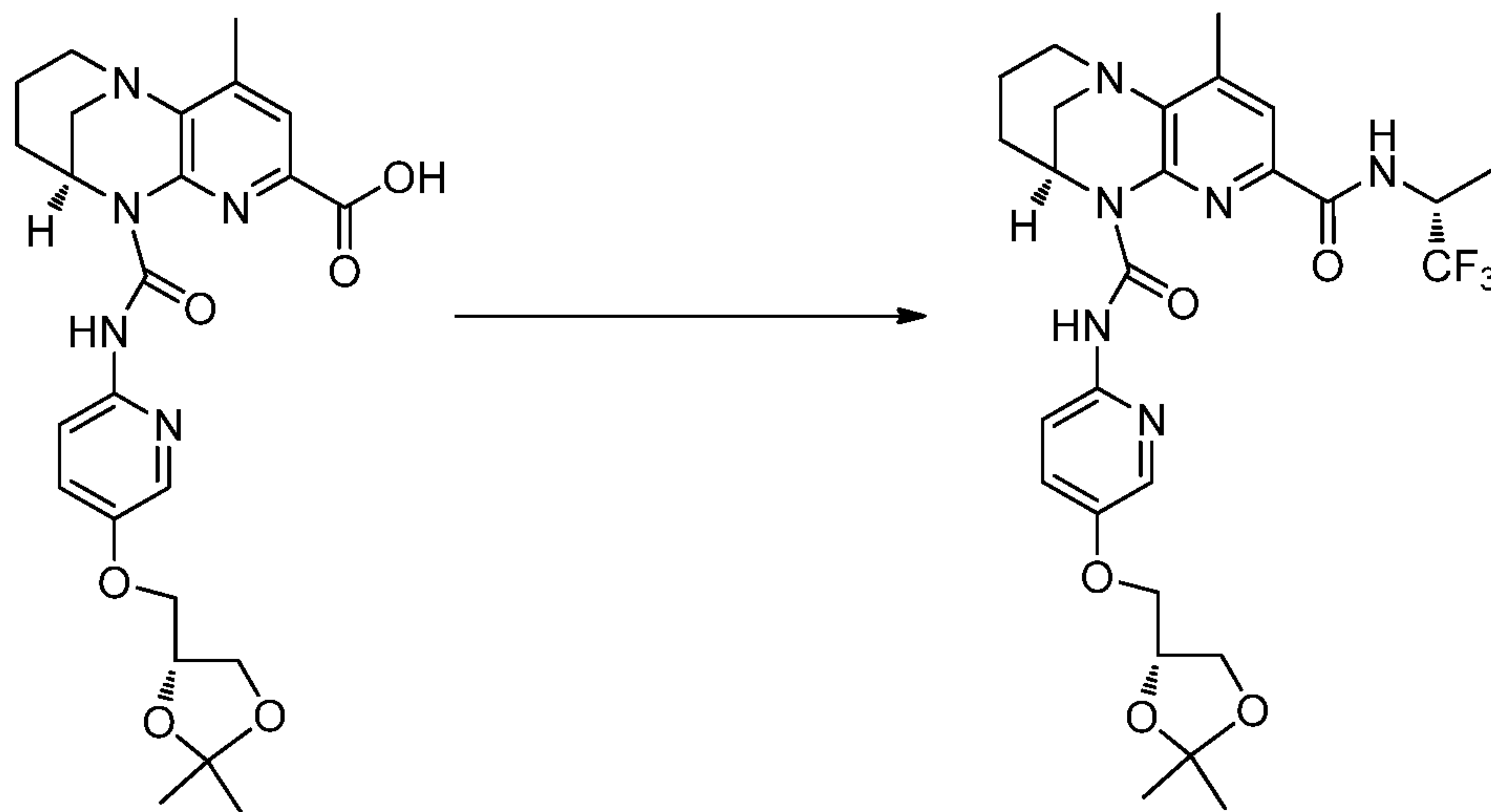
Total No of injections : 70

Instrument details: Make/Model: Thar SFC- 200-NEW-2

LCMS (*m/z*): 545.02 [M+H]⁺. (peak 1)

LCMS (*m/z*): 545.89 [M+H]⁺. (peak 2)

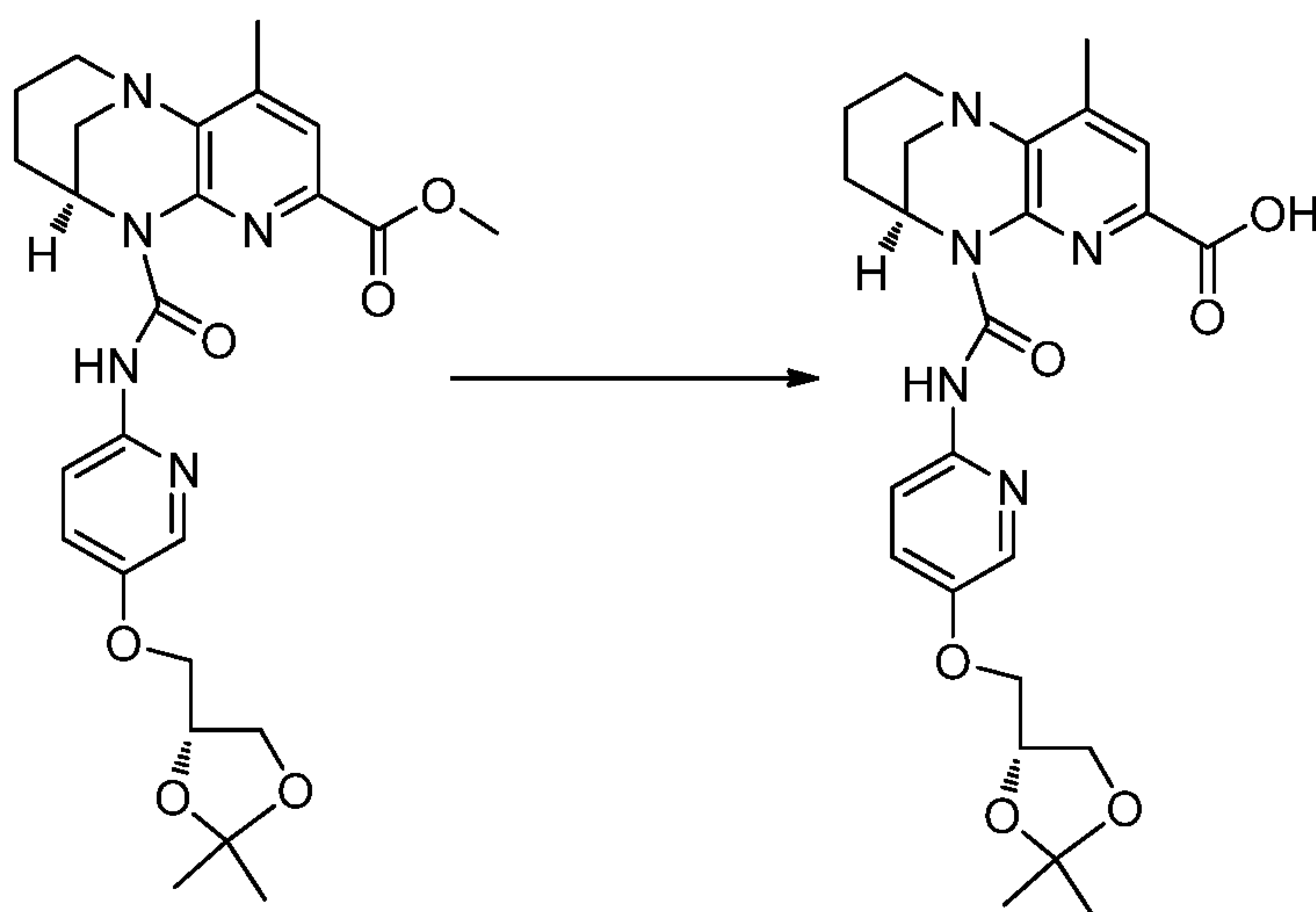
15 **Synthesis of (9S)-N10-(5-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-4-methyl-N2-((R)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide**



To a stirred solution of (9S)-10-((5-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-
 20 2-yl)carbamoyl)-4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-
 b][1,4]diazocine-2-carboxylic acid (400 mg, 0.827 mmol) in N,N-Dimethylformamide
 (DMF) (8 mL) were added DIPEA (0.722 mL, 4.14 mmol), HATU (472 mg, 1.241 mmol)
 and (R)-1,1,1-trifluoropropan-2-amine (140 mg, 1.241 mmol) at 0 °C. The reaction
 mixture was stirred at 27 °C for 3 hr. The reaction mixture was diluted with cold water

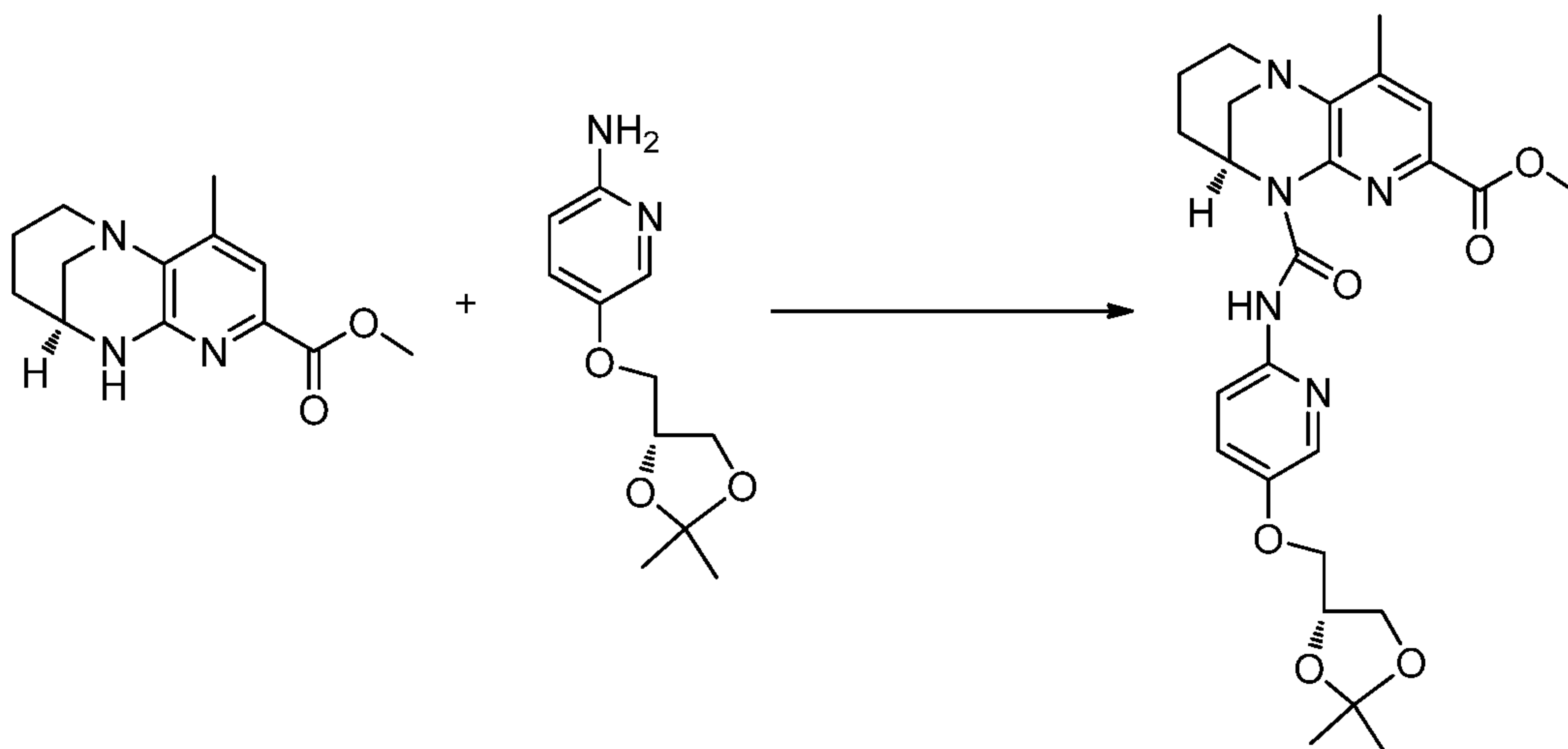
(100 mL) and stirred for 15 min. The precipitated solid was filtered through Buchner Funnel, washed with water and dried under reduced pressure to get crude compound. The crude compound was purified by column chromatography (neutral alumina, eluent: 65-70% ethyl acetate in hexane). Collected fractions were concentrated under reduce pressure to afford desired product (9S)-N10-(5-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-4-methyl-N2-((R)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide (350 mg, 0.580 mmol, 70.1 % yield) as a brown solid, LCMS (m/z): 579.22 $[M+H]^+$.

Synthesis of (9S)-10-((5-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)carbamoyl)-4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid



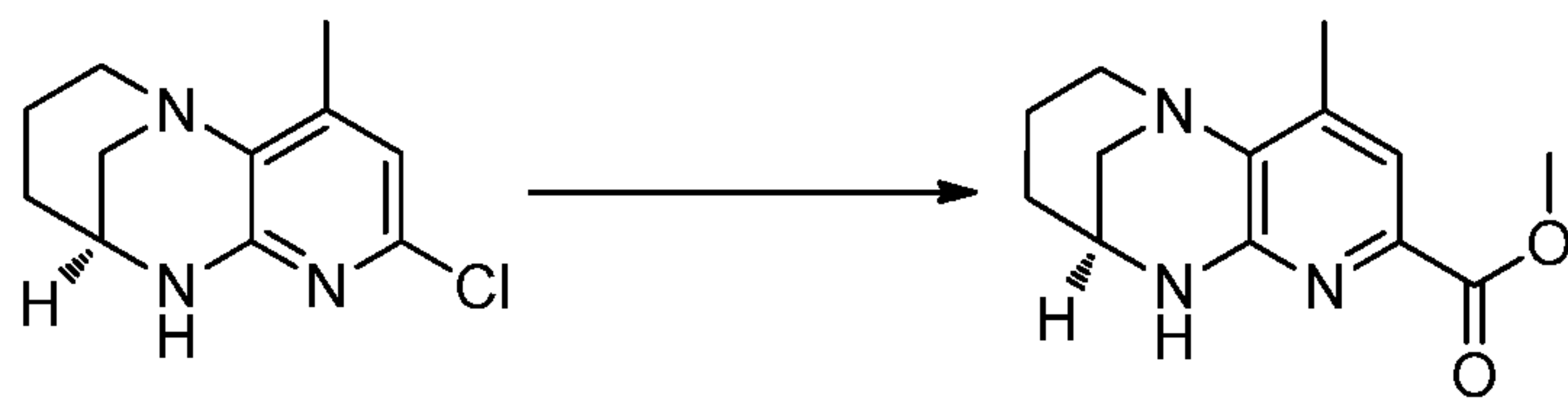
To a stirred solution of (9S)-methyl 10-((5-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)carbamoyl)-4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (450 mg, 0.904 mmol) in Tetrahydrofuran (THF) (20 mL) & Water (3.0 mL) was added LiOH.H₂O (21.66 mg, 0.904 mmol) at 0° C. The reaction mixture was stirred at 27 °C for 6 hr. The reaction mixture solvent was evaporated under reduced pressure completely and acidified with citric acid solution at 0 °C. The aqueous layer was extracted with dichloromethane (2x 50 mL). Organic layer was separated, dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid. The crude compound was triturated with diethyl ether and pentane, LCMS (m/z): 484.28 $[M+H]^+$.

Synthesis of (9S)-methyl 10-((5-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)carbamoyl)-4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate



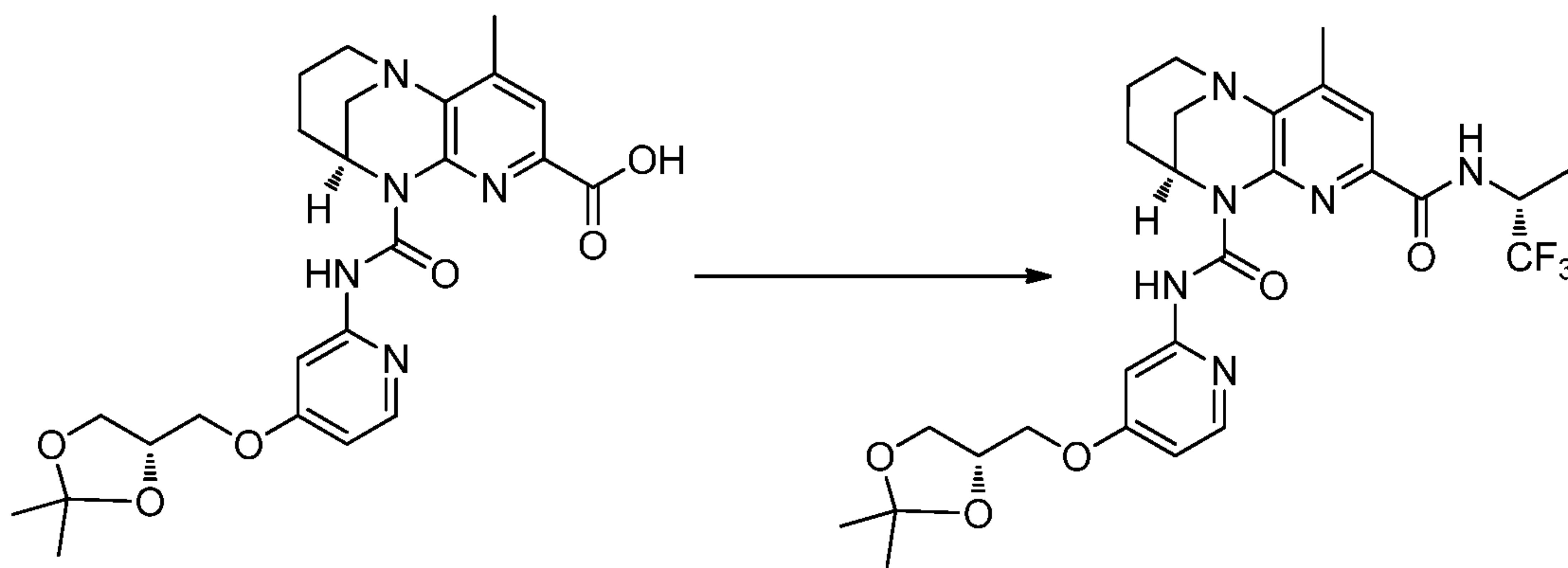
- 5 To a stirred solution of (9S)-methyl 4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (600 mg, 2.426 mmol) in Tetrahydrofuran (THF) (45 mL) were added TEA (2.029 mL, 14.56 mmol) and triphosgene (720 mg, 2.426 mmol) under nitrogen at 28°C. The reaction mixture was stirred at rt for 30 min, then (R)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-
- 10 amine (1088 mg, 4.85 mmol) was added to the reaction mixture. The reaction mixture was stirred 16 hr at 65 °C. The reaction mixture was cooled to room temp, solvent evaporated under reduced pressure completely and was partitioned between water (30 mL) and EtOAc (2 X 40 mL). Organic layer was separated, dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid. TLC eluent: 100% EtOAc/Hexane, R_f
- 15 :0.3, UV active. The crude compound was purified by Grace using C-18 reserval column, Mobile phase A: 0.1% Formic Acid in water; B: ACN, the product was eluted at 70-75% ACN/0.1% Formic Acid in water. The solvent was evaporated and was basified with saturated NaHCO₃. The aqueous layer was extracted with DCM. DCM layer was dried over anhydrous Na₂SO₄, filtered and evaporated to afford pure (9S)-methyl 10-((5-(((R)-
- 20 2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)carbamoyl)-4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (500 mg, 0.936 mmol, 38.6 % yield) as a off white solid, LCMS (*m/z*): 498.48 [M+H]⁺.

Synthesis of (9S)-methyl 4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate



To a stirred solution of (9S)-2-chloro-4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (6 g, 26.8 mmol) in Methanol (100 mL) was degassed for 30 min, then triethylamine (18.69 mL, 134 mmol) and PdCl₂(dppf)-CH₂Cl₂ adduct (1.095 g, 1.341 mmol) were added and filled with 300 psi CO gas. The reaction mixture was stirred at 115 °C for 16 h in steel bomb. The reaction was monitored by TLC. The reaction mixture was concentrated under reduced pressure to afford crude compound. The crude product was dissolved in DCM (50 mL), and was washed with water (10 mL). The DCM layer was dried over anhydrous Na₂SO₄, filtered, and filtrate was evaporated to afford (9S)-methyl 4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (7g, 23.76 mmol, 89 % yield) as a brown solid, LCMS (*m/z*): 248.11 [M+H]⁺.

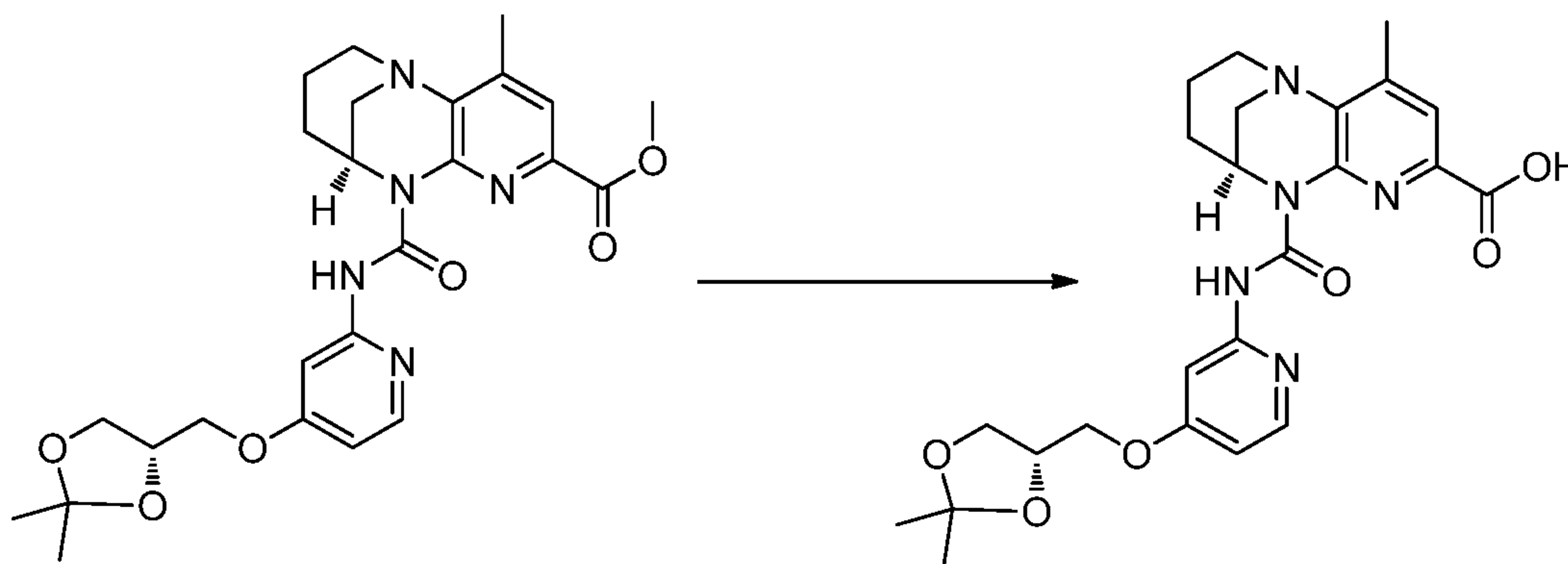
Synthesis of (9S)-N10-(4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-4-methyl-N2-((R)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide



To a stirred solution of (9S)-10-((4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)carbamoyl)-4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (450 mg, 0.931 mmol) in N,N-Dimethylformamide (DMF) (8 mL) were added DIPEA (0.813 mL, 4.65 mmol), HATU (531 mg, 1.396 mmol) and (R)-1,1,1-trifluoropropan-2-amine (158 mg, 1.396 mmol) at 0 °C. The reaction

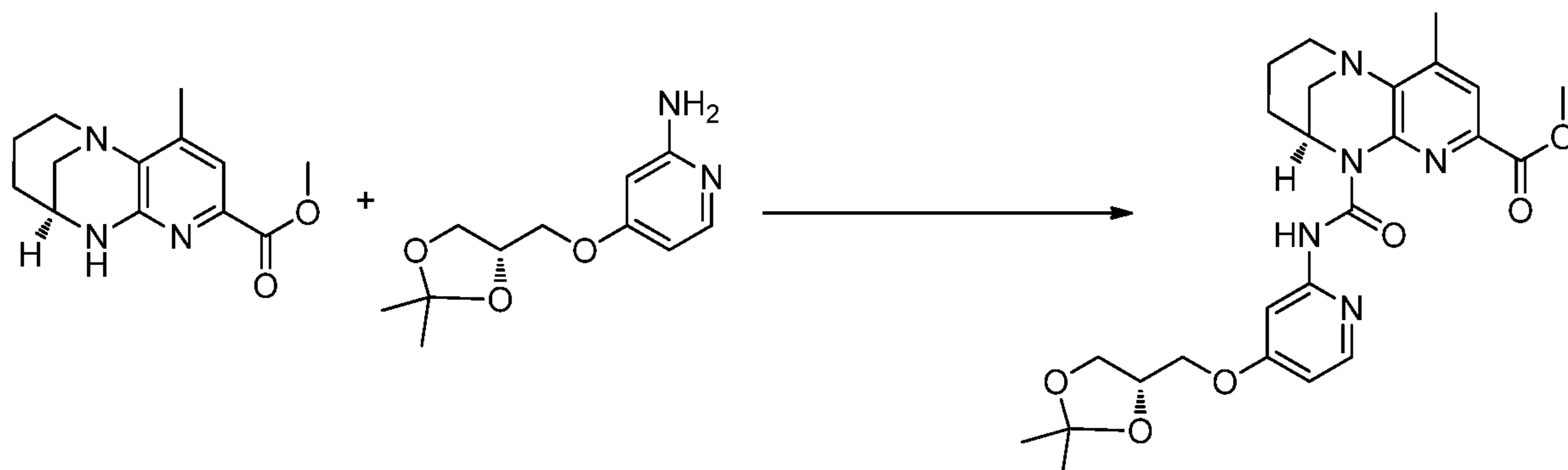
mixture was stirred at 27 °C for 3 hr. The reaction mixture was diluted with cold water (100 mL) and stirred for 15 min. The precipitated solid was filtered through Buchner Funnel, washed with water and dried under reduced pressure to get crude compound. The compound was triturated with ether and to afford (9S)-N10-(4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-4-methyl-N2-((R)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide (330 mg, 0.481 mmol, 51.7 % yield) as an off white solid, LCMS (m/z): 579.32 [M+H]⁺.

Synthesis of (9S)-10-((4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)carbamoyl)-4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid

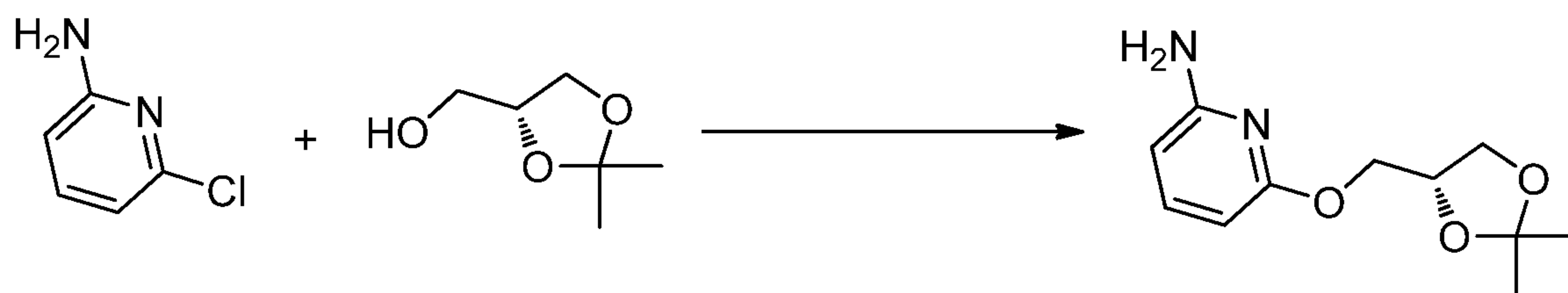


To a stirred solution of (9S)-methyl 10-((4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)carbamoyl)-4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (550 mg, 1.105 mmol) in Tetrahydrofuran (THF) (20.0 mL) & Water (5.00 mL) was added LiOH.H₂O (39.7 mg, 1.658 mmol) at 0° C. The reaction mixture was stirred at 27 °C for 6 hr. The reaction mixture solvent was evaporated under reduced pressure completely and acidified with citric acid solution at 0 °C. The aqueous layer was extracted with dichloromethane(2x 50 mL). Organic layer was separated, dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude. The crude compound was triturated with diethyl ether and pentane to get (9S)-10-((4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)carbamoyl)-4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (450 mg, 0.892 mmol, 81 % yield) as a off white solid, LCMS (m/z):484.30 [M+H]⁺.

Synthesis of (9S)-methyl 10-((4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)carbamoyl)-4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate

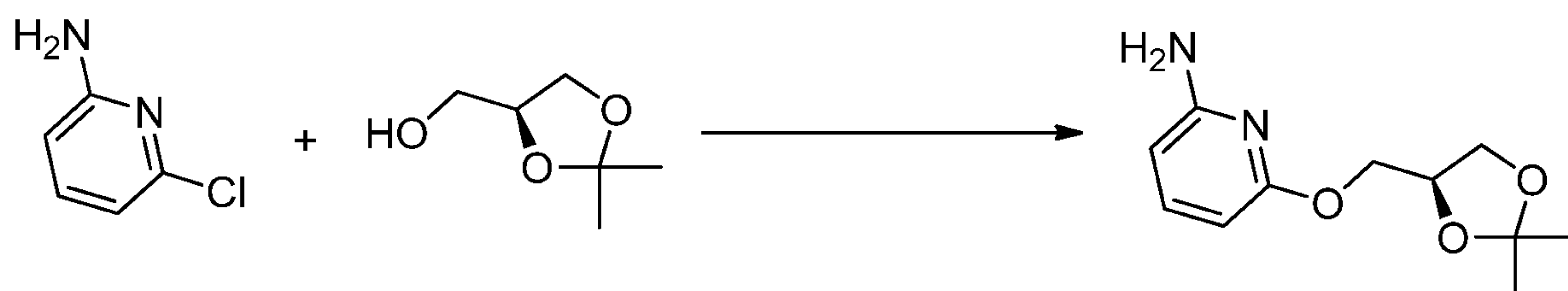


- 5 To a stirred solution of (9S)-methyl 4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (800 mg, 3.24 mmol) in Tetrahydrofuran (THF) (55 mL) were added TEA (2.71 mL, 19.41 mmol) and triphosgene (960 mg, 3.24 mmol) under nitrogen at 28°C. The reaction mixture was stirred at rt for 30 min, then (S)-4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-amine (2176 mg,
- 10 9.71 mmol) was added to the reaction mixture. The reaction mixture was stirred 16 hr at 65 °C. The reaction mixture was cooled to room temp, solvent evaporated under reduced pressure completely and was partitioned between water (30 mL) and EtOAc (2 X 40 mL). Organic layer was separated, dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid. TLC eluent: 100% EtOAc/Hexane, R_f:0.2, UV
- 15 active. The crude compound was purified by Grace using C-18 reserval column, Mobile phase A: 0.1% Formic Acid in water; B: ACN, the product was eluted at 65-70% ACN/0.1% Formic Acid in water . The solvent was evaporated and was basified with saturated NaHCO₃. The aqueous layer was extracted with DCM. DCM layer was dried over anhydrous Na₂SO₄, filtered and evaporated to afford pure (9S)-methyl 10-((4-(((S)-
- 20 2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)carbamoyl)-4-methyl-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylate (650mg, 1.125 mmol, 34.8 % yield) as a off white solid, LCMS (*m/z*): 498.48 [M+H]⁺.

Synthesis of (R)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-amine

To a stirred suspension of NaH (11.67 g, 292 mmol) in N-Methyl-2-pyrrolidone (NMP) (100 mL) under nitrogen at 0°C was added a solution of (R)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (25.7 g, 194 mmol) in N-Methyl-2-pyrrolidone (NMP) (100 mL) dropwise during 10 min at 0°C. After 10 min added a solution of 6-chloropyridin-2-amine (25 g, 194 mmol) in N-Methyl-2-pyrrolidone (NMP) (100 mL) dropwise during 10 min at 0°C. The reaction mixture was heated at 100 °C for 36 hr. TLC indicates small amount starting material along with product.

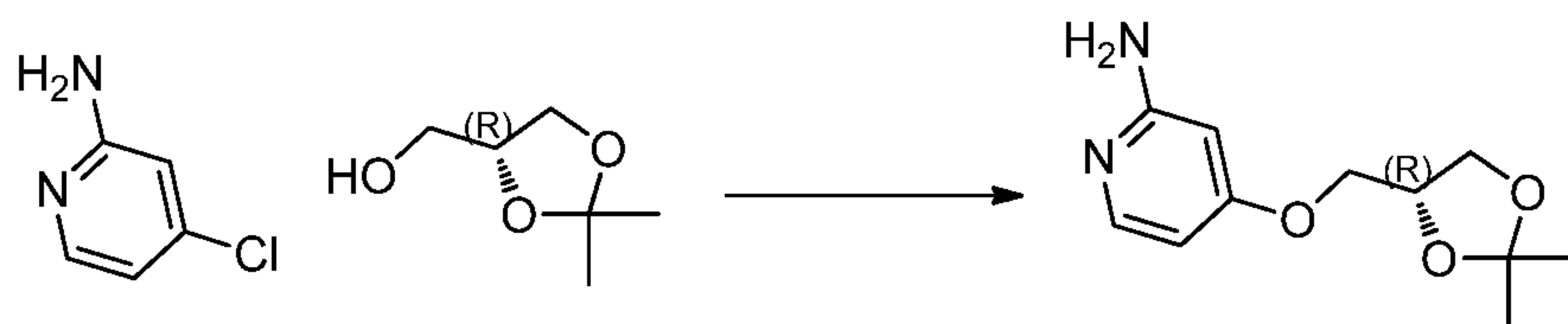
Reaction mixture was poured into ice cold water (600 mL), aqueous layer was extracted with EtOAc (2 x 500 mL). The organic layer was washed with water (3 x 300 mL) to remove excess NMP. The organic layer was dried over Na₂SO₄, filtered and concentrated under reduced pressure to obtain crude product. Crude product was purified by column chromatography using 100-200 silica gel as a eluent (12-15% EtOAc in petether) to obtain (R)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-amine (10 g, 44.6 mmol, 22.93 % yield) as a yellow thick liquid.

Synthesis of (S)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-amine

To a stirred suspension of NaH (62.2 g, 1556 mmol) in N-Methyl-2-pyrrolidone (NMP) (800 mL), under nitrogen at 0°C, was added a solution of (S)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (206 g, 1556 mmol) in N-Methyl-2-pyrrolidone (NMP) (300 mL) dropwise during 2 h. After stirring for another 10 min added a solution of 6-chloropyridin-2-amine (200 g, 1556 mmol) in N-Methyl-2-pyrrolidone (NMP) (300 mL) dropwise during 30 min at 0°C. The reaction mixture was stirred at 120 °C for 48 hr. TLC indicated that starting material was. Reaction mixture was poured into ice cold water (2000 mL), aqueous layer

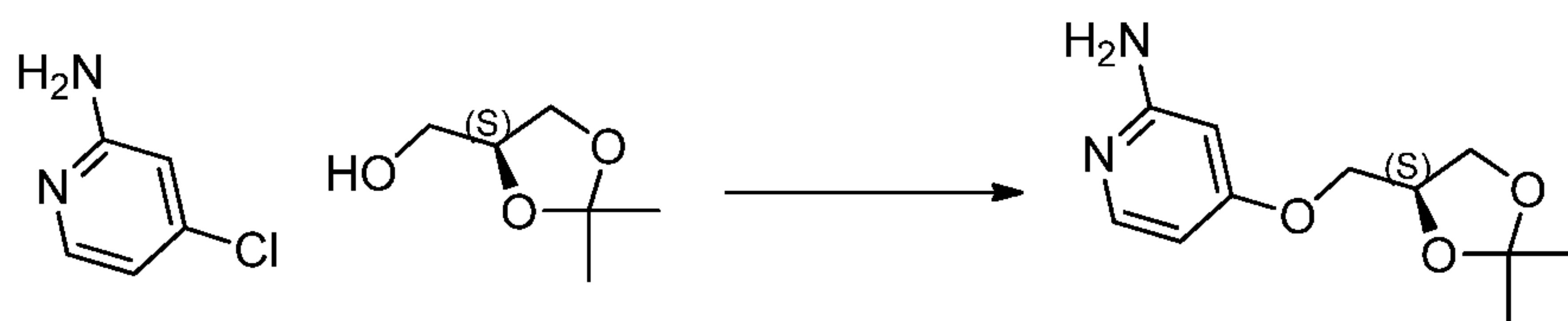
was extracted with EtOAc (3 x 1000 mL). The combined organic layer was washed with water (3 x 1000 mL) to remove excess NMP. The organic layer was dried over Na₂SO₄, filtered and concentrated under reduced pressure to obtain crude product. Crude product was purified by column chromatography using 100-200 silica gel (eluent 12-15% EtOAc in pet ether) to obtain the desired pure product (S)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-amine (75 g, 325 mmol, 20.92 % yield) as a yellow viscous liquid. LCMS (*m/z*): 225 [M+H]⁺.

Synthesis of (R)-4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-amine



To a suspension of (R)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (3.000 g, 22.70 mmol), 4-chloropyridin-2-amine (1.459 g, 11.35 mmol) and sodium (0.522 g, 22.70 mmol) in a sealed tube. The reaction mixture was stirred at 140 °C for 16h. Next, the reaction mixture was cooled to room temperature, dissolved in MeOH and poured in to ice water and extracted with EtOAc. The organic phase was washed with brine solution and dried over sodium sulfate, filtered and evaporated to get crude compound. The crude compound was purified by column chromatography using silica gel and eluted with 2-3% MeOH/DCM to get pure compound (1.1g, 21%), LCMS (*m/z*) 225.2 [M+H]⁺.

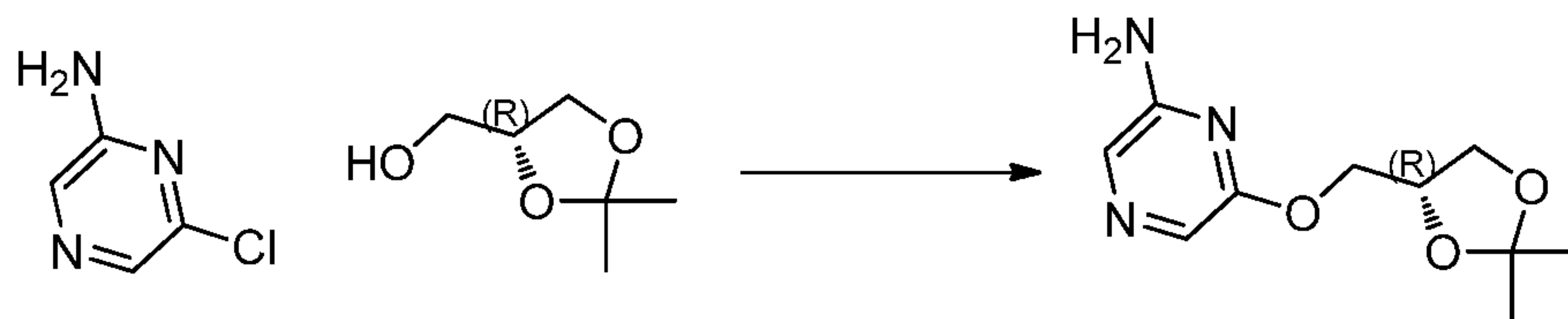
Synthesis of (S)-4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-amine



To a suspension of (S)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (3.000 g, 22.70 mmol), 4-chloropyridin-2-amine (1.459 g, 11.35 mmol) and sodium (0.522 g, 22.70 mmol) in a sealed tube. The reaction mixture was stirred at 140 °C for 16h before being cooled to room temperature, dissolved in MeOH and poured in to ice water and extracted with EtOAc. The organic phase was washed with brine solution and dried over sodium sulfate, filtered and evaporated. The crude material was purified by silica gel column

chromatography eluting with 2-3% MeOH/DCM to give the desired product (1.2g, 22%), LCMS (m/z) 225.2 $[M+H]^+$.

Synthesis of (R)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-amine



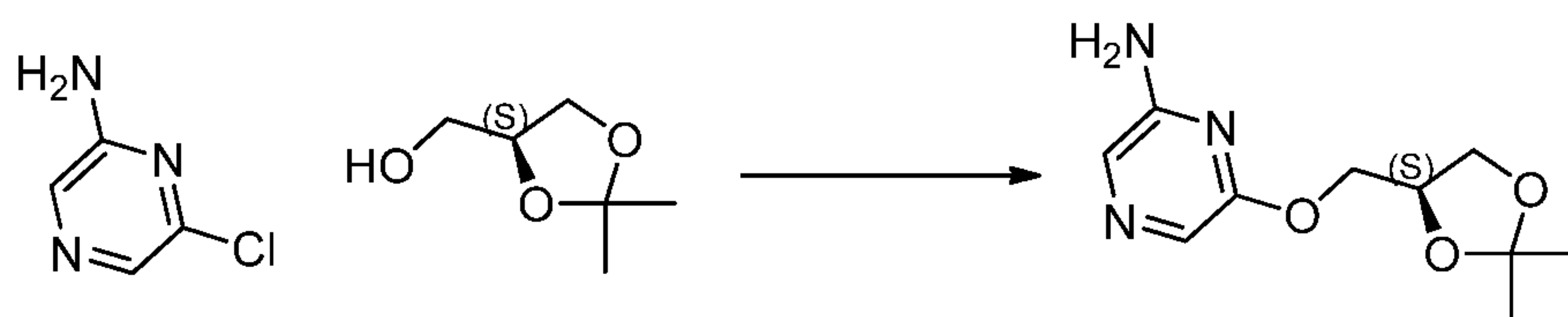
5

To a solution of 6-chloropyrazin-2-amine (5 g, 38.6 mmol), sodium hydride (2.316 g, 57.9 mmol) and (R)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (5.61 g, 42.5 mmol) in Tetrahydrofuran (THF) (50 mL) stirred under nitrogen at 0°C was added reaction mixture was stirred at 80 °C for 16 h. Reaction mixture was quenched with ice cold water and extracted into ethyl acetate. Organic layer dried over Na₂SO₄. Solvent evaporated under reduced pressure to afford the crude product. The crude product was added to a silica gel column and was eluted with DCM/MeOH. Fractions with product were combined and evaporated under reduced pressure to give the required product (2.8g, 11.9 mmol, 31%), LCMS (m/z) 225.9 $[M+H]^+$.

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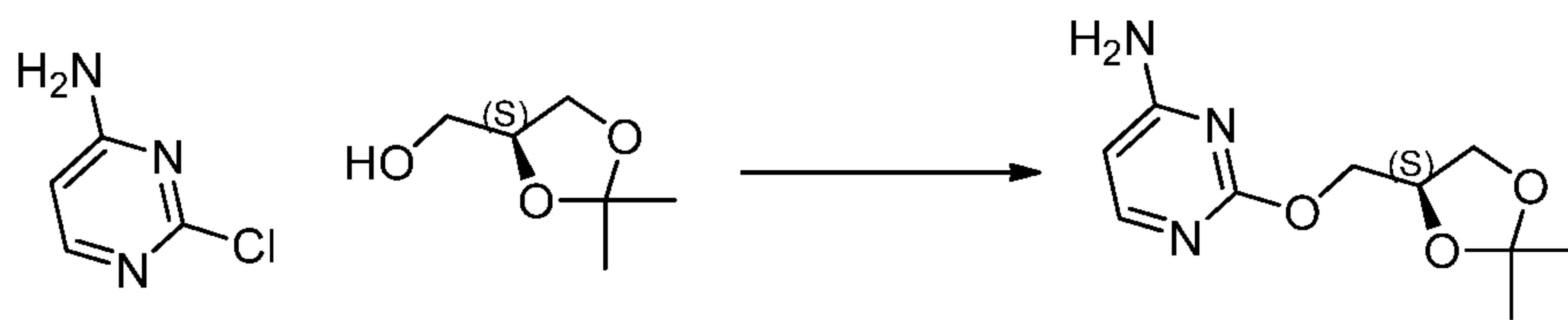
Synthesis of (S)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-amine



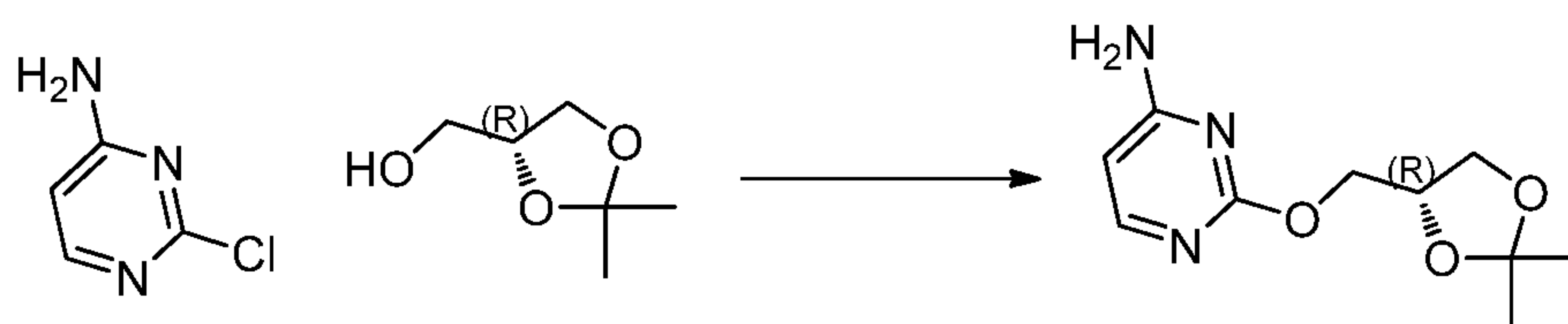
6-chloropyrazin-2-amine (0.980 g, 7.57 mmol), (S)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (2 g, 15.13 mmol) and sodium (0.348 g, 15.13 mmol) were taken in a seal tube and heated at 130 °C for 16 hr and then the reaction mixture was quenched with methanol and ice cold water (100 mL) and extracted with ethyl acetate (5 x 50 mL). The combined organic layers were washed with water, saturated brine solution, dried over anhydrous sodium sulfate, filtered and concentrated to give the product (1 g, 4.26 mmol, 28.2 % yield), LCMS (m/z) 265.1 $[M+H]^+$.

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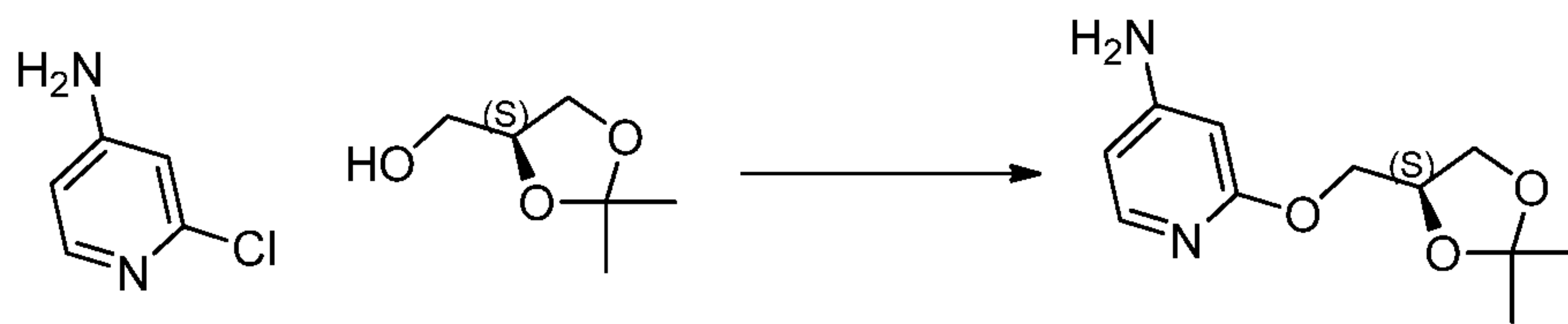
25

Synthesis of (S)-2-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-amine

To suspension of (S)-2,2-dimethyl-1,3-dioxolan-4-ylmethanol (10.20 g, 77 mmol), and NaH (4.63 g, 116 mmol) in tetrahydrofuran (THF) (50 mL) stirred under nitrogen at room temperature was added 2-chloropyrimidin-4-amine (5 g, 38.6 mmol) portion wise over 15 min. The reaction mixture was stirred at 70 °C for 16 hr. Next, the reaction mixture was quenched with solution of aq. NaHCO₃ and then extracted with EtOAc, dried Na₂SO₄ and evaporated. The crude product was added to a silica gel column and was eluted with 50% Hex/EtOAc. Collected fractions were evaporated to give the desired product (3 g, 11.84 mmol, 30.7 % yield) as off white solid, LCMS (*m/z*) 226.2 [M+H]⁺.

Synthesis of (R)-2-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-amine

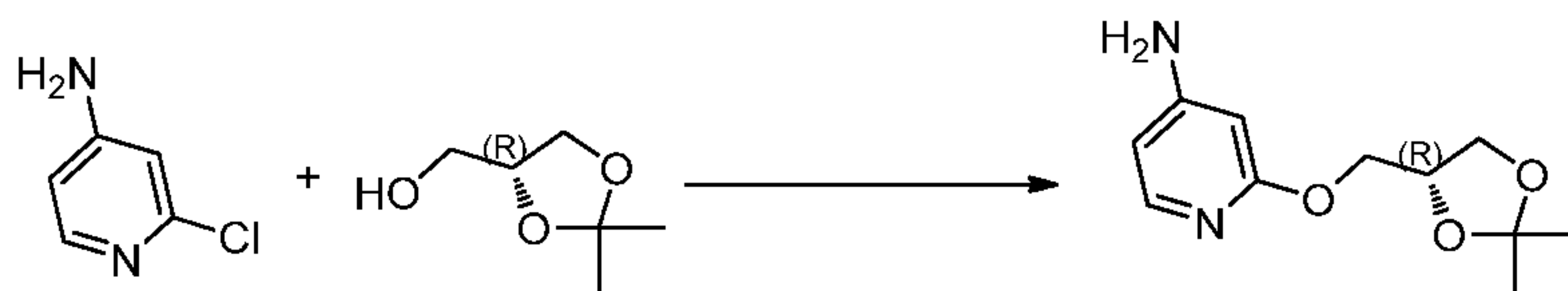
To a solution of sodium hydride (0.817 g, 34.1 mmol) in Tetrahydrofuran (THF) (30 mL) at room temperature was added a solution of (R)-2,2-dimethyl-1,3-dioxolan-4-ylmethanol (3 g, 22.70 mmol) in THF (5 mL) over 1 min and stirred at room temperature for 15 min then add 2-chloropyrimidin-4-amine (2.059 g, 15.89 mmol) portion wise at room temperature. The reaction mixture was stirred at 65 °C for 16h. The reaction mixture was poured in to water and extracted with EtOAc (3 X 100mL). Then the combined organic layer was washed with water, brine solution, dried over sodium sulfate and evaporated to get 4.0 g of crude compound. The crude compound was purified by column chromatography using 100-200 silica gel mesh and eluted with 2-3% MeOH/DCM to get pure compound (2.5g, 10.42 mmol, 46%), LCMS (*m/z*) 226.2 [M+H]⁺.

Synthesis of (S)-2-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-4-amine

To a suspension of 2-chloropyridin-4-amine (1.459 g, 11.35 mmol), (S)-2-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)methanol (3.0 g, 22.70 mmol) was added sodium (0.522 g, 22.70 mmol).

- 5 The reaction mixture was stirred at 140 °C for 16 hr and progress of the reaction was monitored by

The reaction mixture was dissolved in MeOH, poured in to ice water and extracted with EtOAc (3 X 100mL). Then the combined organic layer was washed with water, brine solution, dried over sodium sulfate and evaporated to get 4.0 g of crude compound. The
 10 crude compound was purified by column chromatography using 100-200 silica gel mesh and eluted with 2-3% MeOH/DCM to get (S)-2-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-4-amine (2.5 g, 10.73 mmol, 47.3 % yield), LCMS (m/z) 225.3 $[M+H]^+$.

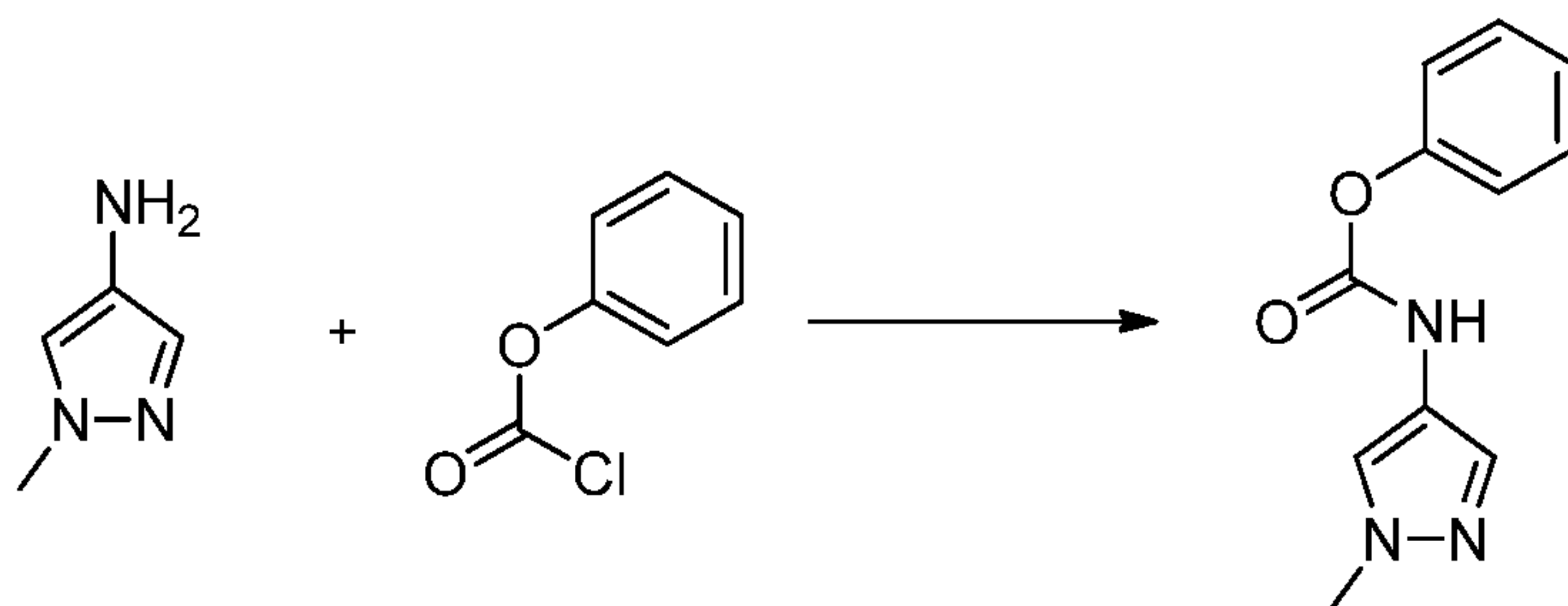
15 Synthesis of (R)-2-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-4-amine

To a solution of 2-chloropyridin-4-amine (4 g, 31.1 mmol), (R)-2-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)methanol (2.056 g, 15.56 mmol) and sodium (0.715 g, 31.1 mmol) in sealed tube at room temperature. The reaction mixture was stirred at 140 °C for 48 hr. The

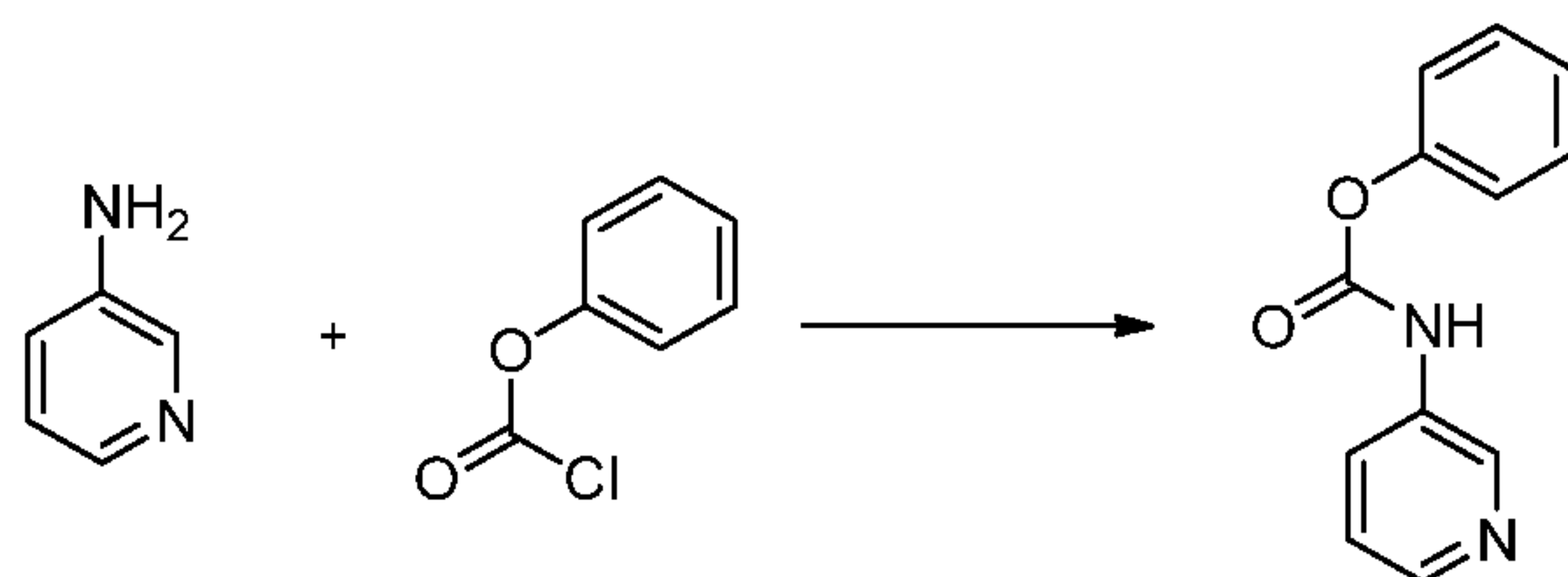
- 20 reaction mixture was cooled to room temp and quenched with MeOH followed by water.

Then reaction mass was extracted with the EtOAc. Then organic layer washed with water followed by brine solution and dried out with sodium sulfate and filtered and distill out completely. The crude product was added to a silica gel column and was eluted with

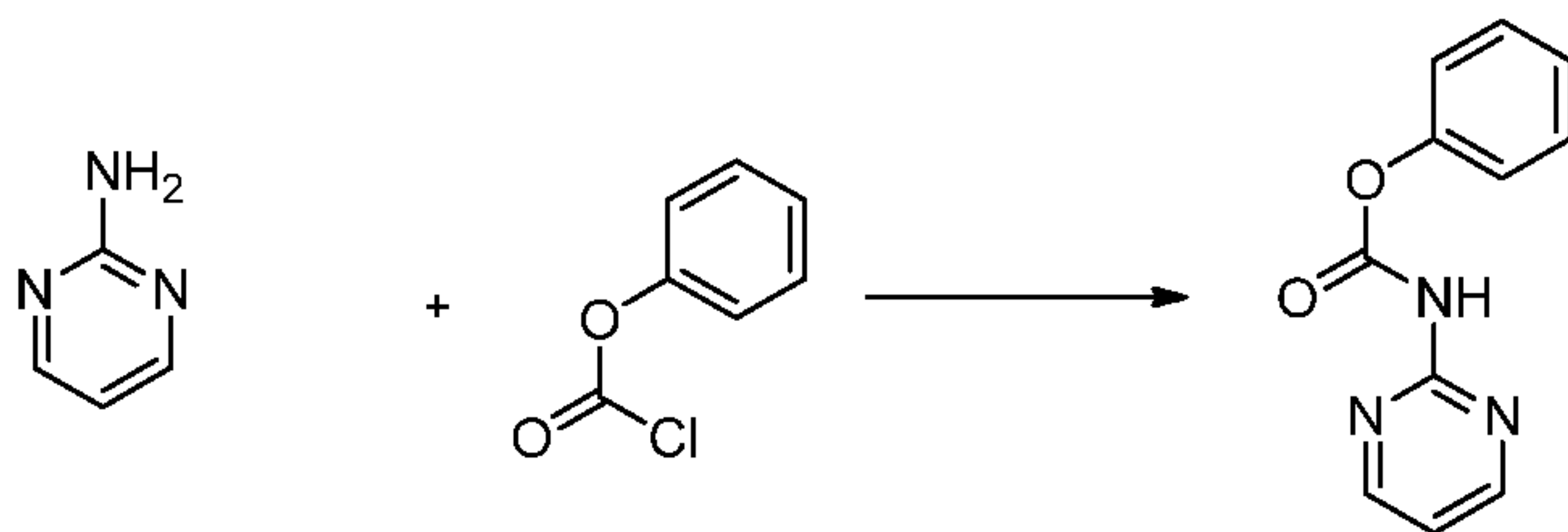
- 25 9.93 mmol, 31.9 % yield), LCMS (m/z) 225.0 $[M+H]^+$.

Synthesis of phenyl (1-methyl-1H-pyrazol-4-yl)carbamate

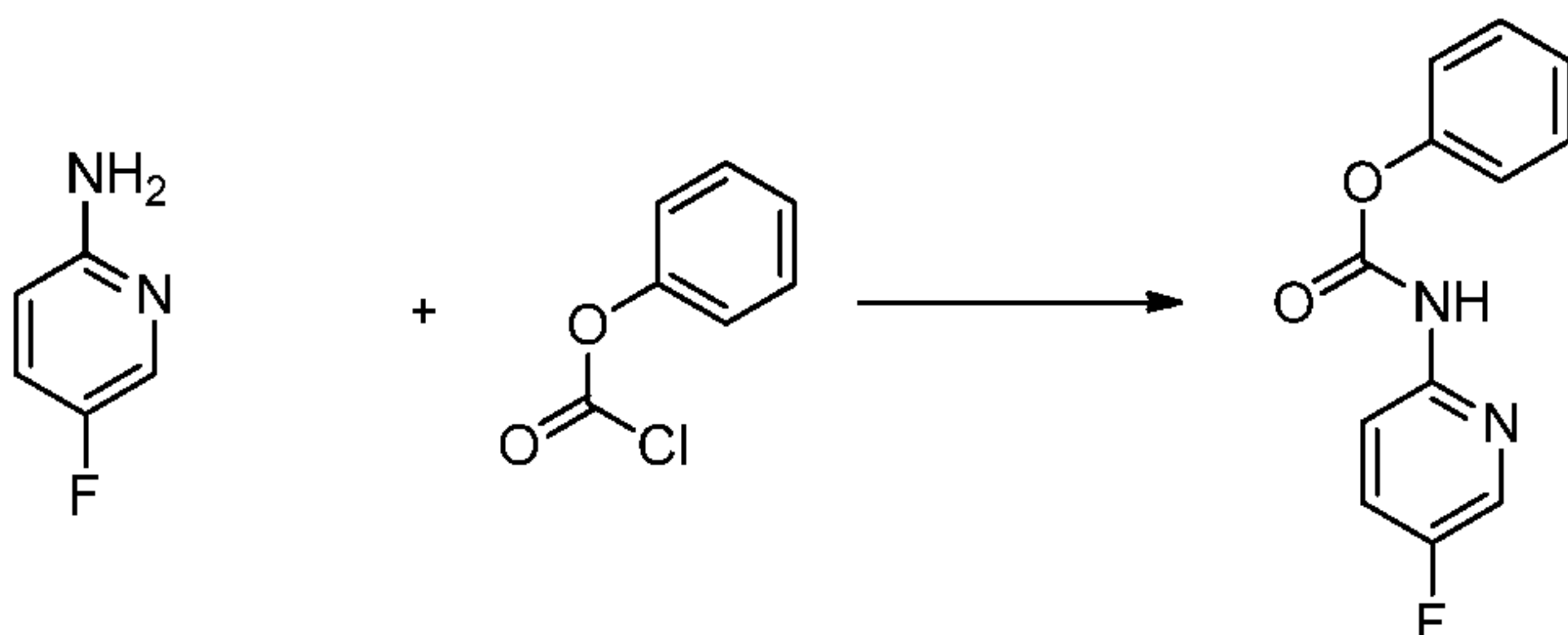
Phenyl carbonochloridate (2.90 g, 18.53 mmol) was added to a stirred solution of pyridine (3.12 mL, 38.6 mmol) in Dichloromethane (DCM) (50 mL) at 0 °C and stirred for 15min
 5 and followed by addition of 1-methyl-1H-pyrazol-4-amine (1.5g, 15.45 mmol) at same temperature. The reaction mixture was stirred at room temperature for 4h. After consumption of starting material (monitored by TLC), ice cold water was added, separated organic layer was washed with water and brine. The organic layer was filtered through sodium sulfate and concentrated to get crude compound. The crude compound was
 10 purified by column chromatography by using 60-120(silica gel) and eluted in 50% ethyl acetate in hexane to afford the desired product (1.6g, 6.41 mmol, 42 % yield) as light brown solid, LCMS (m/z) 218.1 ($M+H$)⁺.

Synthesis of phenyl pyridin-3-ylcarbamate

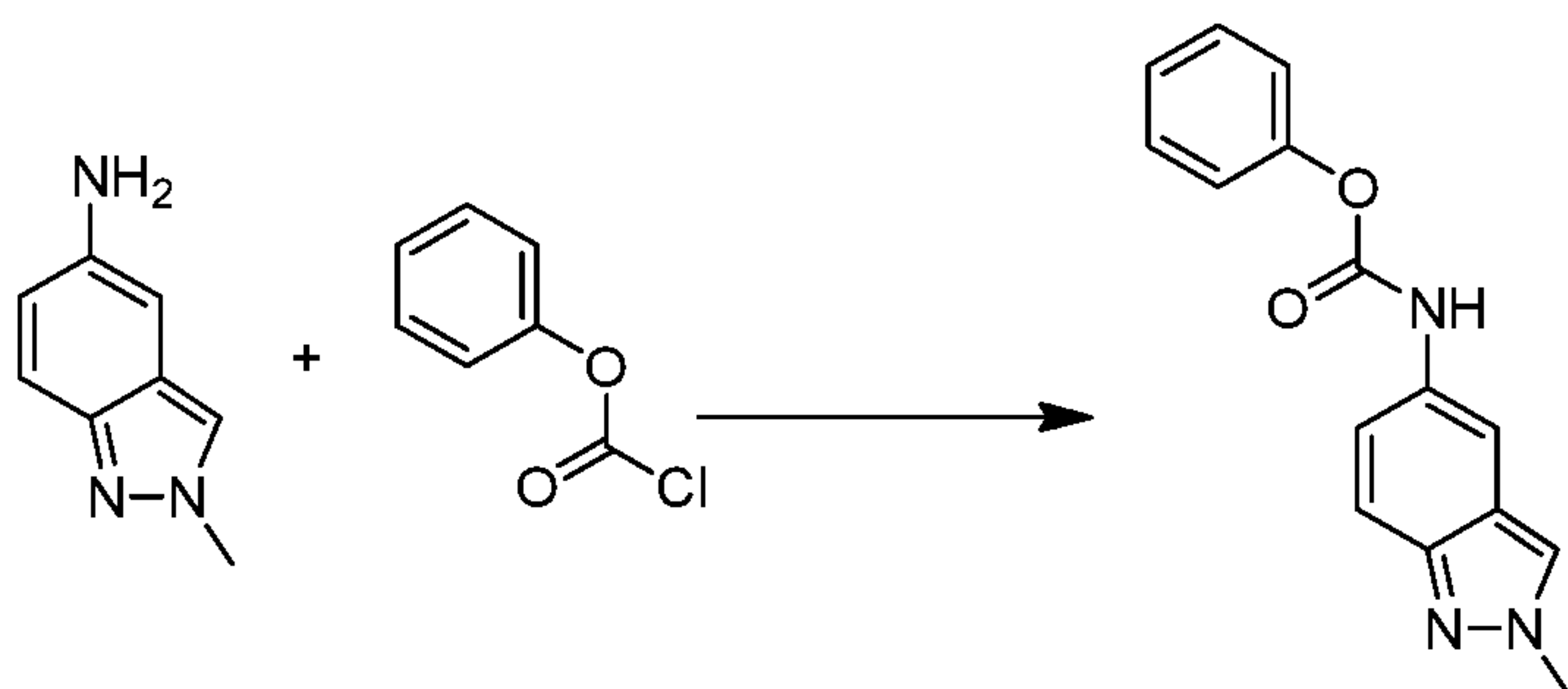
15 To a solution of phenyl carbonochloridate (2.163 g, 13.81 mmol), and pyridine (1.375 mL, 17.00 mmol) in Dichloromethane (DCM) (30 mL) stirred under nitrogen at room temp was added pyridin-3-amine (1.0 g, 10.63 mmol). The reaction mixture was stirred at RT for 30 min. The reaction mixture was quenched with saturated sodium bicarbonate solution. Separated organic layer and the aqueous layer extracted with DCM (50 ml). Combined
 20 DCM layer washed with water and dried out with sodium sulfate, filtered and concentrated under high vacuum to get crude product. The Crude product was added to a silica gel column and was eluted with 20% EtOAc/Hexane. Collected fractions were evaporated to afford the desired product (1.3 g, 6.01 mmol, 57%) as a white solid, LCMS (m/z) 215.1 ($M+H$)⁺.

Synthesis of phenyl pyrimidin-2-ylcarbamate

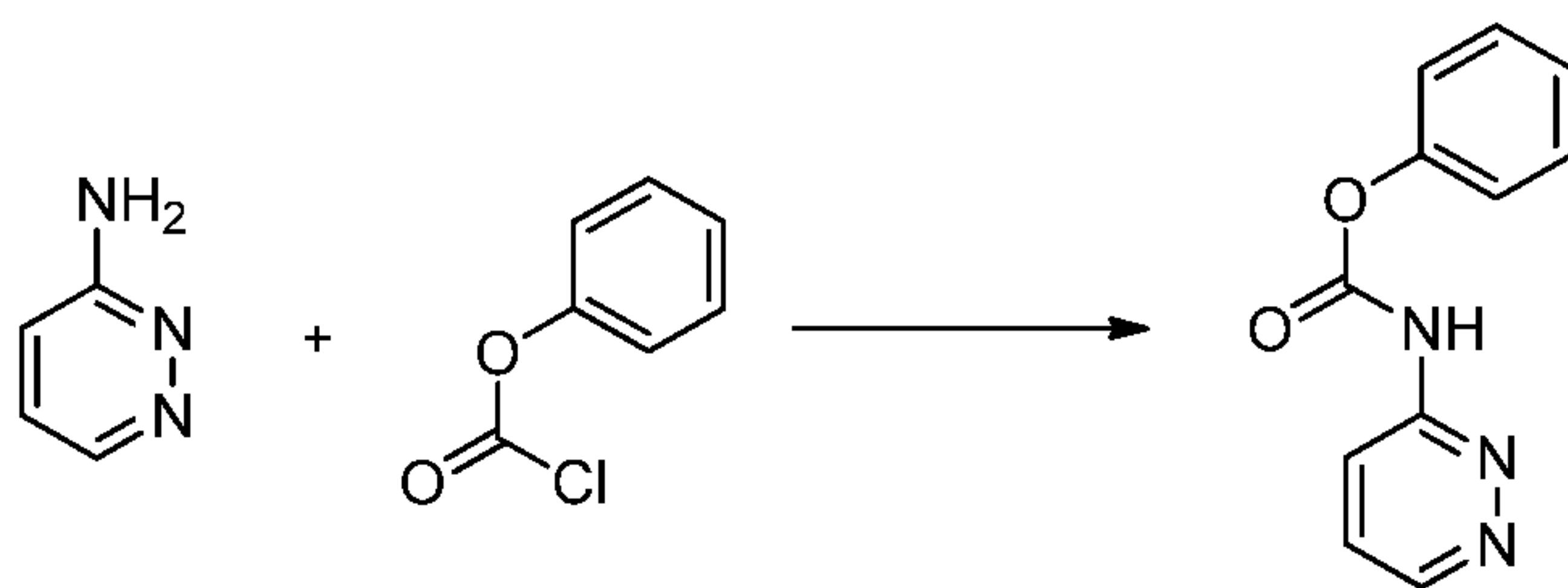
To a solution of phenyl carbonochloridate (2.140 g, 13.67 mmol), and pyridine (1.361 mL, 16.82 mmol) in dichloromethane (DCM) (10 mL) stirred under nitrogen at room temperature was added pyrimidin-2-amine (1.0 g, 10.51 mmol). The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was quenched with saturated sodium bicarbonate solution. Separated organic layer and the aqueous layer extracted with DCM (50 ml). Combined DCM layer washed with water and dried out with sodium sulfate, filtered and concentrated under high vacuum to get crude product. This was added to a silica gel column and was eluted with 20% EtOAc/Hexane. Collected fractions were evaporated to afford the desired product (1.6 g, 6.49 mmol, 61.7 %), LCMS (m/z) 216.3 ($M+H$)⁺.

Synthesis of phenyl (5-fluoropyridin-2-yl)carbamate

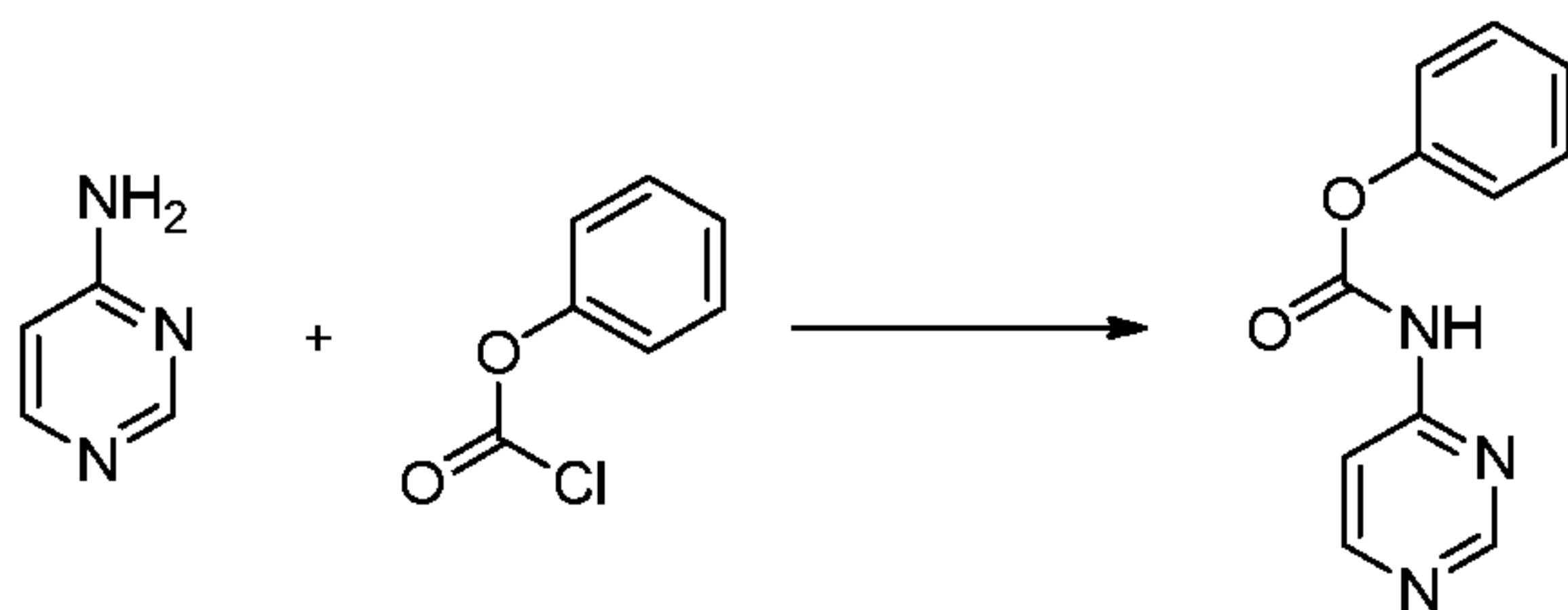
To a solution of phenyl carbonochloridate (1.397 g, 8.92 mmol), and Pyridine (0.721 mL, 8.92 mmol) in dichloromethane (DCM) (40 mL) stirred under nitrogen at room temp was added 5-fluoropyridin-2-amine (1.0 g, 8.92 mmol). The reaction mixture was stirred at RT for 30 min. The reaction mixture was quenched with saturated sodium bicarbonate solution. Separated organic layer and the aqueous layer extracted with DCM (20 ml). Combined organic layer washed with water followed by brine solution and dried out with sodium sulfate, filtered and concentrated under vacuum to give the desired product (1.4 g, 5.94 mmol, 67%), LCMS (m/z) 233.2 ($M+H$)⁺.

Synthesis of phenyl (2-methyl-2H-indazol-5-yl)carbamate

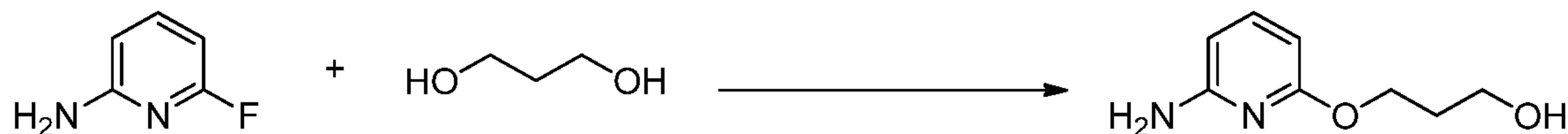
To a solution of phenyl carbonochloridate (1.064 g, 6.79 mmol), and pyridine (0.550 mL, 6.79 mmol) in Dichloromethane (DCM) (40 mL) stirred under nitrogen at room temp was
 5 added 2-methyl-2H-indazol-5-amine (1 g, 6.79 mmol). The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was quenched with saturated sodium bicarbonate solution. Separated organic layer, aqueous layer extracted with DCM (20 ml). Combined organic layer washed with water followed by brine solution and dried out with sodium sulfate and concentrated under vacuum to get phenyl (2-methyl-2H-indazol-5-
 10 yl)carbamate (1.3 g, 4.82 mmol, 70.9 % yield), LCMS (m/z) 268.1 ($M+H$)⁺.

Synthesis of phenyl pyridazin-3-ylcarbamate

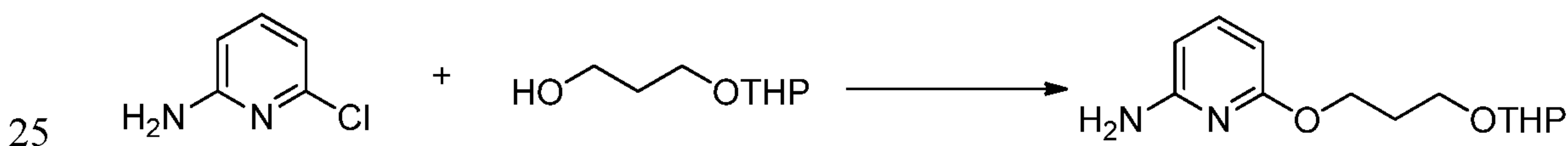
To a solution of phenyl carbonochloridate (1.070 g, 6.83 mmol), pyridine (0.665 g, 8.41
 15 mmol) in dichloromethane (10 ml) stirred under nitrogen at 25°C was added a suspension of pyridazin-3-amine (0.5 g, 5.26 mmol) in dichloromethane (5ml) during 5 min. The reaction mixture was stirred at 25 °C for 1 hr. Next, the organic phase was washed with water 3 mL, saturated brine 3 mL, dried over sodium sulfate and concentrated in vacuo to give the crude product as a white solid. The compound was washed with hexane, dried
 20 under reduced pressure, LCMS (m/z) 216.2 ($M+H$)⁺.

Synthesis of phenyl pyrimidin-4-ylcarbamate

To a solution of phenyl carbonochloridate (1.070 g, 6.83 mmol), pyridine (0.665 g, 8.41 mmol) in DCM (15 ml) stirred under nitrogen at 25°C was added a suspension of
 5 pyrimidin-4-amine (0.5 g, 5.26 mmol) in DCM (5 ml) dropwise during 5 min. The reaction mixture was stirred at 25 °C for 1 hr. The organic phase was washed with water 3 mL, brine 3 mL, dried over sodium sulfate and concentrated under vacuo to give the crude product as a off-white solid. The crude compound was washed with Hexane and then dried under reduced pressure to give the desired product (500 mg, 1.95 mmol, 37%),
 10 LCMS (m/z) 215.9 ($M+H$)⁺.

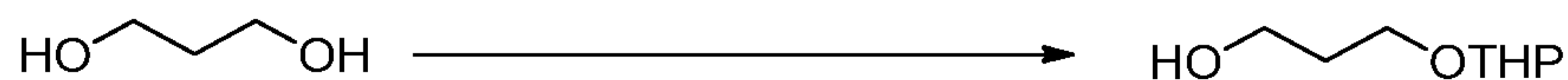
Synthesis of 3-((6-aminopyridin-2-yl)oxy)propan-1-ol

propane-1,3-diol (1.358 g, 17.84 mmol) was added to a stirred solution of NaH (1.070 g, 44.6 mmol) in N-Methyl-2-pyrrolidone (NMP) (5 mL) at 0° C and stirred for 1h and
 15 followed by addition of 6-fluoropyridin-2-amine (1.0 g, 8.92 mmol) and stirred for 2h at 80 °C. Reaction mass was cooled to room temperature, slowly added to ice cold water and diluted with ethyl acetate. The separated organic layer was washed with water and brine. The organic layer was dried over Na₂SO₄, filtered and concentrated to obtain crude
 20 compound. The crude compound was purified by using 100-200 silica gel and eluted in 100% ethyl acetate to afford 3-((6-aminopyridin-2-yl)oxy)propan-1-ol (0.4g, 1.760 mmol, 19.73 % yield) as brown viscous, LCMS (m/z): 169.22 [$M+H$]⁺.

Synthesis of 6-(3-((tetrahydro-2H-pyran-2-yl)oxy)propoxy)pyridin-2-amine

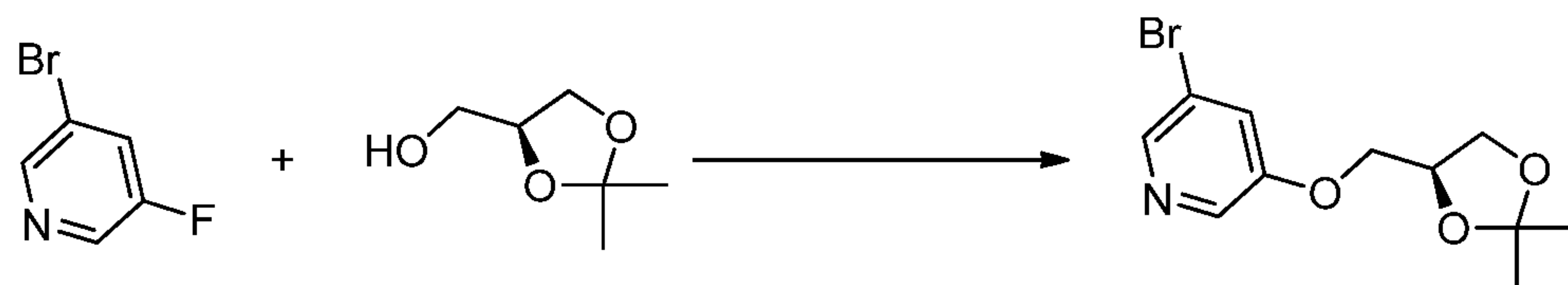
3-((tetrahydro-2H-pyran-2-yl)oxy)propan-1-ol (4.2 g, 26.2 mmol) in 1,4-Dioxane (20 mL) was added to a solution of NaH (1.307 g, 32.7 mmol) in 1,4-Dioxane (20 mL) at 0 °C, and the reaction mixture was stirred for 30 min at 28 °C. 6-chloropyridin-2-amine (2.8g, 21.78 mmol) in 1,4-Dioxane (20 mL) was added to the reaction mixture at 0 °C, and the reaction mixture was stirred for 10 hr at 100 °C. The reaction mixture was partitioned between water (20 mL) and DCM (2 X 25 mL). DCM layer was washed with saturated NaHCO₃ solution, was separated and dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to crude 6-(3-((tetrahydro-2H-pyran-2-yl)oxy)propoxy)pyridin-2-amine (5.5 g, 18.12 mmol, 83 % yield) as brown oil, LCMS (*m/z*): 253.2 [M+H]⁺.

Synthesis of 3-((tetrahydro-2H-pyran-2-yl)oxy)propan-1-ol



p-toluenesulfonic acid monohydrate (0.678 g, 3.57 mmol) was added to a stirred solution of propane-1,3-diol (5.43 g, 71.3 mmol), and 3,4-dihydro-2H-pyran (3g, 35.7 mmol) in Dichloromethane (DCM) (50 mL) at 0 °C. The reaction mixture was stirred for 2h at 28 °C. The reaction mixture was partitioned between water (20 mL) and DCM (2 X 25 mL). DCM layer was washed with saturated NaHCO₃ solution, was separated and dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to crude 3-((tetrahydro-2H-pyran-2-yl)oxy)propan-1-ol (4.2g, 26.2 mmol, 73.5 % yield) as colorless oil.

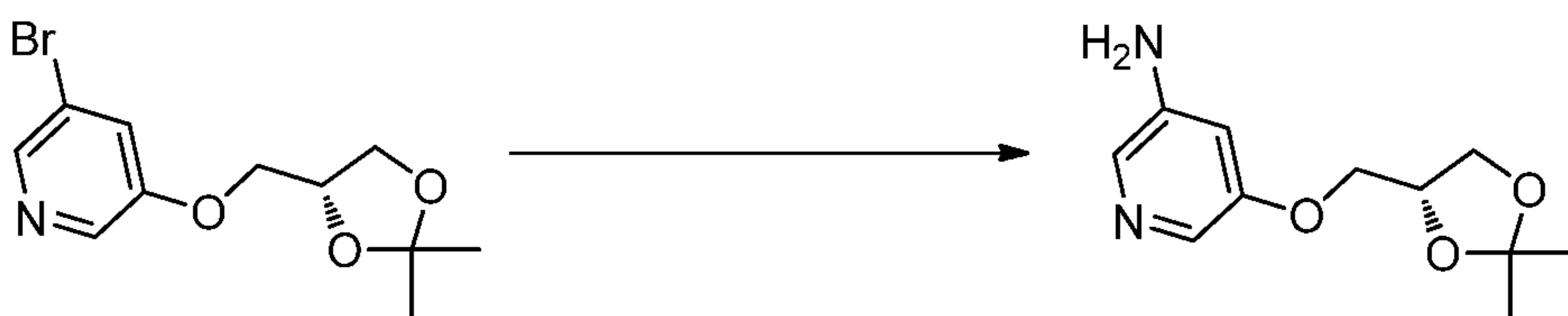
Synthesis of (S)-3-bromo-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridine



Cesium carbonate (37.0 g, 114 mmol) was taken into multi-neck RB. Then flask was cooled to 0 °C and N-Methyl-2-pyrrolidone (NMP) (100 mL) was added slowly over a period of 3 minutes. The resulting reaction mixture was stirred under nitrogen for 15 min. Then (S)-2,2-dimethyl-1,3-dioxolan-4-ylmethanol (10 g, 76 mmol) was added dropwise over a period of 5 min at 0 °C. This suspension was stirred at room temperature °C for 1 h. Suspension became pale yellow solution after added 3-bromo-5-fluoropyridine (7.62 mL, 73.9 mmol). The resulting solution was stirred at 75 °C for 24 hr. Reaction progress was

monitored by TLC 40% EtOAc in Hexane. TLC indicated consumption of SM and formation of new spot after 24 h. The reaction mass was cooled to room temperature, diluted with water (500 mL). The aqueous layer was extracted with ethyl acetate (2X300 mL). The organic layer was washed with brine (250 mL), dried over Na₂SO₄ filtered, concentrated under reduced pressure to afford brown oil. The crude product was purified by column chromatography over 100-200 mesh size silica gel. Column was eluted with a gradient of EtOAc/Hexane. Desired compound was eluted with 20% EtOAc in Hexane. Compound fractions containing pure compound were concentrated under reduced pressure to afford (S)-3-bromo-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridine (10 g, 34.0 mmol, 44.9 % yield) as pale yellow viscous oil, LCMS (*m/z*): 289.99 [M+H]⁺.

Synthesis of (R)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-amine



(R)-3-bromo-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridine (50g, 174 mmol), liquor ammonia (25 mL, 1155 mmol) were taken in a sealed tube. Then added copper(II) sulfate (5.54 g, 34.7 mmol) at 0 °C. The resulting blue solution was heated to 120 °C for 2 hr. The reaction progress was monitored by TLC 10% MeOH in DCM, TLC indicated formation of new spot and consumption of SM after 24 h. After completion, The reaction mass was cooled to room temperature. The reaction mass was brought to pH 10 with 20% NaOH, saturated with NaCl, extracted with ethyl acetate (30 mL* 2). The combined organic layer was washed with brine (20 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to afford crude brown solid, which was triturated with diethyl ether and stirred for 4 hours then filtered to afford (R)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-amine (35.4 g, 146 mmol, 84 % yield) as pale brown solid, LCMS (*m/z*): 225.29 [M+H]⁺.

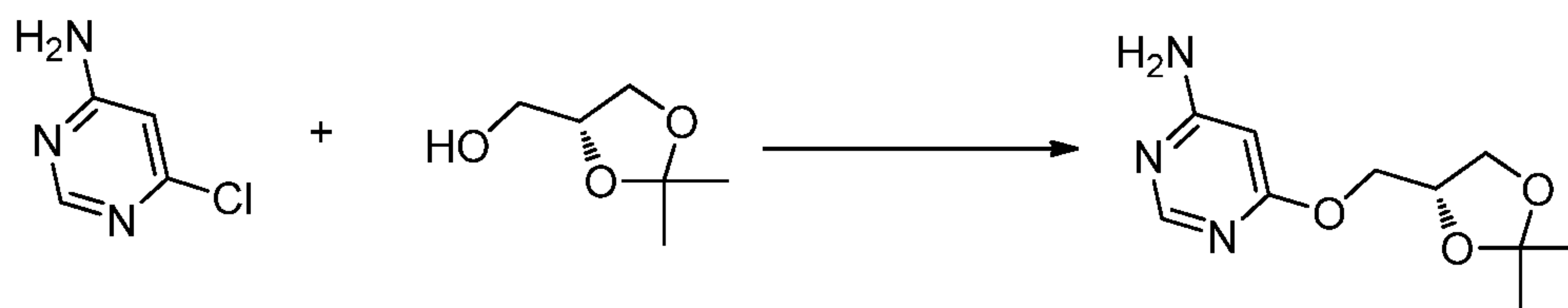
Synthesis of (S)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-amine



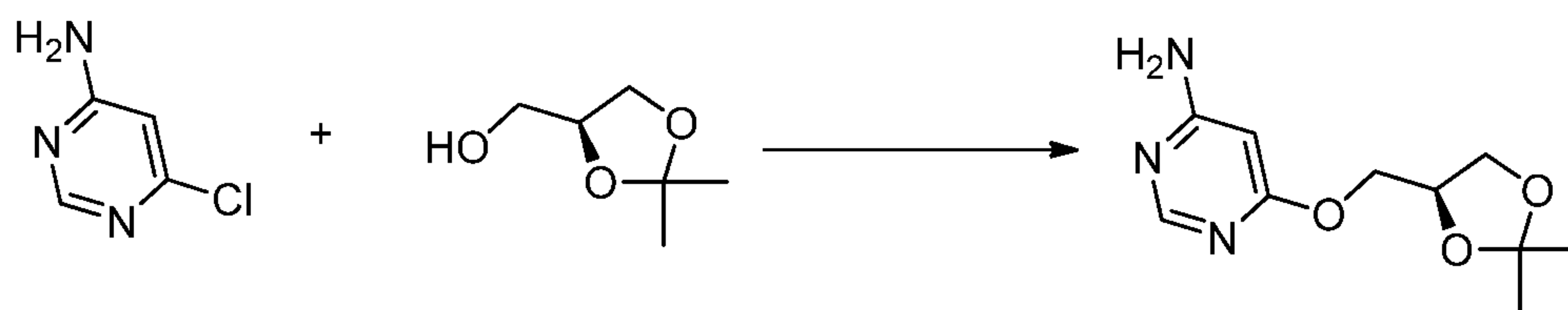
(S)-3-bromo-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridine (10 g, 34.7 mmol), liquor ammonia (100 mL, 4621 mmol) were taken in a sealed tube. The resulting brown solution was heated to 120 °C for 24 hr. The reaction progress was monitored by TLC 10% MeOH in DCM, TLC indicated formation of new spot and consumption of SM after 24 h.

5 After completion, The reaction mass was cooled to room temperature. The reaction mass was brought to pH 10 with 20% NaOH, saturated with NaCl, extracted with ethyl acetate (30 mL* 2). The combined organic layer was washed with brine (20 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to afford the (S)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-amine (6 g, 25.8 mmol, 74.2 % yield) as an
10 pale brown solid, LCMS (*m/z*): 225.10 [M+H]⁺.

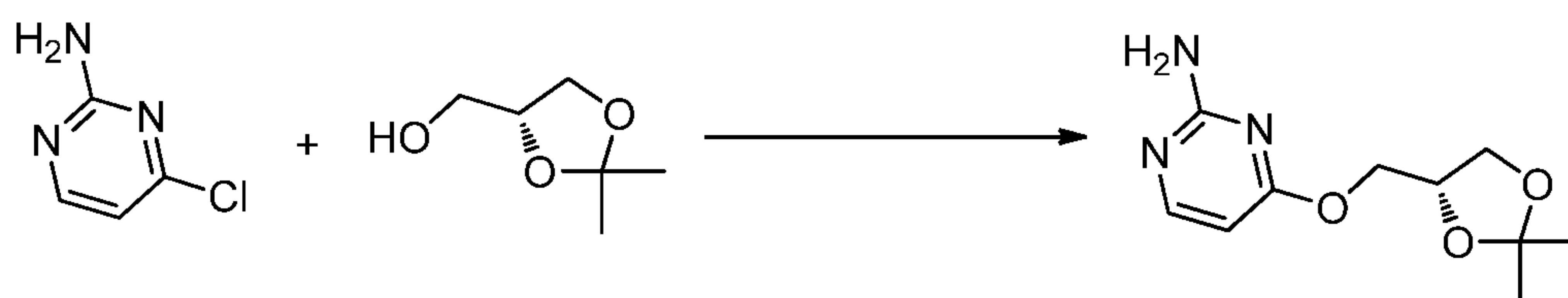
Synthesis of (R)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-amine



To a suspension of NaH (11.35 g, 473 mmol) in THF (100 mL) was added dropwise a
15 solution of (R)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (25 g, 189 mmol) in THF (150 mL) under Nitrogen at 0°C. The resulting suspension was stirred at rt for 1h. 6-chloropyrimidin-4-amine (19.61 g, 151 mmol) was added to the reaction mixture portion wise at rt and the resulting suspension was heated to 90 °C for 48 hr. After the completion of reaction (monitored by TLC, it shows little bit of starting and new spot observed at
20 polar), reaction mixture was poured into ice water (500 mL) and aqueous layer was extracted with EtOAc (2 X 1000 mL). Combined organics dried over Na₂SO₄, filtered and concentrated under reduced pressure to get light brown solid (crude). Crude material was purified by silica gel column (100-200, 3%MeOH in DCM). Fractions containing pure compound were combined and concentrated to afford the desired product (R)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-amine (13 g, 53.9 mmol, 28.5 % yield)
25 as an off-white solid and also get the impure compound (10 g). LCMS (*m/z*):226.17 (M+H)⁺.

Synthesis of (S)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-amine

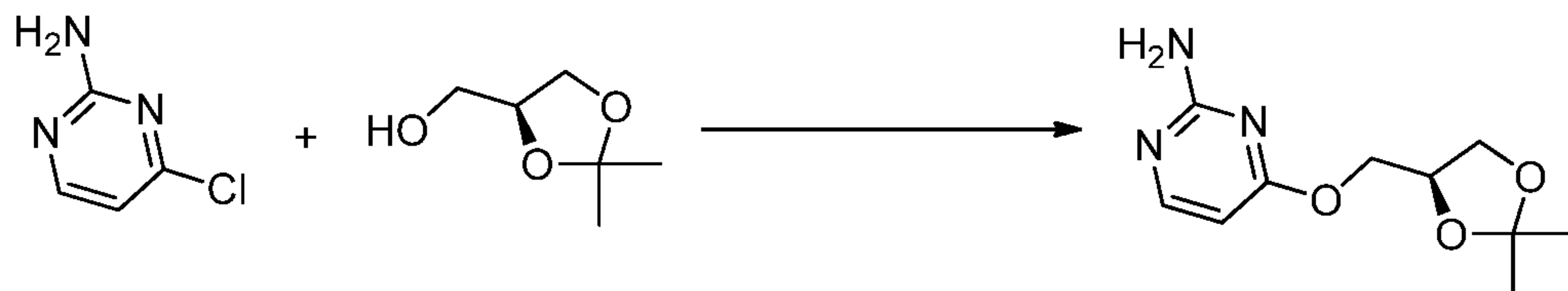
To a suspension of NaH (9.08 g, 378 mmol) in THF (150 mL) was added drop wise a solution of (S)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (20 g, 151 mmol) in THF (200 mL) under Nitrogen at 0°C, and the resulting suspension was stirred at rt for 1h. 6-chloropyrimidin-4-amine (15.68 g, 121 mmol) was added to the reaction mass portion wise at rt and the resulting suspension was heated to 90 °C for 48 hr. After the completion of reaction (monitored by TLC, starting material completely consumed and new spot observed at polar), reaction mass was poured into ice water (200 mL) and extracted with ethyl acetate (2X400 ml). Combined organics dried over Na₂SO₄, filtered and concentrated under reduced pressure to get light brown solid. The obtained solid was stirred in diethyl ether (200 ml) for 30 min filtered and dried under vacuum to get (S)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-amine (13 g, 57.3 mmol, 37.9 % yield) as a light brown solid, LCMS (*m/z*): 225.96 [M+H]⁺.

Synthesis of (R)-4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-amine

To a suspension of NaH (9.08 g, 378 mmol) in THF (150 mL) was added dropwise a solution of (R)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (20 g, 151 mmol) in THF (250 mL) under Nitrogen at 0°C. The resulting suspension was stirred at rt for 1h. 4-chloropyrimidin-2-amine (15.68 g, 121 mmol) was added to the reaction mixture portion wise at rt and the resulting suspension was heated to 90 °C for 48 hr. After the completion of reaction (monitored by TLC, starting completely consumed and new spot observed at polar), reaction mixture was poured into ice water (250 mL) and aqueous layer was extracted with EtOAc (2 X 300 mL). Combined organics dried over Na₂SO₄, filtered and concentrated under reduced pressure to get pale yellow liquid (crude). Obtained crude material was purified by column (100-200 silica gel) by using 0-50%EtOAc-petether to get

(R)-4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-amine (13 g, 57.0 mmol, 37.7 % yield) as pale yellow solid, LCMS (m/z): 226.20 $[M+H]^+$.

Synthesis of (S)-4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-amine



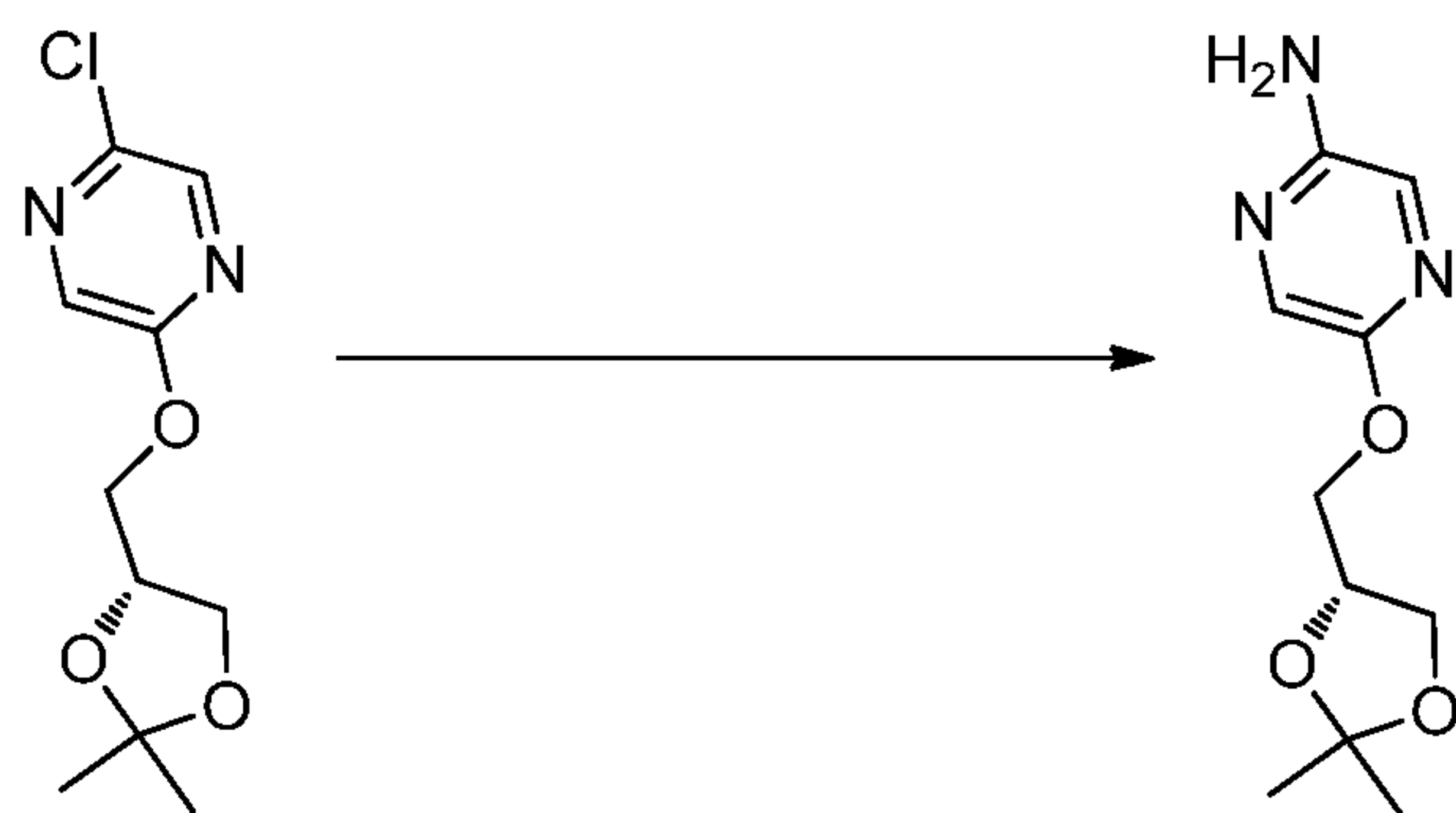
5

To a suspension of NaH (8.25 g, 189 mmol) in 1,4-Dioxane (200 mL) was added dropwise a solution of (S)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (10 g, 76 mmol) in 1,4-Dioxane (50 mL) under Nitrogen at 0°C. The resulting suspension was stirred at rt for 1h. 4-chloropyrimidin-2-amine (7.84 g, 60.5 mmol) was added to the reaction mixture portion wise at rt and the resulting suspension was heated to 90 °C for 48 hr. The reaction mixture was cooled to 28 °C and was partitioned between water (200 mL) and EtOAc (200 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to get crude (TLC eluent: Neat ethyl acetate R_f0.3; UV active). The crude compound was purified by column chromatography (100-200 mesh silica gel, eluted at 60% Ethyl acetate in hexane) to afford (S)-4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-amine (8.0 g, 35.4 mmol, 46.8 % yield) as pale yellow solid LCMS (m/z) 226.30 $(M+H)^+$.

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Synthesis of (R)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-amine



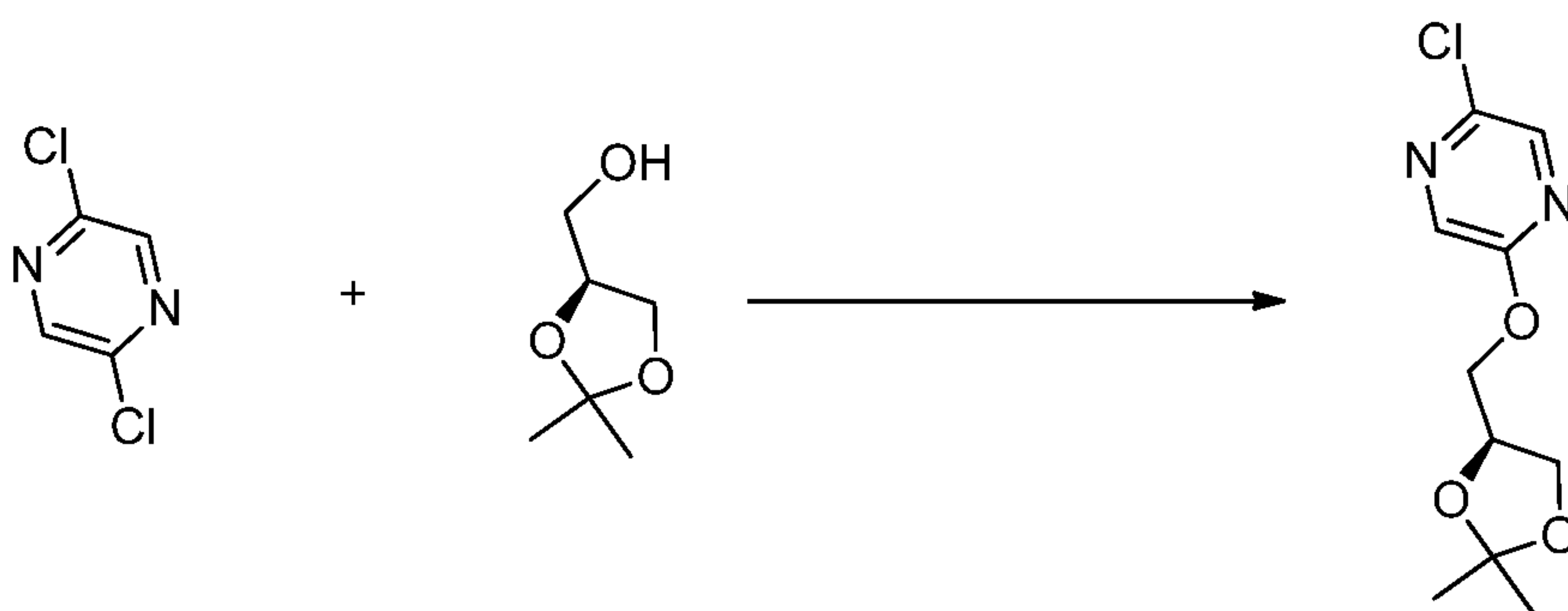
20

To a stirred solution of (R)-2-chloro-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazine (12 g, 49.0 mmol) in Tetrahydrofuran (THF) (20 mL) was added ammonium hydroxide (300 mL, 1926 mmol) and copper(II) sulfate (1.566 g, 9.81 mmol) in a sealed tube. Reaction mixture was stirred at 120 °C for 18 hr. Progress of the reaction was monitored by TLC, TLC indicates formation of polar spot along with un-reacted SM. Reaction

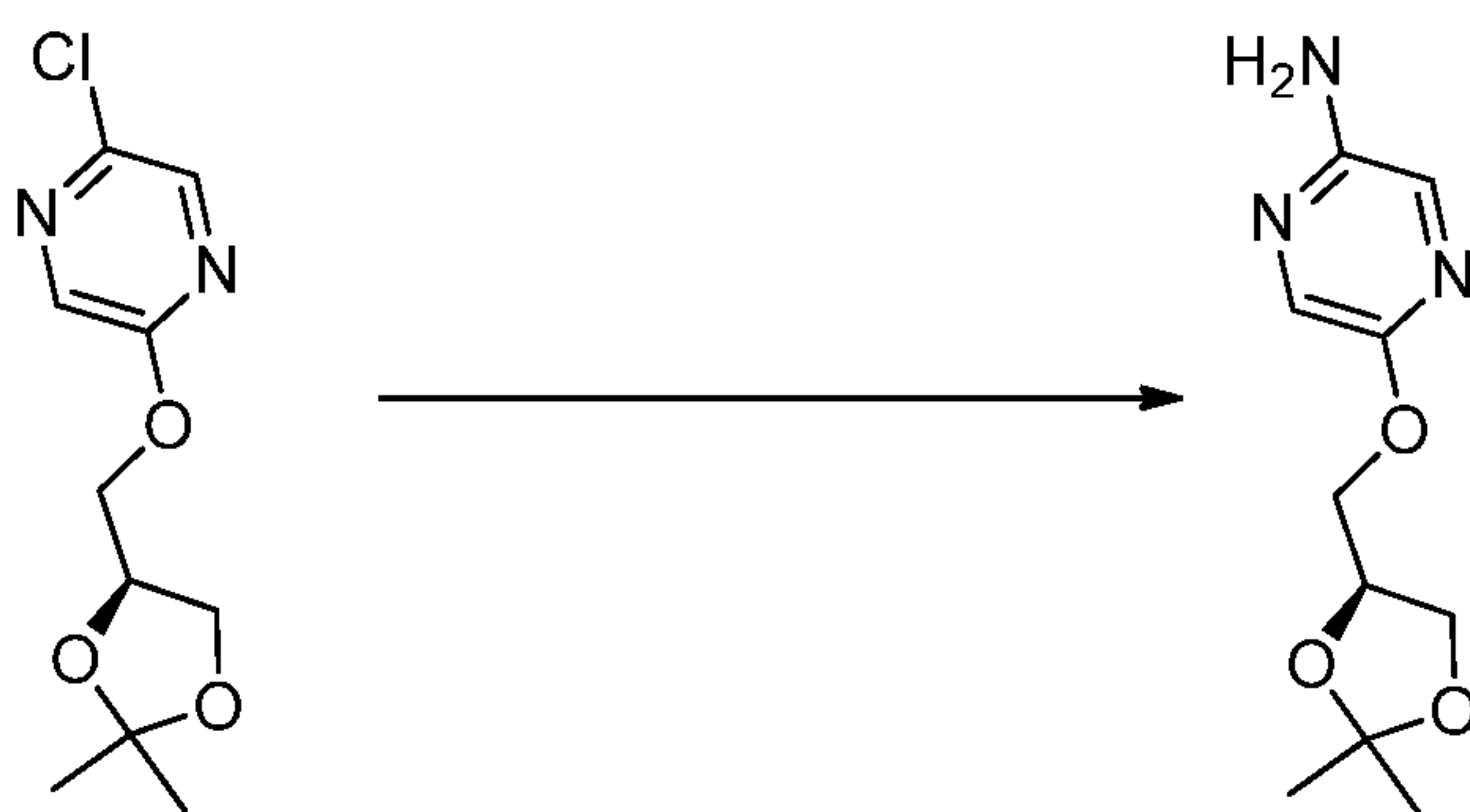
25

mixture was diluted with water (300 mL), extracted with EtOAc(3x 200mL), organic layers were combined and washed with water (100 mL), brine solution (100 mL), organic layer dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to afford (R)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-amine (10 g, 3.97 mmol, 8.09 % yield) as a yellow oily crude compound, LCMS (*m/z*): 226.13 (M+H)⁺.

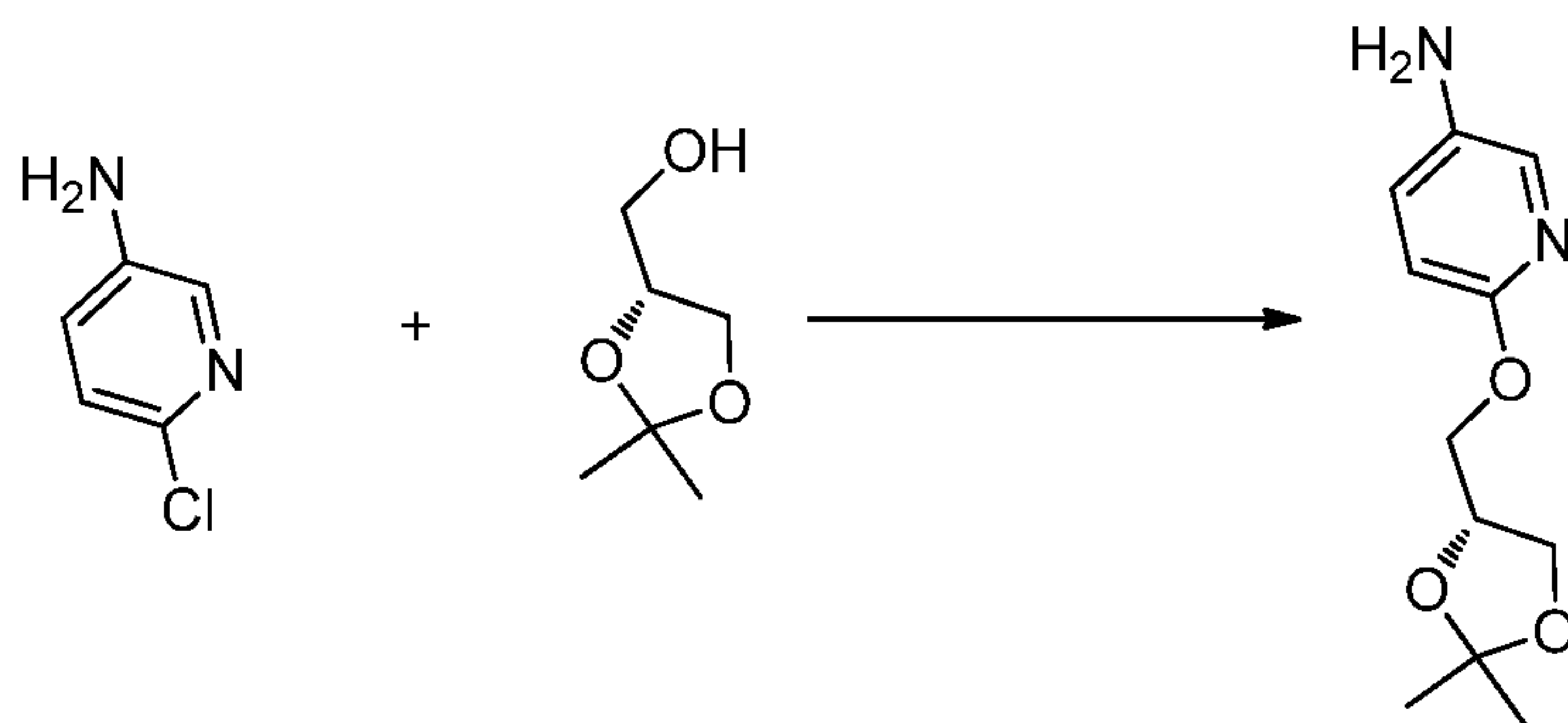
Synthesis of (S)-2-chloro-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazine



To a suspension of (S)-2,2-dimethyl-1,3-dioxolan-4-ylmethanol (8.87 g, 67.1 mmol), in N,N-Dimethylformamide (DMF) (50 mL) stirred under nitrogen at 0°C was added cesium carbonate (32.8 g, 101 mmol), the resulting reaction mixture was stirred at 0 °C for 1 hr. To this added 2,5-dichloropyrazine (10 g, 67.1 mmol). The resulting reaction mixture was stirred at 100 °C for 6 hr. Progress of the reaction was monitored by TLC. TLC indicated starting material was consumed to form new polar spot with 0.3 R_f. The reaction mass was cooled to rt, added water(100mL) and extracted with Ethyl acetate(100mL). The organic layer was washed with water(100mLX2). The organic layer was dried over Na₂SO₄ and filtered and concentrated to get crude as light brown liquid. The crude product was added to a silica gel (60-120) column and was eluted with Hex/EtOAc. Collected fractions: 30%EtOAc in Hexane the product was eluted. Concentrated the product fractions to afford (S)-2-chloro-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazine (12 g, 47.7 mmol, 71.0 % yield) as light brown liquid, LCMS (*m/z*): 244.90 [M+H]⁺.

Synthesis of (S)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-amine

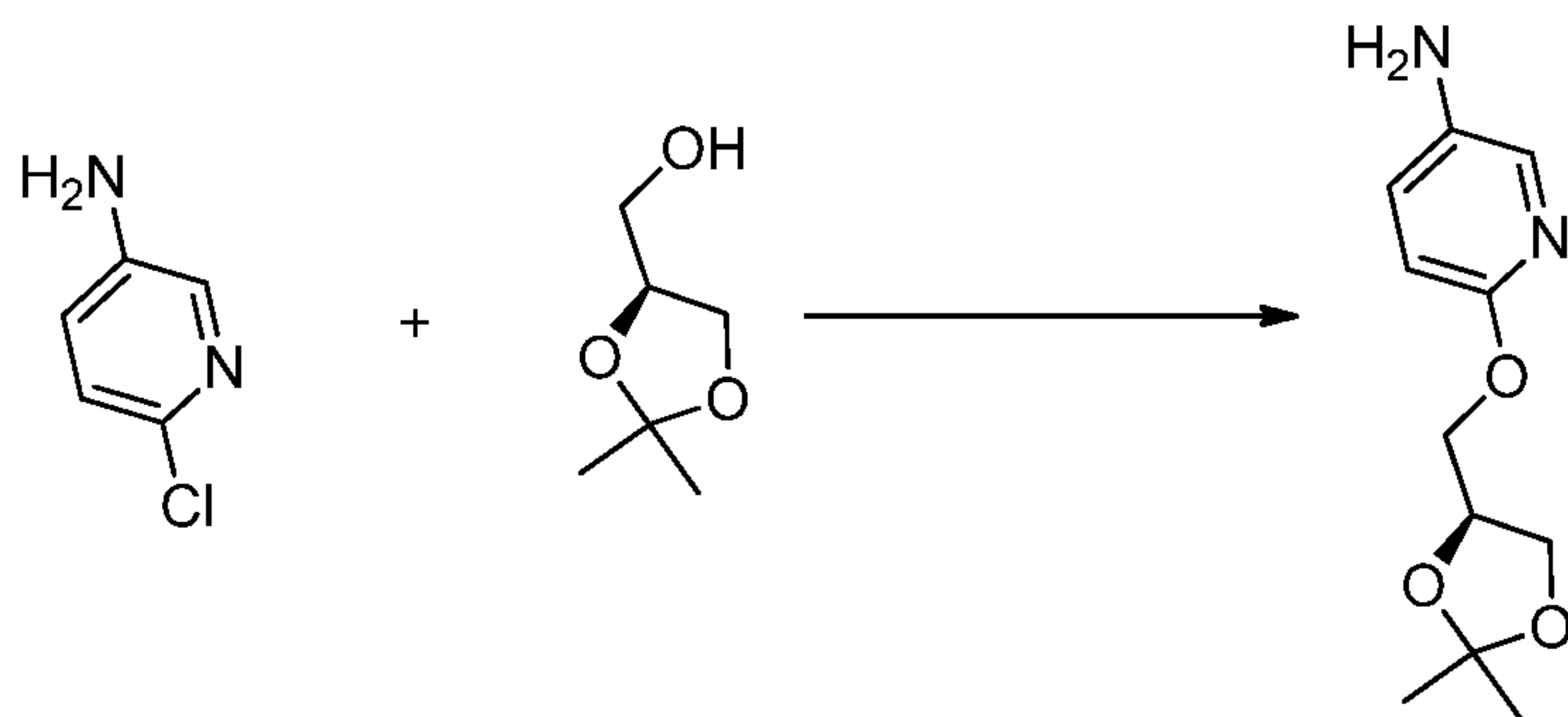
To a solution of (S)-2-chloro-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazine (10 g, 40.9 mmol), in Tetrahydrofuran (THF) (10 mL) stirred at room temp was added ammonium hydroxide (63.7 mL, 409 mmol) and copper(II) sulfate (3.26 g, 20.44 mmol) at rt. The reaction mixture was stirred in sealed tube at 130 °C for 2days. Progress of the reaction was monitored by TLC. TLC indicated starting material was consumed. Cooled the reaction mass to rt, diluted with water(100mL), Extracted with ethyl acetate (250mLX2). The organic layer was dried over Na₂SO₄, filtered and concentrated to get crude compound as brown sticky compound. The crude product was added to a silica gel column and was eluted with DCM/EtOAc. Collected fractions: 50%EtOAc in petether the product was eluted. Concentrated the product fractions to afford (S)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-amine (2 g, 8.77 mmol, 21.46 % yield)(N35119-51-A2) as light brown solid. NMR: in CDCl₃ consistent with, LCMS (*m/z*): 226.09 [M+H]⁺.

Synthesis of (R)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-amine

(R)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (27.8 g, 210 mmol) was added to a stirred solution of KOtBu (45.8 g, 408 mmol) in NMP (200 mL) at 0 °C then stirred at RT for 1 h and cooled to 0 °C, 6-chloropyridin-3-amine (15 g, 117 mmol) was added and heated to

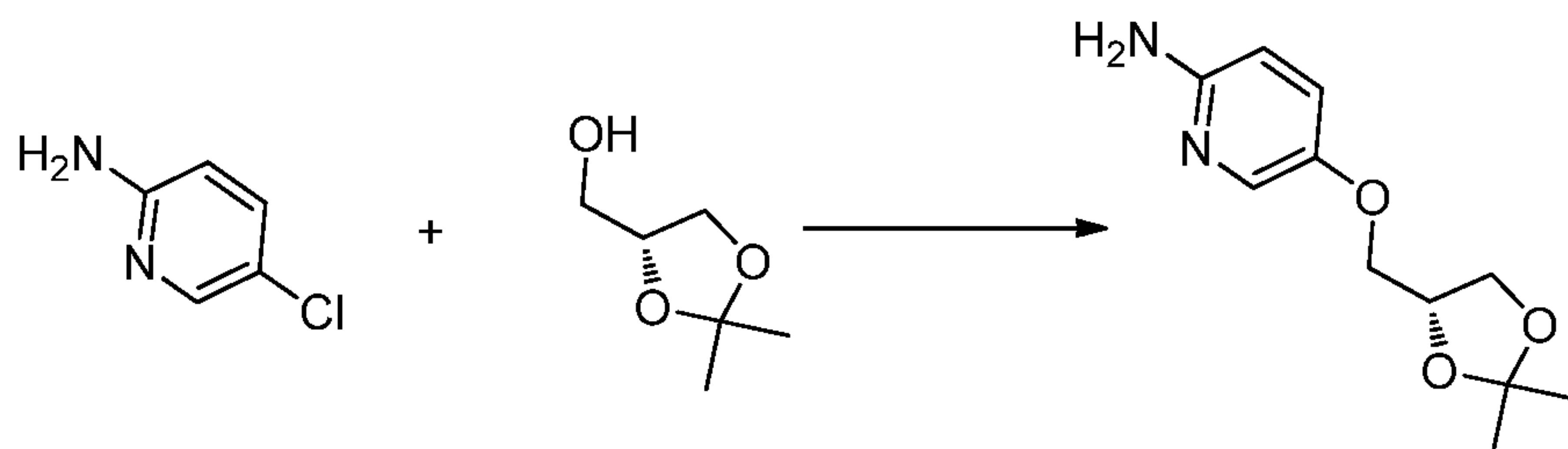
110 °C for 144 h. The reaction mixture cooled to RT and partitioned between water (500 mLX2) and EtOAc (200 mL x 4). Organic layers were separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to get crude and purified by column chromatography (using 100-200 silica gel, column eluted at 50 % ethyl acetate in hexane) to afford the (R)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-amine (8 g, 35.1 mmol, 30.1 % yield) as brown oil, LCMS (*m/z*): 225.16 [M+H]⁺.

Synthesis of (S)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-amine



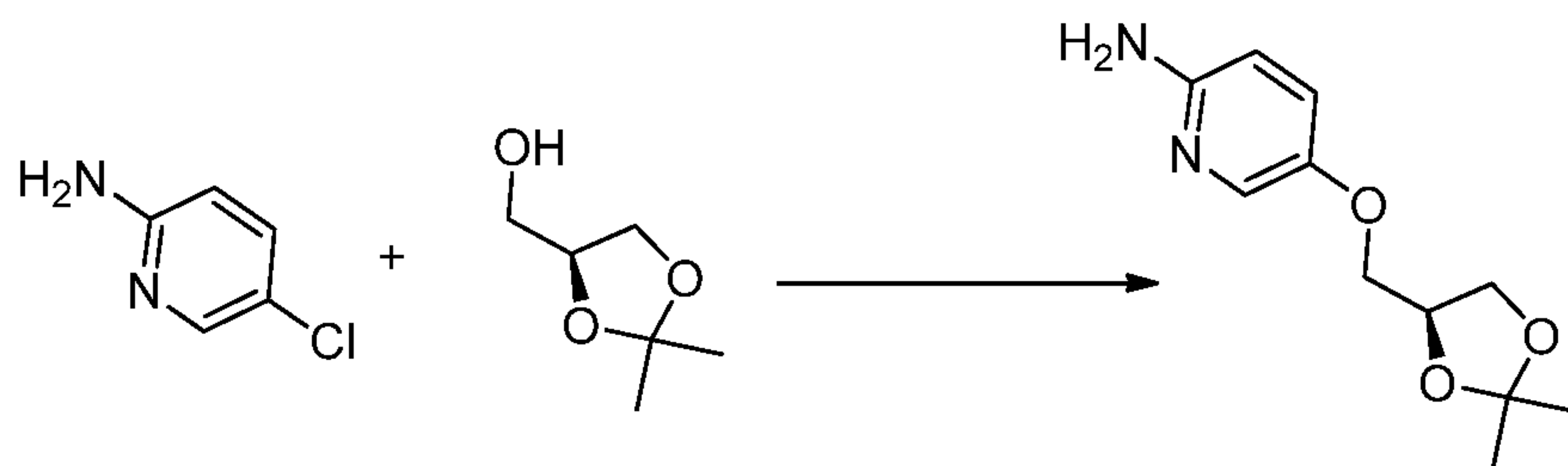
(S)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (18.50 g, 140 mmol) was added to a stirred solution of KOtBu (30.5 g, 272 mmol) in NMP (600 mL) at 0 °C then stirred at RT for 1 h and cooled to 0 °C, 6-chloropyridin-3-amine (10.0 g, 78 mmol) was added and heated to 110 °C for 88h. The reaction mixture cooled to RT and partitioned between water (50 mLX2) and EtOAc (100 mL x 2). Organic layers were separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to get crude compound as a gum.(TLC: Eluent: 100% ethyl acetate, R_f 0.5; UV active:). The crude product was purified by flash column chromatography (silica-gel: 100-200 mesh) eluted with 50% EtOAc in hexane to afford (S)-6-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-amine (10.0 g, 41.7 mmol, 53.6 % yield) as a dark sticky mass, LCMS (*m/z*) 225.0 (M+H)⁺.

Synthesis of (R)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-amine

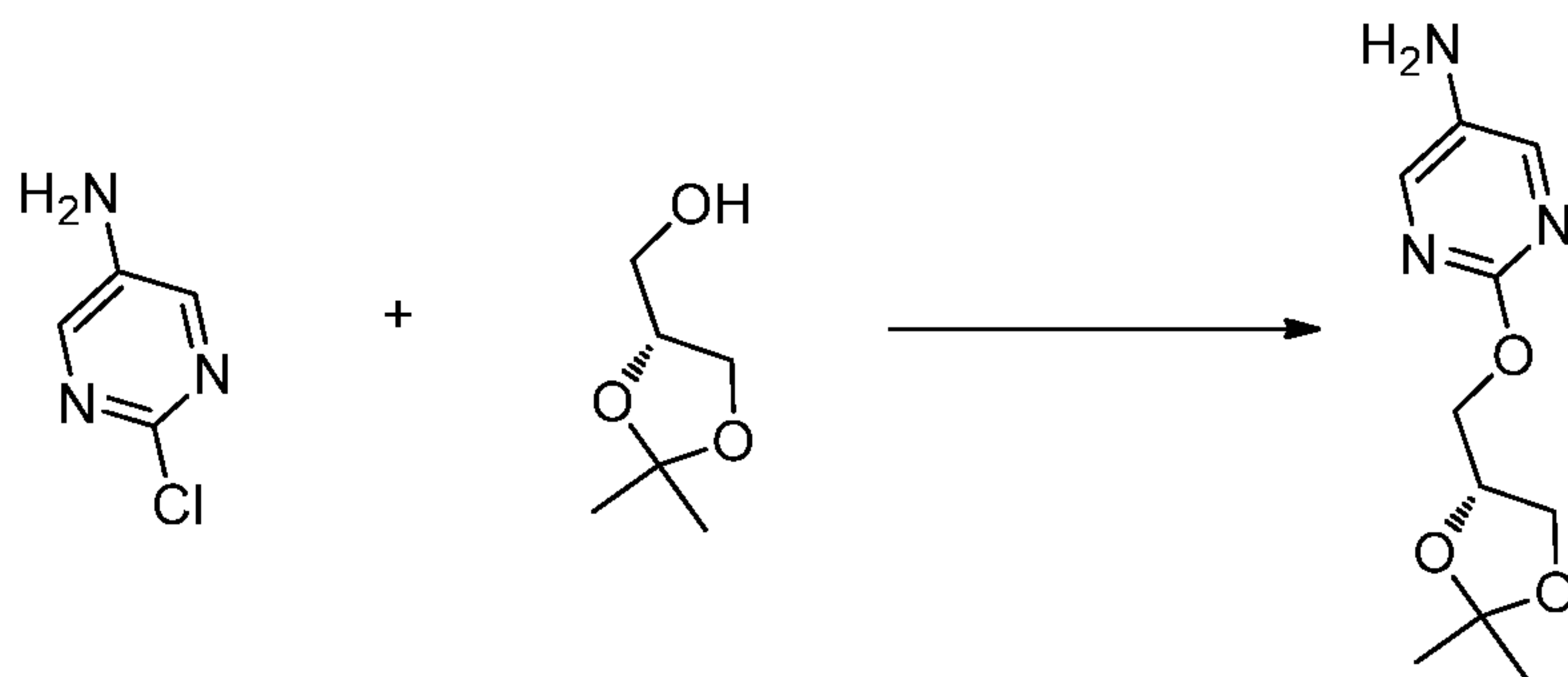


(R)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (30.7 g, 232 mmol) was added to a stirred solution of KOtBu (70.1 g, 624 mmol) in NMP (800 mL) at 0 °C then stirred at RT for 1 h and cooled to 0 °C then 5-fluoropyridin-2-amine (20 g, 178 mmol) was added and heated to 110 °C for 114 h. The reaction mixture cooled to RT and partitioned between water (500 mLX2) and EtOAc (500 mL x 4). Organic layers were separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to get crude compound, then it was purified by column chromatography (using 100-200 silica gel, column eluted at 80% ethyl acetate in hexane) to afford the (R)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-amine (10 g, 40.1 mmol, 22.50 % yield) as a brown oil, LCMS: 225.0 (M+H).

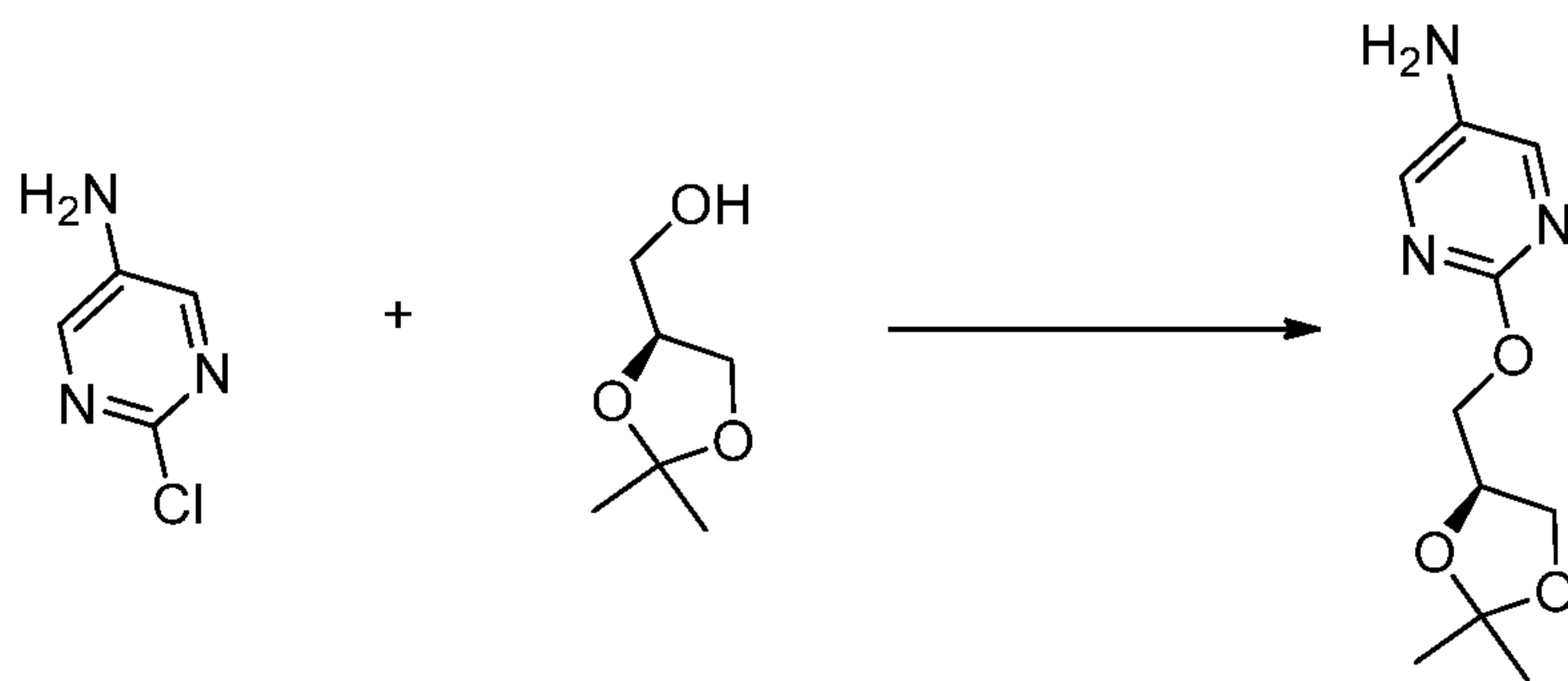
Synthesis of (S)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-amine



NaH (12.84 g, 268 mmol) was added to a stirred solution of (S)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (31.8 g, 241 mmol) in Dimethyl Sulfoxide (DMSO) (100 mL) at 0 °C then stirred at RT for 1 h and cooled to 0 °C, 5-fluoropyridin-2-amine (15.0 g, 134 mmol) was added and heated to 110 °C for 60 h. The reaction mixture cooled to RT and partitioned between water (50 mL) and EtOAc (100 mL). Organic layers were separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to get crude compound (TLC: Eluent: 100% ethyl acetate, R_f0.5; UV active), The crude product was purified by flash column chromatography (silica-gel: 100-200 mesh) eluted with 50% EtOAc in hexane to afford (S)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-amine (7.2 g, 32.1 mmol, 23.99 % yield) as a pale yellow sticky, LCMS (*m/z*): 225.1 (M+H)⁺.

Synthesis of (R)-2-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-5-amine

Tetrahydrofuran (75 mL) was added to NaH (5.56 g, 232 mmol) at 0 °C, (R)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (12.46 mL, 100 mmol) in Tetrahydrofuran (50 mL) was added to the reaction mixture at 0 °C, and the reaction mixture was stirred for 1h at 28°C. 2-chloropyrimidin-5-amine (10 g, 77 mmol) in Tetrahydrofuran (25 mL) was added and stirred for 16 hr at 70 °C. The reaction mixture was quenched with cold water (30 mL) and extracted with ethyl acetate(3 x 80 mL). The organic layer was washed with water (2 X 50 mL) and saturated brine solution (50 mL), dried over anhydrous Na₂SO₄, filtered and concentrated. The crude compound was purified by column chromatography (Neutral alumina) product was eluted with 40-45% Ethyl acetate in Hexane to afford (R)-2-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-5-amine (6.5 g, 28.3 mmol, 36.6 % yield) as pale yellow solid, LCMS (*m/z*): 226.0 [M+H]⁺.

15 Synthesis of (S)-2-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-5-amine

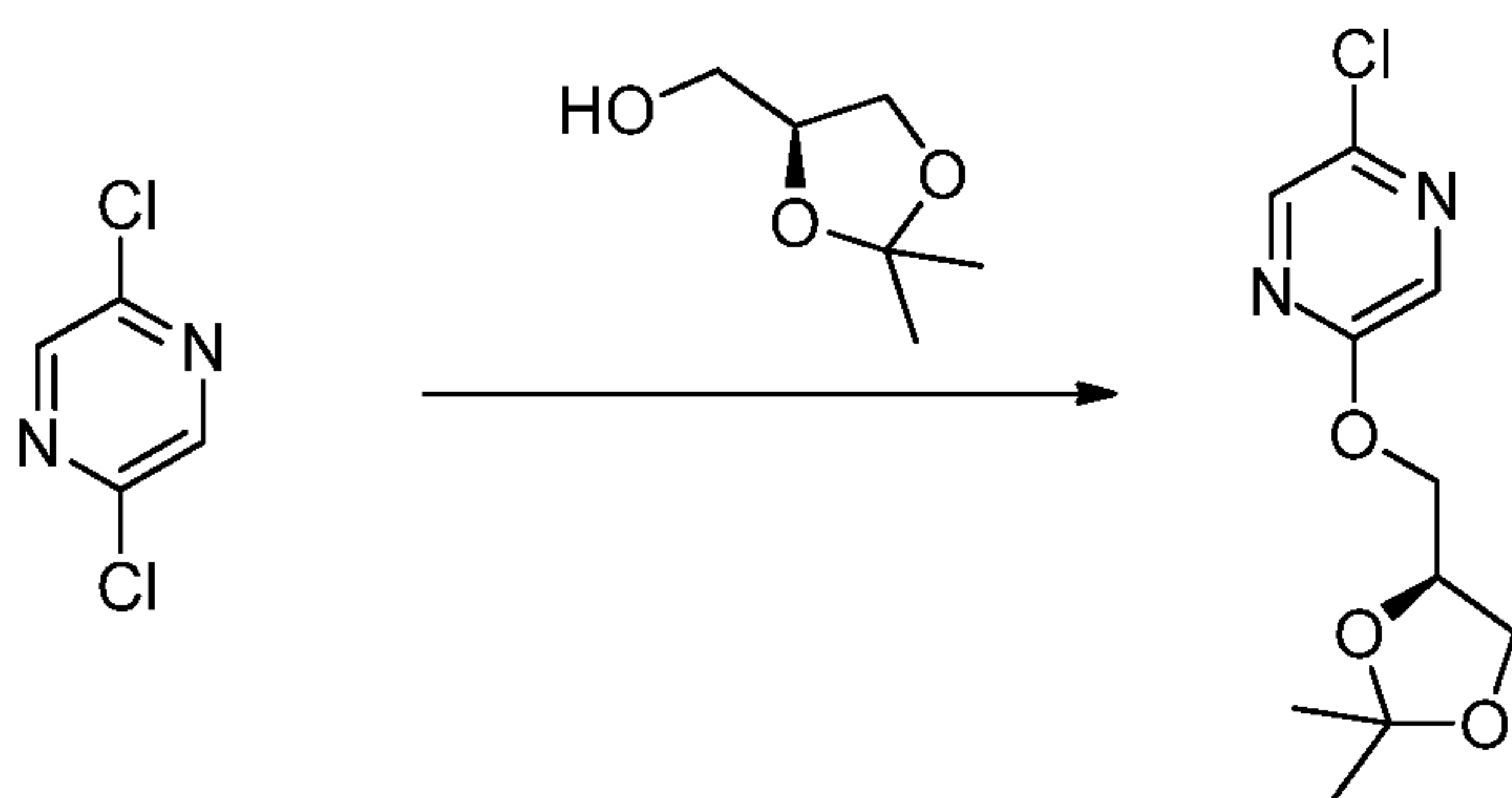
To a suspension of NaH (6.17 g, 154 mmol) in THF (100 ml) was added (S)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (13.26 g, 100 mmol) in THF (50 ml) was added to the reaction mixture at 0 °C, and the reaction mixture was stirred for 1h at 25°C.to this 2-chloropyrimidin-5-amine (10 g, 77 mmol) in THF (50 ml) and was added at 0°C and slowly heated to 80 °C and stirred for 16 hr at 80 °C. After completion of the reaction, reaction mixture was quenched with the ammonium chloride (10 ml) and extracted with

the ethyl acetate (3x20 ml). The organic layer was separated and washed with the brine and dried over Na₂SO₄, filtered it and concentrated under reduced pressure to get the crude.

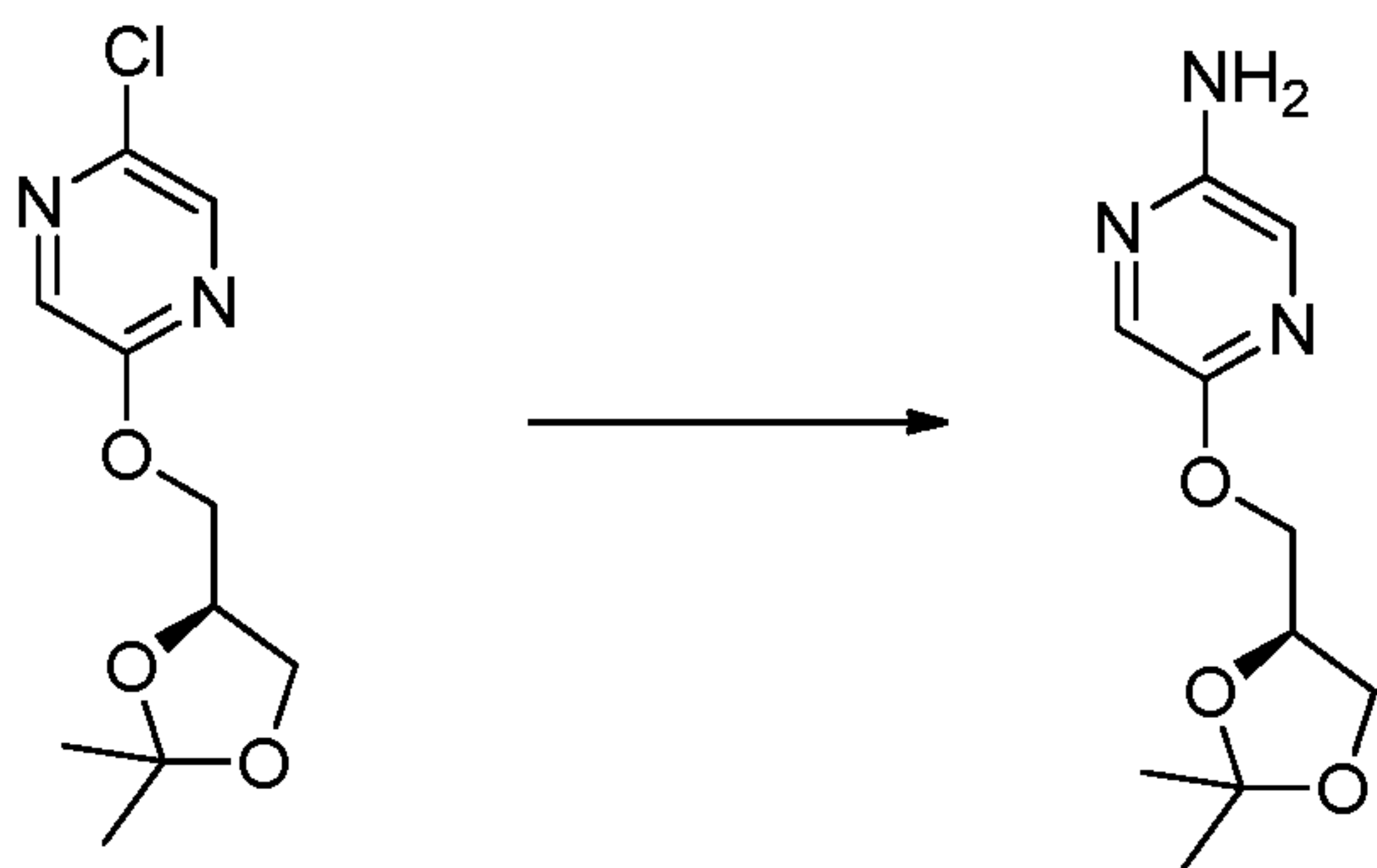
This crude was triturated with the diethyl ether to get (S)-2-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-5-amine (5.0 g, 19.77 mmol, 25.6 % yield) as a brown solid, LCMS

5 (*m/z*): 226.1 [M+H]⁺.

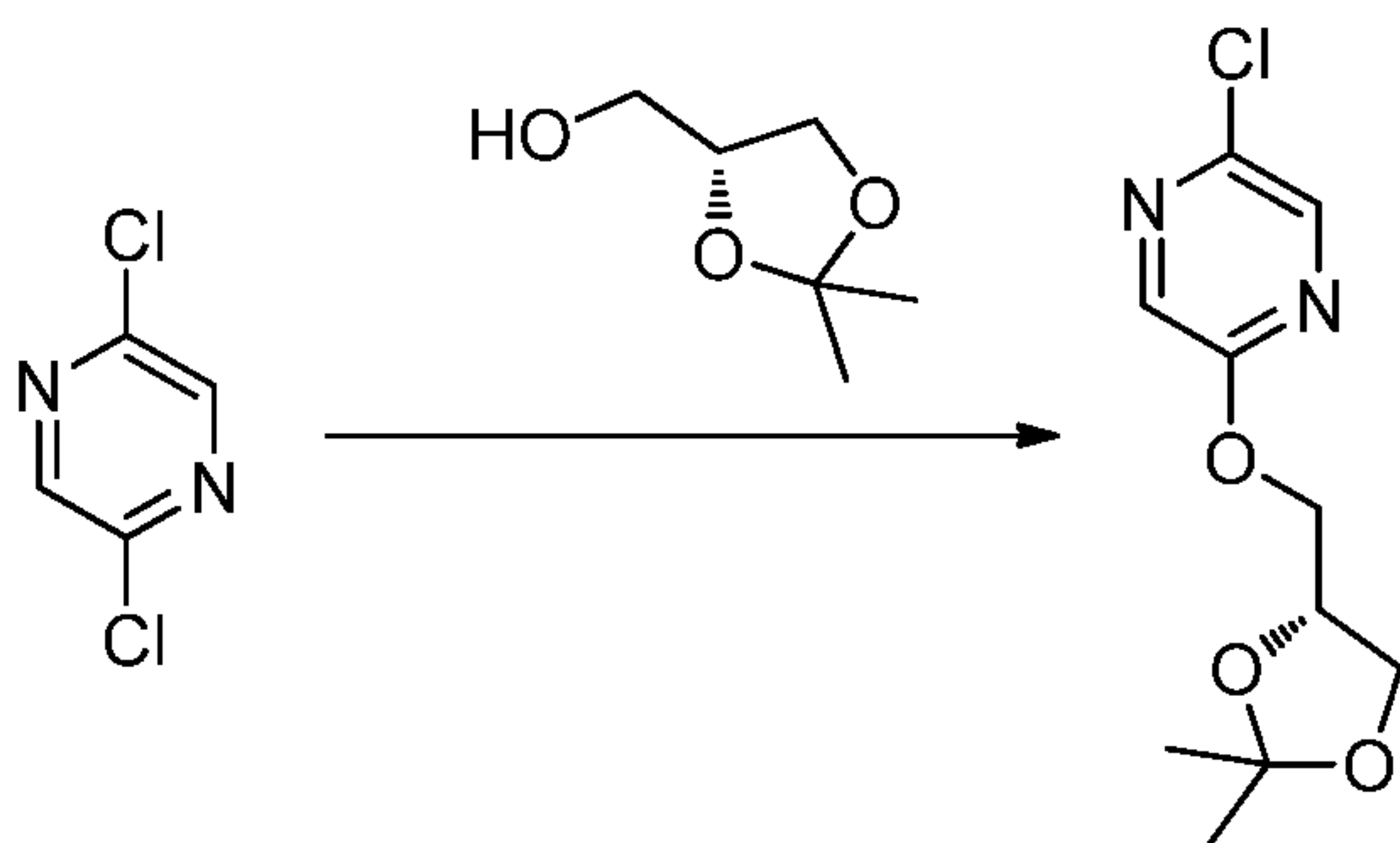
Synthesis of (S)-2-chloro-5-((2, 2-dimethyl-1, 3-dioxolan-4-yl) methyl) pyrazine



- 10 To a stirred solution of cesium carbonate (492 g, 1510 mmol) in DMF (1000 mL) was added (S)-2, 2-dimethyl-1, 3-dioxolan-4-yl) methanol (133 g, 1007 mmol) at 0 °C. The resulting reaction mixture was stirred at room temperature for 30 min. Then a solution of 2, 5-dichloropyrazine (150 g, 1007 mmol) in DMF (500 mL) was added at 0 °C and the resulted reaction mixture was stirred at 100 °C for 4 h. (TLC System: 20% Ethyl acetate in
- 15 Petether, R_f: 0.5, UV active). The reaction mixture was diluted with ice cold water (500 mL), extracted with EtOAc (3 x 300 mL). The combined organic layer was washed with water (2 x 200 mL) and brine solution (100 mL), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to obtain crude compound. The crude compound was purified by flash column chromatography (silica gel: 100-200 mesh, eluent: 10 %
- 20 EtOAc in Hexane) to afford the desired product (S)-2-chloro-5-((2, 2-dimethyl-1, 3-dioxolan-4-yl) methoxy) pyrazine (200 g, 768 mmol, 76 % yield) as a yellow liquid. LCMS (*m/z*): 245.1 [M+H]⁺.

Synthesis of (S)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-amine

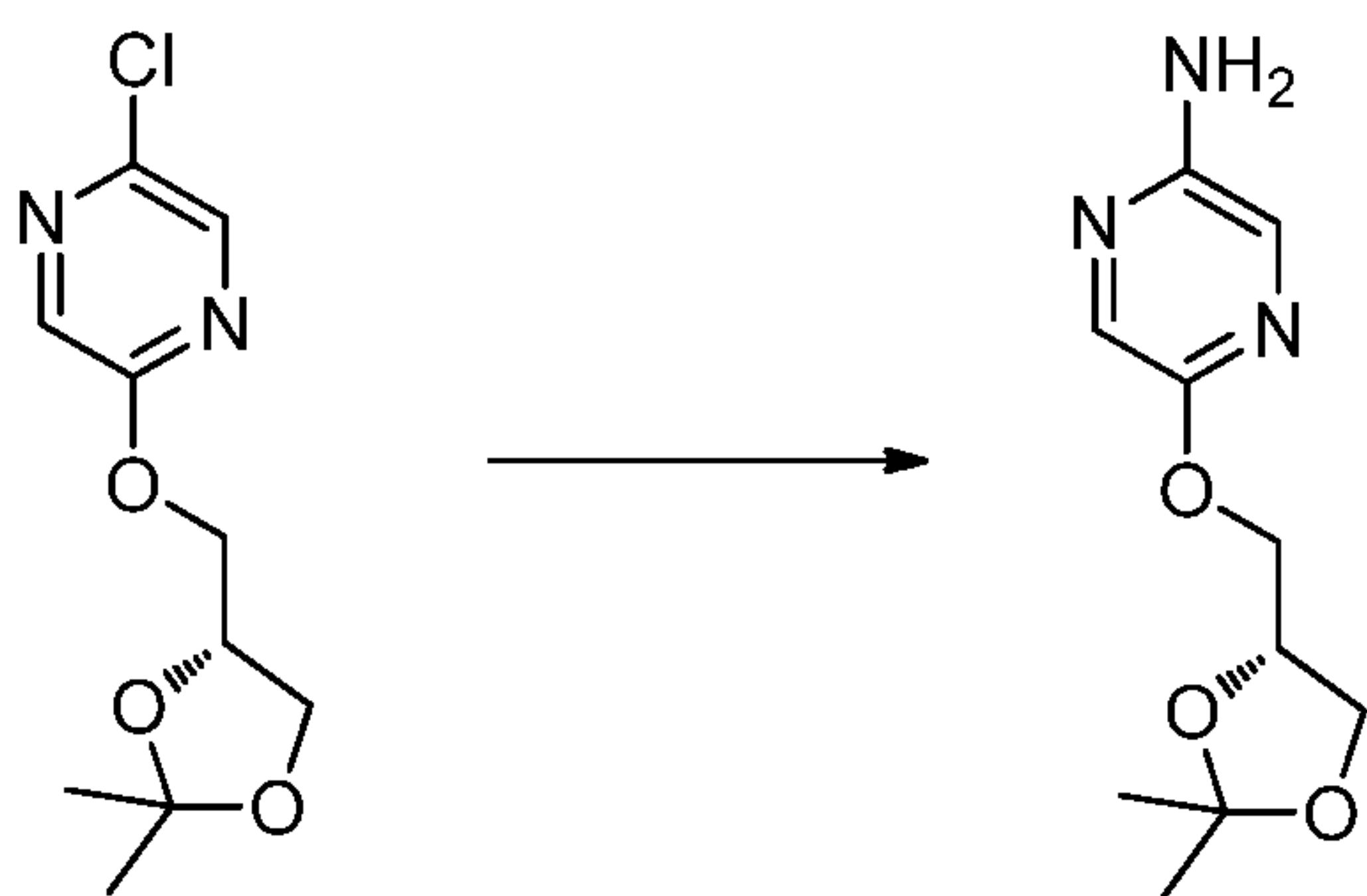
To a stirred solution of (S)-2-chloro-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazine (120 g, 490 mmol) in THF (30 mL) were added ammonium hydroxide (1000 mL, 6420 mmol) and copper(II) sulfate (15.66 g, 98 mmol) in a sealed tube and the resulting reaction mixture was stirred at 120 °C for 48 h (TLC System: 50% Ethyl acetate in Petether, R_f : 0.4, UV active). The reaction mixture was diluted with water (300 mL), extracted with EtOAc (3x 500 mL). The combined organic layer was washed with water (200 mL) and brine solution (200 mL), dried over anhydrous Na_2SO_4 , filtered and concentrated under reduced pressure to get crude compound. The crude was purified by flash column chromatography (using 100-200 mesh silicagel and eluted the compound with 40% EtOAc in Hexane) to afford the desired product (S)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-amine (65 g, 280 mmol, 57.2 % yield) as a yellow crystal solid. LCMS (m/z): 226.13 $[\text{M}+\text{H}]^+$.

Synthesis of (R)-2-chloro-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazine

To a stirred suspension of cesium carbonate (32.8 g, 101 mmol) in DMF (100 mL) was added (R)-2-chloro-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazine (8.87 g, 67.1 mmol) at 0 °C and stirred at room temperature for 30 min. Then 2,5-dichloropyrazine (10 g, 67.1 mmol) was added and the resulting reaction mixture was stirred at 100 °C for 4 h. (TLC System: 20%

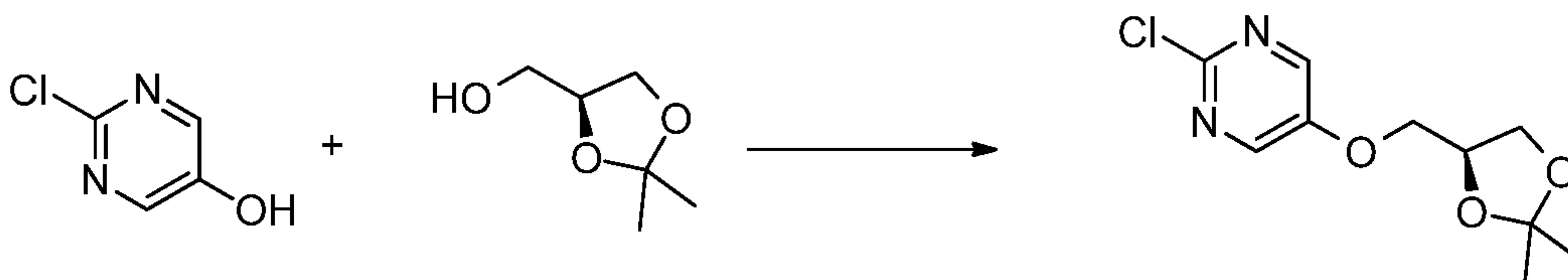
Ethyl acetate in Hexane, R_f : 0.5, UV active). The reaction mixture was diluted with ice cold water (200 mL), extracted with EtOAc (3 x 100 mL). The combined organic layer was washed with water (2x50 mL) and brine solution (50 mL), dried over anhydrous Na_2SO_4 , filtered and concentrated under reduced pressure to afford (*R*)-2-chloro-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazine (12 g, 43.8 mmol, 65.3 % yield) as a yellow oily compound. LCMS (m/z): 244.99 $[\text{M}+\text{H}]^+$.

Synthesis of (*R*)-5-((2, 2-dimethyl-1, 3-dioxolan-4-yl) methoxy) pyrazin-2-amine



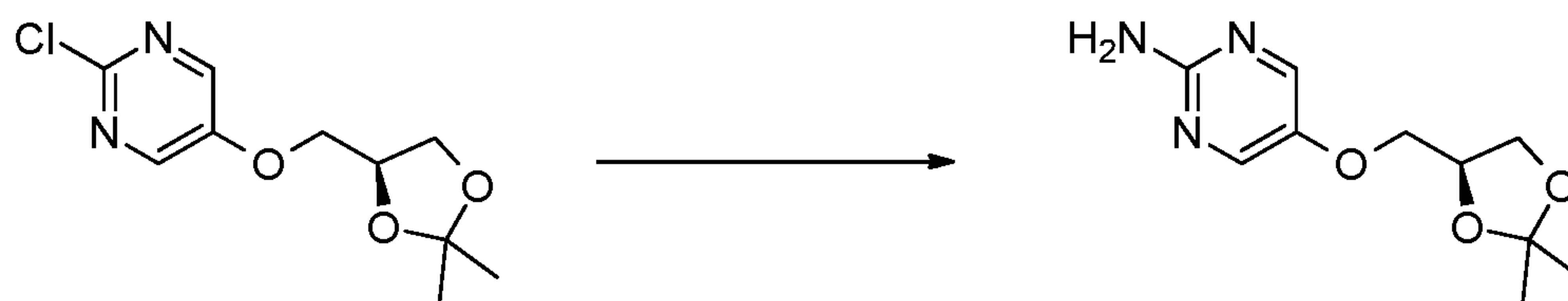
To a stirred solution of (*R*)-2-chloro-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazine (8 g, 32.7 mmol) in Tetrahydrofuran (10 mL) was added ammonium hydroxide (400 mL, 2568 mmol) and copper(II) sulfate (1.044 g, 6.54 mmol) in a sealed tube and the reaction mixture was stirred at 120 °C for 48 h. (TLC System: 50% Ethyl acetate in Hexane, R_f : 0.4, UV active). The reaction mixture was diluted with water (200 mL), extracted with EtOAc (3x 50 mL). The combined organic layer was washed with water (50 mL), brine solution (50 mL), dried over anhydrous Na_2SO_4 , filtered and concentrated under reduced pressure to get crude compound. The crude was purified by flash column chromatography (using 100-200 mesh silicagel and eluted the compound with 40% EtOAc in Hexane), pure fraction were collected and concentrated under reduced pressure to afford (*R*)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-amine (2 g, 8.65 mmol, 26.4 % yield) as a yellow crystal solid. LCMS (m/z): 226.10 $[\text{M}+\text{H}]^+$.

Synthesis of (*S*)-2-chloro-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidine



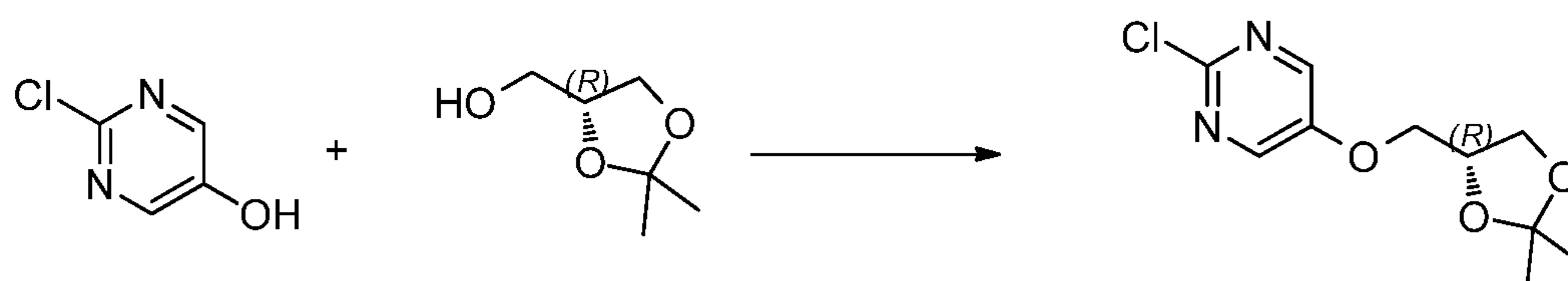
To a stirred solution of 2-chloropyrimidin-5-ol (13 g, 100 mmol) in THF (100 mL) at 0 °C was added (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (13.16 g, 100 mmol), triphenylphosphine (32.7 g, 124 mmol) followed by DEAD (19.71 mL, 124 mmol) and reaction was stirred at RT for 4 h. (TLC eluting system: 30% EtOAc in pet ether; R_f 0.5; UV active). The reaction mixture was quenched with water (50 mL) and extracted into EtOAc (2x75 mL). Organic layer was separated and dried over anhydrous Na_2SO_4 , filtered and filtrate was evaporated to give crude product. The crude was purified by chromatography (Silicagel, eluent: 20% EtOAc in hexane) to afford (*S*)-2-chloro-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidine (20g, 79 mmol, 79 % yield) as an off white solid. LCMS (m/z): 245.10; $[\text{M}+\text{H}]^+$.

Synthesis of (*S*)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-amine



A mixture of (*S*)-2-chloro-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidine (10 g, 40.9 mmol) and aq.ammonia (66.3 ml, 1226 mmol) in a sealed tube was heated at 120 °C for 24 h. (TLC eluting system: 100% EtOAc; R_f 0.2; UV active). The reaction mixture was cooled to RT, quenched with water (50 mL) and extracted into EtOAc (2x75 mL). Organic layer was separated, dried over anhydrous sodiumsulphate, filtered and filtrate was evaporated to give crude product as yellow solid. The crude compound was triturated with n-pentane (50 mL) to afford (*S*)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-amine (6.6g, 28.6 mmol, 70.0 % yield) as an off white solid. LCMS (m/z): 226.17; $[\text{M}+\text{H}]^+$.

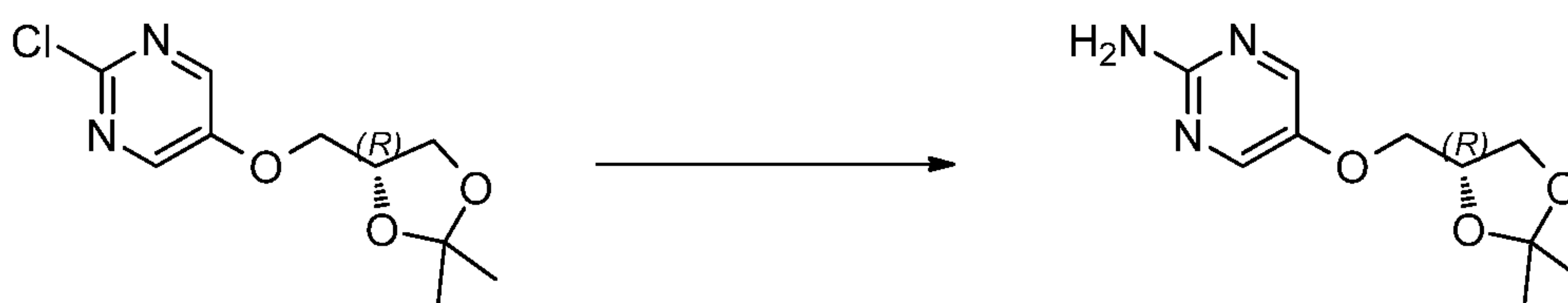
Synthesis of (*R*)-2-chloro-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidine



To a stirred solution of 2-chloropyrimidin-5-ol (20 g, 153 mmol) in THF (100 mL) at 0 °C was added (*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (24.30 g, 184 mmol),

triphenylphosphine (50.2 g, 192 mmol) followed by DEAD (30.3 mL, 192 mmol) and the reaction was stirred at RT for 12 h. (TLC eluting system: 70% EtOAc in pet ether; R_f 0.5; UV active). The reaction mixture was quenched with water (100 mL) and extracted into EtOAc (200 mL). Organic layer was separated and dried over anhydrous Na_2SO_4 , filtered and filtrate was evaporated to give crude product. The crude was purified by chromatography (Silicagel, eluent: 35% EtOAc in hexane) to afford (*R*)-2-chloro-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidine (23 g, 91 mmol, 59.5 % yield) as a white solid. LCMS (m/z): 245.06; $[\text{M}+\text{H}]^+$.

10 Synthesis of (*R*)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-amine

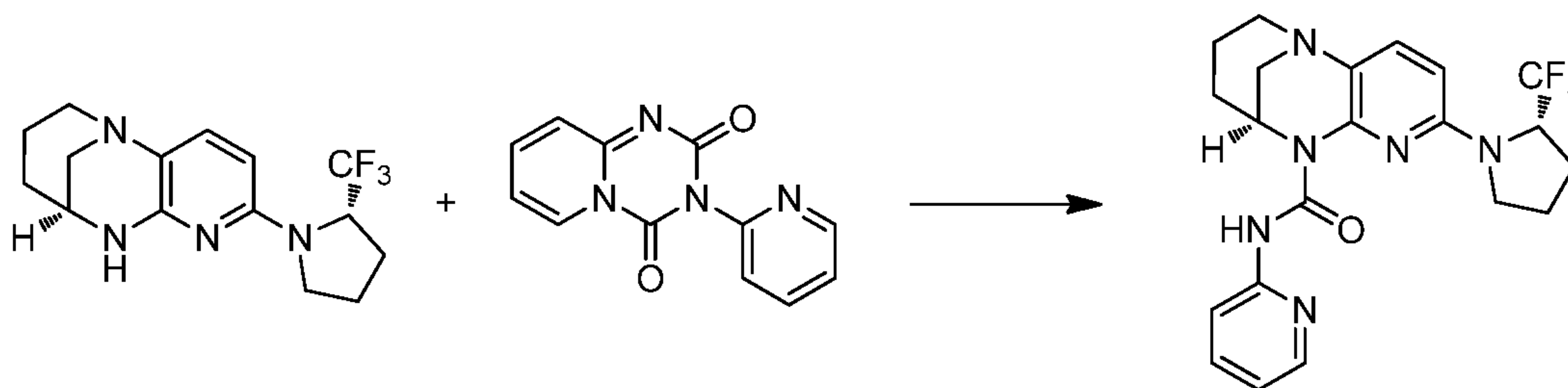


A mixture of (*R*)-2-chloro-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidine (5 g, 20.44 mmol) and aq. ammonia (50 mL, 924 mmol) in a sealed tube was heated 120 °C for 48 h. (TLC eluting system: 100% EtOAc; R_f 0.2; UV active). The reaction mixture was cooled to RT, quenched with water (50 mL) and extracted into DCM (2x75 mL). Organic layer was separated, dried over anhydrous Na_2SO_4 , filtered and filtrate was evaporated to afford (*R*)-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-amine (2.7 g, 11.5 mmol, 57.5 % yield) as a pale yellow solid. LCMS (m/z): 226.02; $[\text{M}+\text{H}]^+$.

20 COMPOUND EXAMPLES

Example 1

Synthesis of (9*S*)-*N*-(pyridin-2-yl)-2-((*S*)-2-(trifluoromethyl)pyrrolidin-1-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide



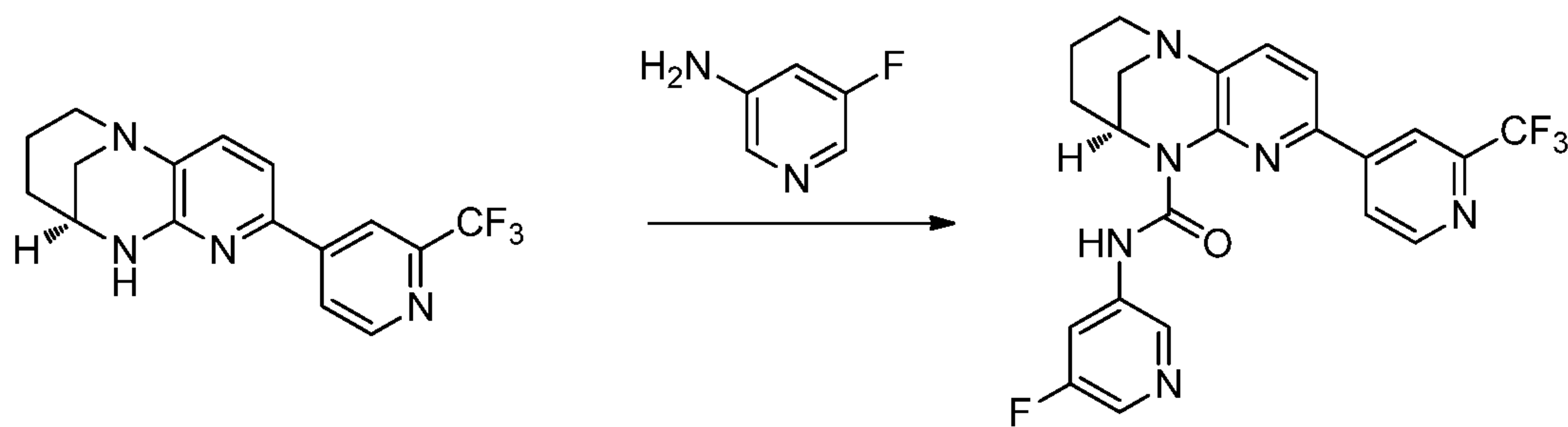
NaH (0.254 g, 10.57 mmol) was added to a stirred solution of of (*9S*)-2-((*S*)-2-

(trifluoromethyl)pyrrolidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (0.550 g, 1.761 mmol) in Tetrahydrofuran (THF) (30 mL) stirred under nitrogen at room temp. The reaction mixture was stirred at room temp for 30 minutes. Then 3-(pyridin-2-yl)-2H-pyrido[1,2-a][1,3,5]triazine-2,4(3H)-dione (0.635 g, 2.64 mmol) was added at room temp. Then reaction mixture was stirred at 65 °C for 24 hr. The reaction mixture was cooled to 28 °C and was partitioned between water (10 mL) and EtOAc (25 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid (TLC eluent: 100% EtOAc: R_f 0.3; UV active). The crude was purified by column chromatography using (100-200 mesh) silica gel and was eluted with 75-80% EtOAc in Hexane to afford pure (9S)-N-(pyridin-2-yl)-2-((S)-2-(trifluoromethyl)pyrrolidin-1-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (0.275 g, 0.631 mmol, 35.8 % yield) as off-white solid, LCMS (*m/z*): 433.2 [M+H]⁺.

¹H NMR (400 MHz, DMSO-*d*₆): δ ppm 13.60 (s, 1 H), 8.20 - 8.29 (m, 1 H), 8.09 (dt, *J*=8.33, 0.88 Hz, 1 H), 7.72 - 7.81 (m, 1 H), 7.37 (d, *J*=8.55 Hz, 1 H), 7.05 (ddd, *J*=7.29, 4.88, 0.99 Hz, 1 H), 6.45 (d, *J*=8.55 Hz, 1 H), 5.11 - 5.19 (m, 1 H), 4.74 (br s, 1 H), 3.91 (dt, *J*=10.19, 5.21 Hz, 1 H), 3.50 - 3.62 (m, 1 H), 3.27 (d, *J*=1.75 Hz, 1 H), 3.11 - 3.22 (m, 1 H), 3.01 - 3.08 (m, 1 H), 2.81 (br d, *J*=13.37 Hz, 1 H), 2.07 - 2.28 (m, 4 H), 1.94 - 2.02 (m, 1 H), 1.81 - 1.92 (m, 1 H), 1.21 - 1.40 (m, 2 H)

Example 2

Synthesis of (9S)-N-(5-fluoropyridin-3-yl)-2-(2-(trifluoromethyl)pyridin-4-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



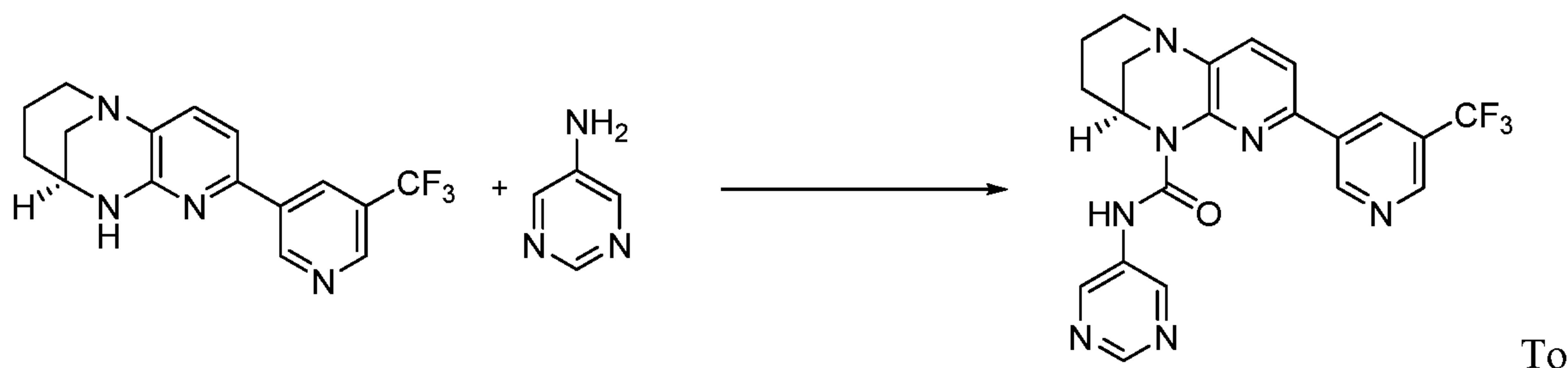
To a stirred solution of (9S)-2-(2-(trifluoromethyl)pyridin-4-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (500 mg, 1.561 mmol) in THF (15 mL) were added triethylamine (0.653 mL, 4.68 mmol) and triphosgene (232 mg, 0.780 mmol) at 30 °C and stirred at room temperature for 1 h. Then 5-fluoropyridin-3-amine (525 mg, 4.68 mmol) was added at 30 °C and reaction was heated at 70 °C for 16 h. The THF evaporated

under reduced pressure and the obtained residue was diluted with water and extracted with CH₂Cl₂ (3x50 mL). The combined organic layer was washed with water, brine, dried over Na₂SO₄ and solvent was evaporated under reduced pressure to obtain the crude compound and it was purified by flash column chromatography (silica-gel: 100-200 mesh, eluent: 3% MeOH in DCM) to afford (9*S*)-*N*-(5-fluoropyridin-3-yl)-2-(2-(trifluoromethyl)pyridin-4-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (200 mg, 0.4366 mmol, 35% yield) as an off-white solid (TLC: R_f = 0.3, 10% MeOH in EtOAc), LCMS (*m/z*): 459.25[M+H]⁺.

¹H NMR (400 MHz, DMSO-*d*₆): δ ppm 13.19 (s, 1 H), 8.93 (d, *J*=5.04 Hz, 1 H), 8.47 (s, 1 H), 8.37 (s, 1 H), 8.33 - 8.21 (m, 2 H), 8.03 (d, *J*=11.18 Hz, 1 H), 7.88 (d, *J*=8.11 Hz, 1 H), 7.78 (d, *J*=8.11 Hz, 1 H), 4.83 (s, 1 H), 3.43 (d, *J*=12.06 Hz, 1 H), 3.25 (s, 2H), 2.90 (d, *J*=13.81 Hz, 1 H), 2.11 - 1.84 (m, 2 H), 1.33 (s, 2 H).

Example 3

Synthesis of (9*S*)-*N*-(pyrimidin-5-yl)-2-(5-(trifluoromethyl)pyridin-3-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide

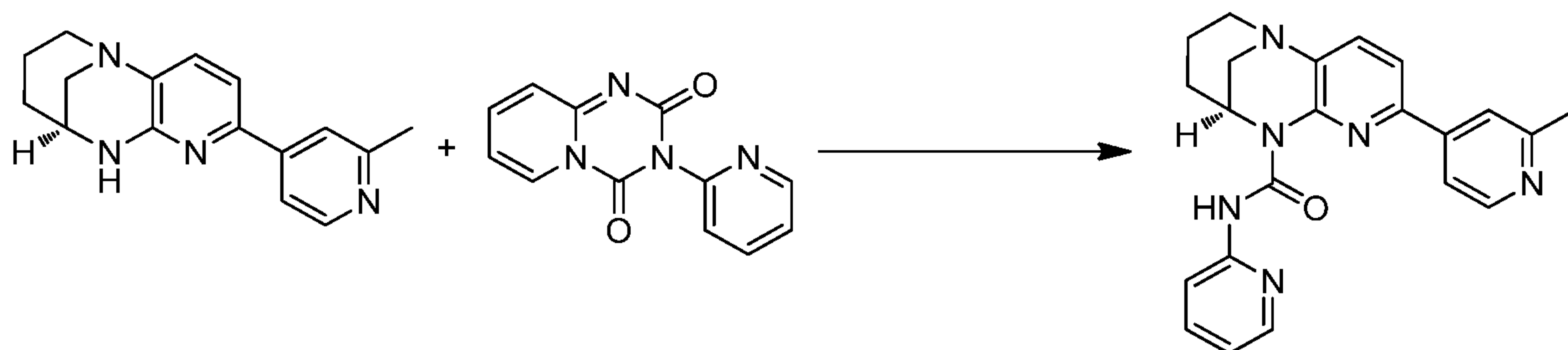


a solution of (9*S*)-2-(5-(trifluoromethyl)pyridin-3-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (0.6 g, 1.873 mmol), triethylamine (0.783 mL, 5.62 mmol) in THF (12 mL) was added triphosgene (0.278 g, 0.937 mmol) at 0 °C and stirred at RT for 30 min. Then Pyrimidine-5-amine was added and the reaction mixture was stirred at 65 °C for 16 h. The solvent was removed under reduced pressure to obtain the crude and diluted with dichloromethane, washed with water and brine solution and dried over anhydrous Na₂SO₄, evaporated the organic layer under reduced pressure to obtain the crude product. The crude mixture was purified by flash column chromatography (silica-gel: 100-200 mesh, eluted with 2% MeOH in CH₂Cl₂) to afford (9*S*)-*N*-(pyrimidin-5-yl)-2-(5-(trifluoromethyl)pyridin-3-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (459 mg, 1.016 mmol, 54.2 % yield) as a white solid (TLC: 80% EtOAc in Hexane, R_f = 0.5), LCMS (*m/z*): 442.2 [M+H]⁺.

¹H NMR (400 MHz, DMSO-*d*₆): δ ppm 13.19 (s, 1 H), 9.48 (d, *J*=2.19 Hz, 1 H), 9.08 (s, 1H), 8.96 (s, 1 H), 8.88 (s, 1 H), 8.71 (s, 1 H), 7.85 (d, *J*=8.11 Hz, 1 H), 7.77 (d, *J*=7.89 Hz, 1 H), 4.84 (s, 1 H), 3.43 (dd, *J*=13.59, 1.97 Hz, 1 H), 3.33 (s, 2 H), 2.90 (d, *J*=13.81 Hz, 2 H), 1.95 (d, *J*=10.30 Hz, 2 H), 1.33 (m, 2 H).

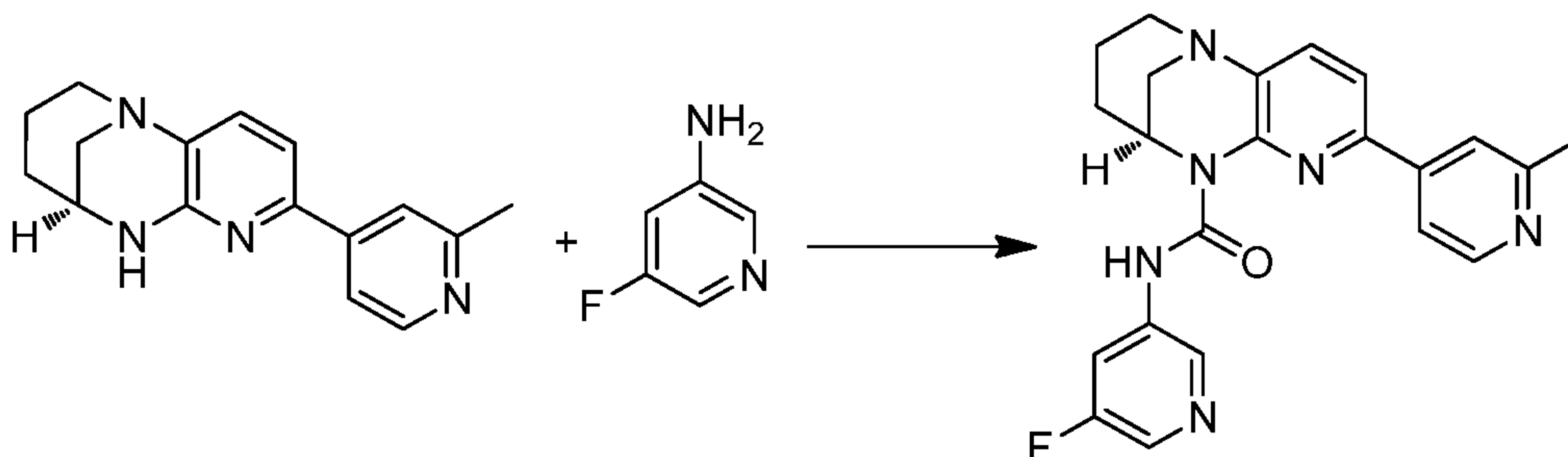
5 Example 4

Synthesis of (9S)-2-(2-methylpyridin-4-yl)-N-(pyridin-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



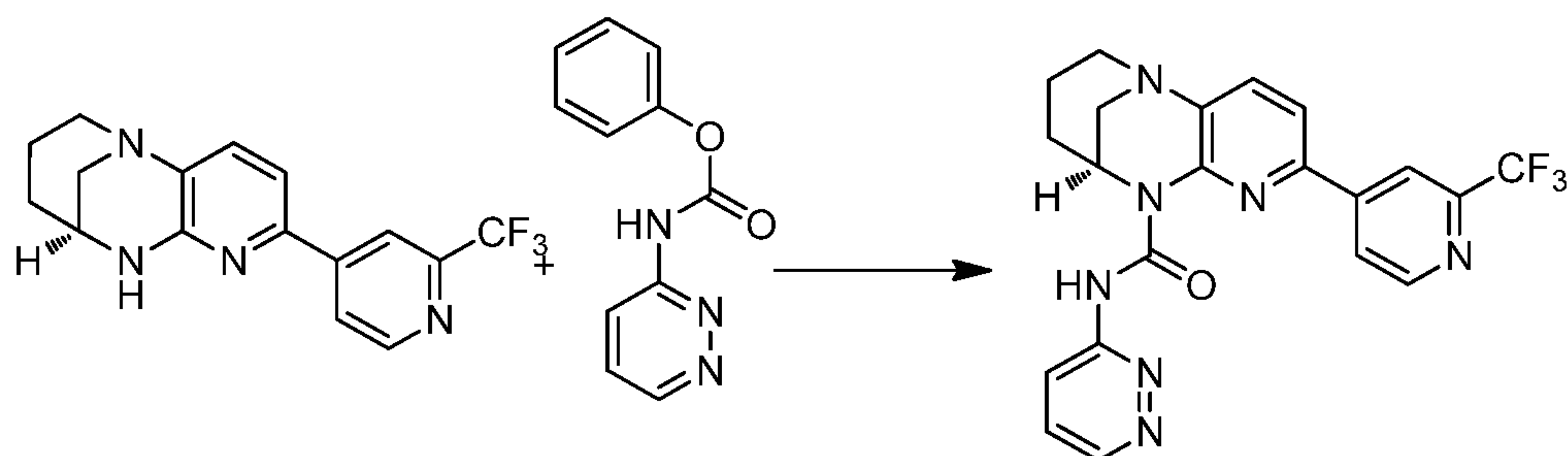
NaH (0.297 g, 12.39 mmol) was added to a stirred solution of (9S)-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (0.550 g, 2.065 mmol) in Tetrahydrofuran (THF) (30 mL) stirred under nitrogen at room temperature. Then the reaction mixture was stirred at room temperature for 30 minutes. 3-(pyridin-2-yl)-2H-pyrido[1,2-a][1,3,5]triazine-2,4(3H)-dione (0.744 g, 3.10 mmol) was added at was added at room temperature. Then the reaction mixture was stirred at 65 °C for 24 hr. The reaction mixture was cooled to 28 °C and was partitioned between water (20 mL) and EtOAc (50 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid (TLC eluent: 70% EtOAc in Hexane: R_f = 0.3; UV active). The crude was purified by column chromatography using (100-200 mesh) silica gel and was eluted with 50-60% EtOAc in Hexane to afford pure (9S)-2-(2-methylpyridin-4-yl)-N-(pyridin-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (0.292 g, 0.751 mmol, 36.4 % yield) as a pale yellow solid, LCMS (*m/z*): 387 [M+H]⁺.

¹H NMR (400 MHz, DMSO-*d*₆): δ ppm 13.99 (s, 1 H), 8.59 (d, *J*=5.48 Hz, 1 H), 8.41 - 8.45 (m, 1 H), 8.27 (s, 1 H), 8.18 (d, *J*=8.33 Hz, 1 H), 7.97 (br d, *J*=3.73 Hz, 1 H), 7.82 - 7.86 (m, 2 H), 7.71 (d, *J*=7.89 Hz, 1 H), 7.11 - 7.16 (m, 1 H), 4.86 (br s, 1 H), 3.40 (br d, *J*=13.37 Hz, 1 H), 3.29 (s, 2 H), 2.91 (br d, *J*=13.15 Hz, 1 H), 2.64 (s, 3 H), 1.93 - 2.05 (m, 2 H), 1.32 (br s, 2 H)

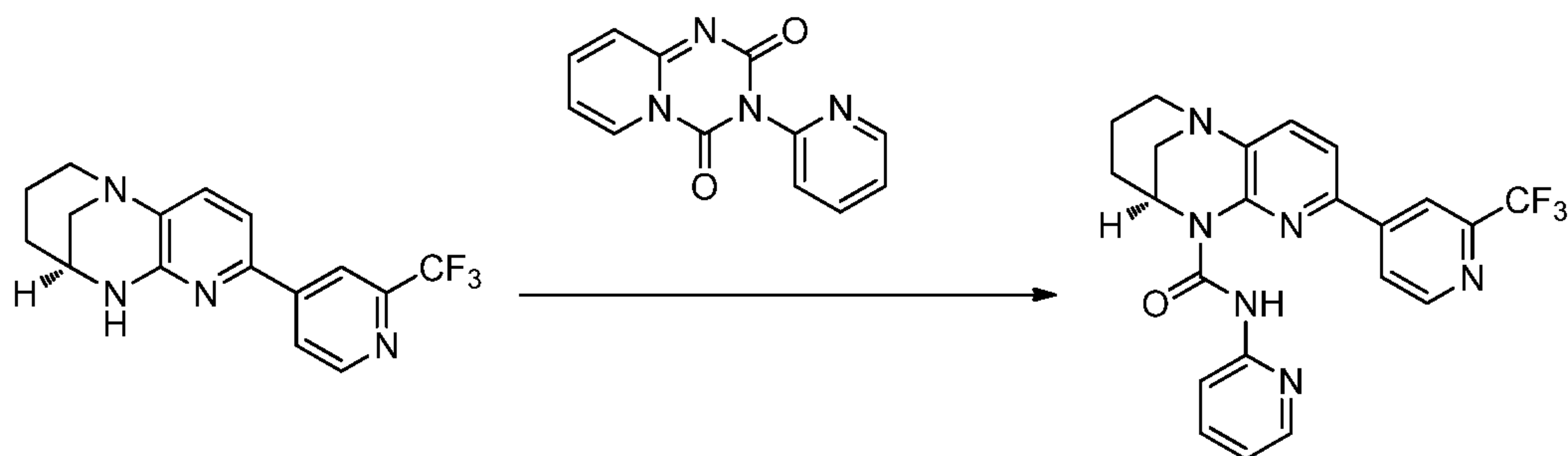
Example 5**Synthesis of (9S)-N-(5-fluoropyridin-3-yl)-2-(2-methylpyridin-4-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide**

- 5 To a solution of (9S)-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (0.6 g, 2.253 mmol) in Tetrahydrofuran (THF) (30 mL) stirred under nitrogen at room temperature was added triethylamine (1.884 mL, 13.52 mmol) and triphosgene (0.669 g, 2.253 mmol). Then reaction mixture was stirred at rt for 30 minutes. Next, 5-fluoropyridin-3-amine (0.758 g, 6.76 mmol) was added at rt. and the
- 10 reaction mixture was stirred at 65 °C for 16 hr. The reaction mixture was cooled to room temp. The reaction mixture concentrated under reduced pressure and then partitioned between water (20 mL) and Dichloromethane (50 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid (TLC eluent: 100% EtOAc: R_f-0.4; UV active). The crude was purified by
- 15 column chromatography using (100-200 mesh) silica gel and was eluted with 100% EtOAc to afford pure (9S)-N-(5-fluoropyridin-3-yl)-2-(2-methylpyridin-4-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (0.314 g, 0.770 mmol, 34.2 % yield) as a pale yellow solid, LCMS (*m/z*): 405.3 [M+H]⁺.

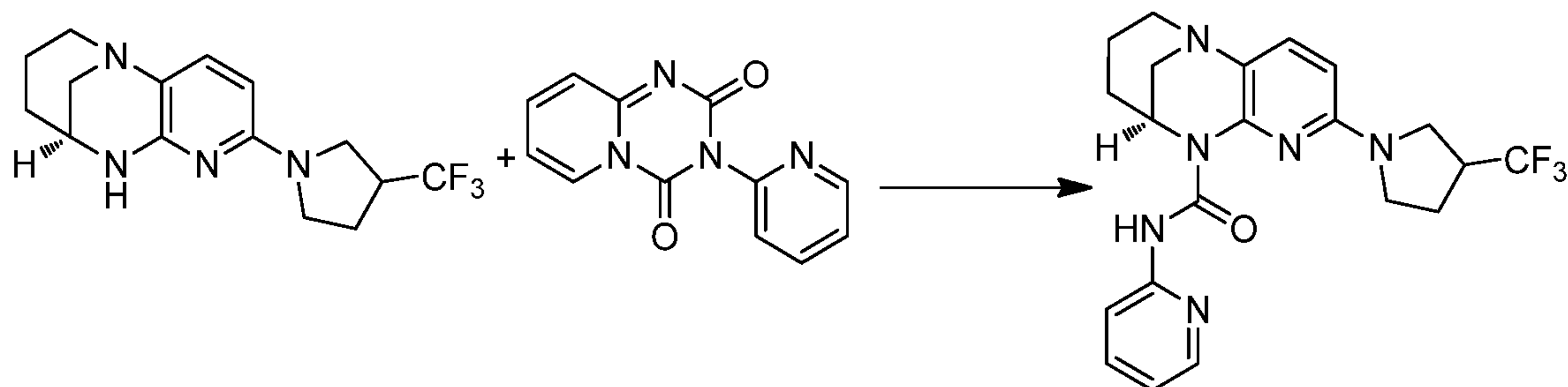
¹H NMR (400 MHz, DMSO-*d*₆): δ 13.71 (s, 1H), 8.74 - 8.55 (m, 1H), 8.44 (d, *J* = 1.7 Hz, 1H), 8.29 (d, *J* = 2.6 Hz, 1H), 8.18 - 7.99 (m, 1H), 7.81 (d, *J* = 1.7 Hz, 1H), 7.73 (d, *J* = 2.8 Hz, 3H), 4.82 (s, 1H), 3.39 (d, *J* = 1.9 Hz, 1H), 3.30 (s, 2H), 2.92 (s, 1H), 2.57 (s, 3H), 2.01 (s, 2H), 1.32 (s, 2H).

Example 6**Synthesis of (9*S*)-*N*-(pyridazin-3-yl)-2-(2-(trifluoromethyl)pyridin-4-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

- 5 To a stirred solution of (9*S*)-2-(2-(trifluoromethyl)pyridin-4-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (0.4 g, 1.249 mmol), phenyl pyridazin-3-ylcarbamate (0.806 g, 3.75 mmol) in THF (8 mL) was added DMAP (0.458 g, 3.75 mmol) at 25°C under nitrogen. The reaction mixture was heated at 80 °C for 16 h. Allowed to cool to RT and the solvent was removed in vacuo to obtain crude was diluted with CH₂Cl₂
- 10 and washed with water, brine solution, dried over anhydrous Na₂SO₄. The organic layer was concentrated in vacuo to obtain the crude compound. The crude mixture was purified by flash column chromatography (silica-gel: 100-200 mesh, eluent: 4% MeOH in CH₂Cl₂) to afford (9*S*)-*N*-(pyridazin-3-yl)-2-(2-(trifluoromethyl)pyridin-4-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (320 mg, 0.711 mmol, 56.9 %
- 15 yield) as a white solid (TLC: 80% EtOAc in Hexane R_f: 0.6), LCMS (*m/z*): 442.2 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆): δ 14.27 (s, 1H), 8.98 (dd, *J* = 4.7, 1.5 Hz, 1H), 8.90 (d, *J* = 5.1 Hz, 1H), 8.59 - 8.48 (m, 2H), 8.38 (dd, *J* = 9.0, 1.5 Hz, 1H), 8.01 (d, *J* = 8.1 Hz, 1H), 7.79 (d, *J* = 8.1 Hz, 1H), 7.71 (dd, *J* = 9.0, 4.7 Hz, 1H), 5.25 - 4.49 (m, 1H), 3.42 (dd, *J* = 13.7, 1.9 Hz, 1H), 3.30 (s, 2H), 2.94 (dt, *J* = 13.8, 2.5 Hz, 1H), 2.02 (m, 2H), 1.34 (q, *J* =
- 20 5.2, 4.4 Hz, 2H).

Example 7**Synthesis of (9*S*)-*N*-(pyridin-2-yl)-2-(2-(trifluoromethyl)pyridin-4-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

- 5 To a solution of (9*S*)-2-(2-(trifluoromethyl)pyridin-4-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (0.250 g, 0.780 mmol) in THF (5 mL) was added sodium hydride (0.156 g, 3.90 mmol) at RT in one charge. The reaction mixture was stirred at RT for 30 min. Then 3-(pyridin-2-yl)-2*H*-pyrido[1,2-*a*][1,3,5]triazine-2,4(3*H*)-dione (0.562 g, 2.341 mmol) was added and stirred the reaction mixture at 65 °C for 16 h.
- 10 Allowed to cool to room temperature and quenched it with ice cold water, extracted with ethyl acetate (3x50 mL). The combined organic layer was washed with water, saturated brine solution, dried over sodium sulfate and concentrated under reduced pressure to obtain crude product. The crude mixture was purified by flash column chromatography (silica-gel: 100-200 mesh, eluent: 2% MeOH in CH₂Cl₂) to afford (9*S*)-*N*-(pyridin-2-yl)-2-(2-(trifluoromethyl)pyridin-4-yl)-8,9-dihydro-6*H*-5,9-methano pyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (302 mg, 0.681 mmol, 87 % yield) as a white solid (TLC: 70% EtOAc in Hexane, R_f: 0.5), LCMS (*m/z*): 441.3 [M+H]⁺.
- 15 ¹H NMR (400 MHz, DMSO-*d*₆): δ 13.85 (s, 1H), 8.94 (d, *J* = 5.2 Hz, 1H), 8.64 (dd, *J* = 1.7, 0.9 Hz, 1H), 8.55 - 8.50 (m, 1H), 8.34 (ddd, *J* = 4.8, 2.0, 0.9 Hz, 1H), 8.15 (dt, *J* = 8.3, 1.0 Hz, 1H), 7.98 (d, *J* = 8.1 Hz, 1H), 7.86 - 7.79 (m, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.12 (ddd, *J* = 7.3, 4.9, 1.0 Hz, 1H), 4.87 (t, *J* = 2.6 Hz, 1H), 3.41 (dd, *J* = 13.6, 1.9 Hz, 1H), 3.30 (s, 2H), 2.92 (d, *J* = 14.0 Hz, 1H), 2.09 - 1.86 (m, 2H), 1.34 (d, *J* = 8.5 Hz, 2H).
- 20

Example 8**Synthesis of (9S)-N-(pyridin-2-yl)-2-(3-(trifluoromethyl)pyrrolidin-1-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide**

- 5 NaH (0.277 g, 11.53 mmol) was added to a solution of (9S)-2-(3)pyrrolidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-metha-(trifluoromethyl nopyrido[2,3-b][1,4]diazocine (0.6 g, 1.921 mmol) in Tetrahydrofuran (THF) (20 mL) and stirred for 30 min. Then 3-(pyridin-2-yl)-2H-pyrido[1,2-a][1,3,5]triazine-2,4(3H)-dione (0.692 g, 2.88 mmol) was added at room temp. Then reaction mixture was stirred at 65 °C for 24 h. The reaction mixture was
- 10 cooled to RT and was partitioned between water (20 mL) and EtOAc (50 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid (TLC eluent: 70% EtOAc in Hexane: R_f 0.3; UV active). The crude was purified by column chromatography using (100-200 mesh) silica gel and was eluted with 50-60% EtOAc in hexane to afford mixture of stereoisomers
- 15 (9:1) which on further SFC purification obtained pure (9S)-N-(pyridin-2-yl)-2-(3-(trifluoromethyl)pyrrolidin-1-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (0.422 g, 0.973 mmol, 50.6 % yield) as an off-white solid, LCMS (*m/z*): 433.3 [M+H]⁺.

Analytical SFC Conditions: Peak-I Column/dimensions: Chiralcel OX-H (250 X

20 4.6)mm,5u

% CO₂ : 60.0%

% Co solvent : 40.0% (0.5% DEA IN MeOH)

Total Flow : 4.0g/min

Back Pressure : 100 bar

25 Temperature : 26.8°C

UV : 263nm

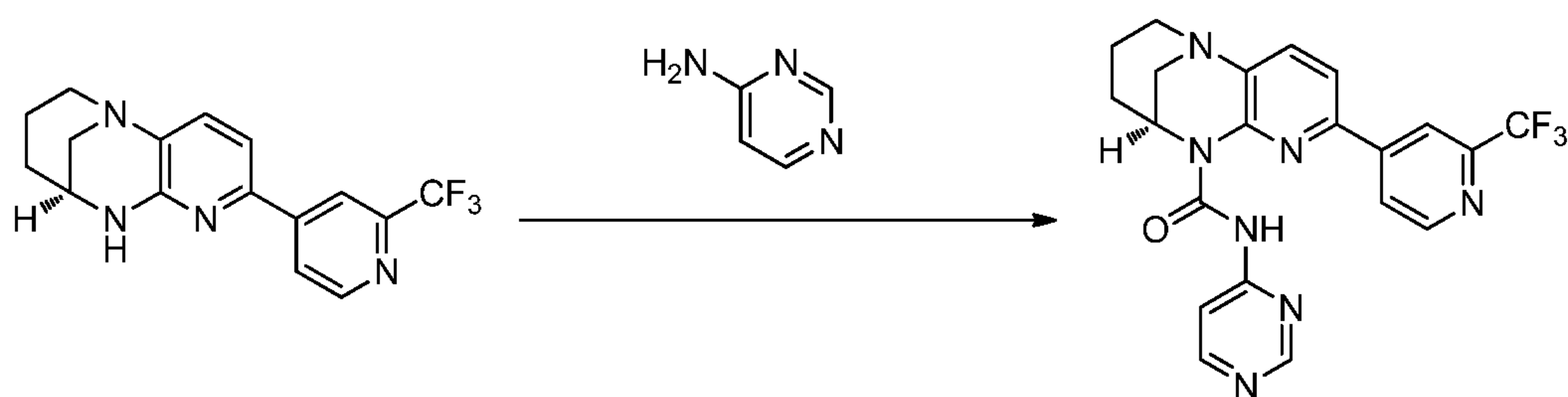
¹H NMR (400 MHz, DMSO-*d*₆): δ 13.81 (s, 1H), 8.23 - 8.18 (m, 1H), 8.11 (dt, *J* = 8.3, 1.0 Hz, 1H), 7.76 (ddd, *J* = 8.5, 7.4, 1.9 Hz, 1H), 7.32 (d, *J* = 8.5 Hz, 1H), 7.04 (ddd, *J* = 7.3,

4.9, 1.0 Hz, 1H), 6.24 (d, $J = 8.5$ Hz, 1H), 4.71 (s, 1H), 3.96 (d, $J = 8.5$ Hz, 1H), 3.78 (d, $J = 7.0$ Hz, 1H), 3.74 (s, 1H), 3.66 (d, $J = 4.1$ Hz, 2H), 3.59 - 3.38 (m, 1H), 3.26 (d, $J = 1.9$ Hz, 1H), 3.12 (dd, $J = 12.7, 3.5$ Hz, 1H), 3.05 (s, 1H), 2.80 (d, $J = 13.4$ Hz, 1H), 2.41 - 2.29 (m, 1H), 2.15 (dd, $J = 12.7, 8.1$ Hz, 1H), 1.96 (s, 1H), 1.83 (s, 2H), 1.31 (s, 2H).

5

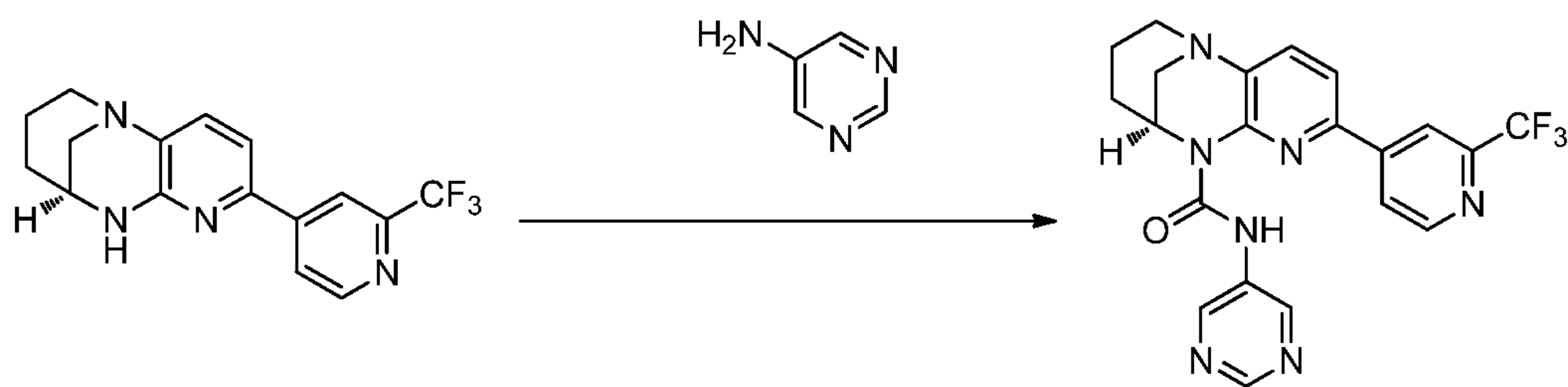
Example 9

Synthesis of (9*S*)-*N*-(pyrimidin-4-yl)-2-(2-(trifluoromethyl)pyridin-4-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide

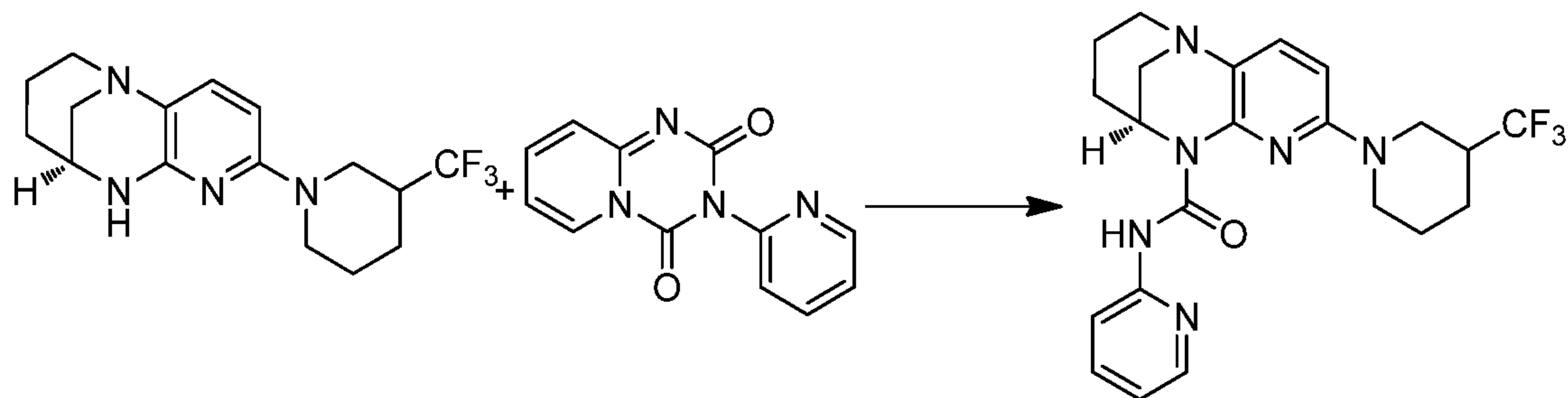


10 (9*S*)-2-(2-(trifluoromethyl)pyridin-4-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (0.5 g, 1.561 mmol), triethylamine (0.653 mL, 4.68 mmol) in THF (10 mL) stirred under nitrogen at 25°C was added bis(trichloromethyl) carbonate (0.232 g, 0.780 mmol) in one charge. The reaction mixture was stirred at 25 °C for 30 min. Then pyrimidin-4-amine (0.445 g, 4.68 mmol) was added in a portion, reaction was stirred at 65
15 °C for 16 h. Allowed to cool to RT and solvent was removed under vacuo, crude was diluted with DCM, washed with water, brine solution, dried over sodium sulfate and concentrated under vacuum to obtain crude compound. The crude mixture was purified by prep HPLC to afford (9*S*)-*N*-(pyrimidin-4-yl)-2-(2-(trifluoromethyl)pyridin-4-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (199 mg, 0.446
20 mmol, 28.6% yield) as a white solid (TLC: 70%EtOAc in Hexane, R_f : 0.6), LCMS (m/z): 442.3 $[M+H]^+$.

¹H NMR (400 MHz, DMSO-*d*₆): δ 14.18 (s, 1H), 8.97 (d, $J = 5.1$ Hz, 1H), 8.85 (d, $J = 1.2$ Hz, 1H), 8.69 (d, $J = 5.8$ Hz, 1H), 8.60 (dd, $J = 1.9, 0.8$ Hz, 1H), 8.50 (dd, $J = 5.1, 1.7$ Hz, 1H), 8.12 (dd, $J = 5.8, 1.3$ Hz, 1H), 8.02 (d, $J = 8.1$ Hz, 1H), 7.80 (d, $J = 8.1$ Hz, 1H), 4.93 - 4.73 (m, 1H), 3.42 (dd, $J = 13.7, 1.9$ Hz, 1H), 3.32 (d, $J = 8.2$ Hz, 2H), 2.93 (dt, $J = 13.9, 2.5$ Hz, 1H), 2.12 - 1.83 (m, 2H), 1.33 (d, $J = 7.8$ Hz, 2H).

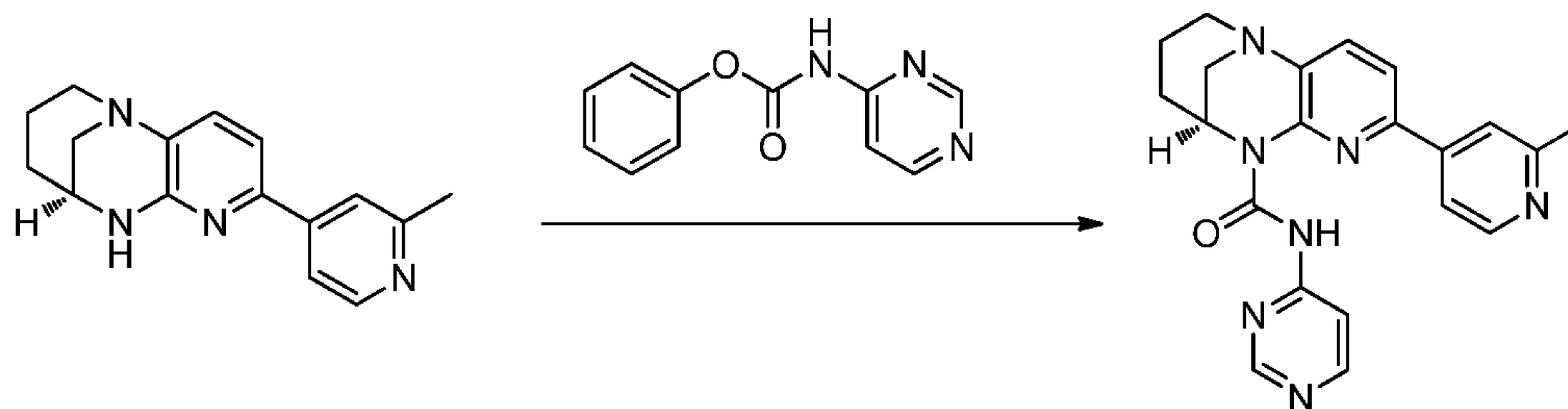
Example 10**Synthesis of (9*S*)-*N*-(pyrimidin-5-yl)-2-(2-(trifluoromethyl)pyridin-4-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

- 5 To a solution of (9*S*)-2-(2-(trifluoromethyl)pyridin-4-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (0.5 g, 1.561 mmol), triethylamine (0.653 mL, 4.68 mmol) in THF (10 mL) was added bis(trichloromethyl) carbonate (0.232 g, 0.780 mmol) at 25 °C under nitrogen atmosphere and stirred at RT for 30 min. Then pyrimidin-5-amine (0.445 g, 4.68 mmol) was added and heated the reaction mixture at 65 °C for 16 h.
- 10 Allowed to cool to RT and solvent was removed on rota-vapour, the crude was diluted with CH₂Cl₂ (20ml) and washed with water (5ml), brine solution (5ml) followed by dried over sodium sulfate. The organic solvent was evaporated under reduced pressure to obtain the crude product. The crude mixture was purified by prep-HPLC (formic acid, ACN 25%) to afford (9*S*)-*N*-(pyrimidin-5-yl)-2-(2-(trifluoromethyl)pyridin-4-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (250 mg, 0.56 mmol, 45% yield) as an off white solid (TLC: 80% EtOAc in Hexane, *R_f*: 0.5), LCMS (*m/z*): 442.3 [M+H]⁺.
- 15 ¹H NMR (400 MHz, DMSO-*d*₆): δ 13.07 (s, 1H), 9.08 - 8.72 (m, 4H), 8.38 (dd, *J* = 1.7, 0.9 Hz, 1H), 8.28 (dd, *J* = 5.2, 1.6 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 4.85 (t, *J* = 2.7 Hz, 1H), 3.43 (dd, *J* = 13.7, 1.9 Hz, 1H), 3.33 (d, *J* = 10.1 Hz, 2H), 2.91 (d, *J* = 13.7 Hz, 1H), 2.15 - 1.79 (m, 2H), 1.33 (s, 2H).
- 20

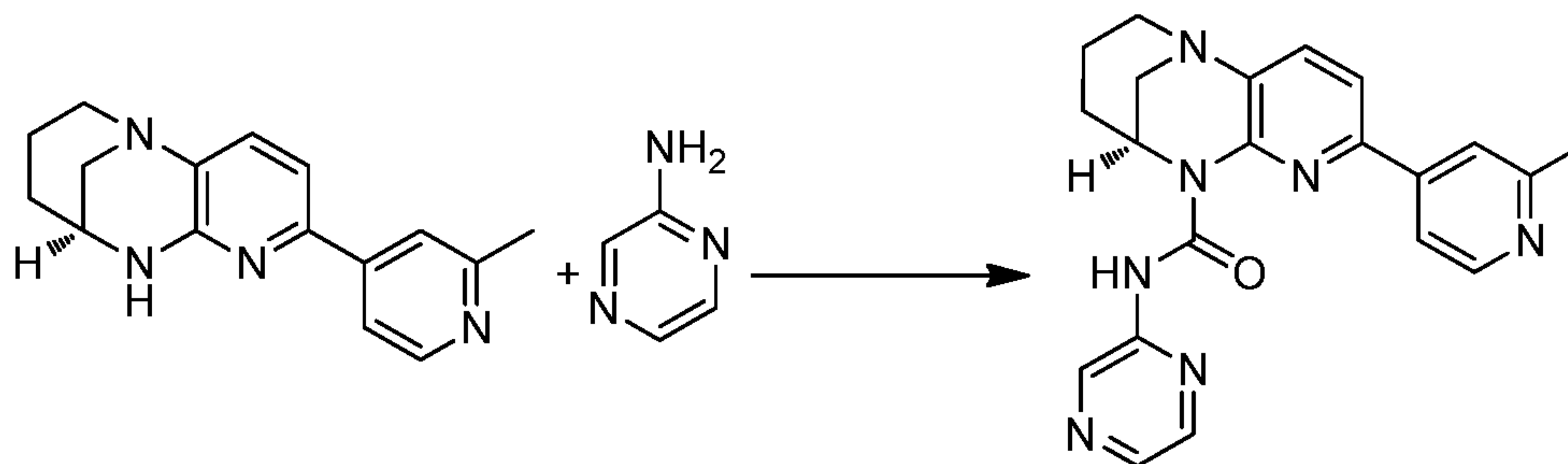
Example 11**Synthesis of (9S)-N-(pyridin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide**

- 5 NaH (0.110 g, 4.60 mmol) was added to a stirred solution of (9S)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (Peak-II from the intermediate SFC separation, 0.250 g, 0.766 mmol) in Tetrahydrofuran (THF) (20 mL) stirred under nitrogen at room temp. The reaction mixture was stirred at room temp for 30 minutes. Then 3-(pyridin-2-yl)-2H-pyrido[1,2-a][1,3,5]triazine-2,4(3H)-dione (0.276 g, 1.149 mmol) was added at room temp°C. Then reaction mixture was stirred at 65 °C for 24 hr. Reaction was cooled to room temperature and was partitioned between water (10 mL) and EtOAc (50 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid (TLC eluent: 70% EtOAc in Hexane: R_f 0.3; UV active). The crude was purified by column chromatography using (100-200 mesh) silica gel and was eluted with 50-60% EtOAc in Hexane to afford pure (9S)-N-(pyridin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (0.2 g, 0.439 mmol, 57.3 % yield) as a off-white solid, LCMS (*m/z*): 447.2 [M+H]⁺.
- 20 ¹H NMR (400 MHz, DMSO-*d*₆): δ 13.50 (s, 1H), 8.32 - 8.18 (m, 1H), 8.16 - 8.03 (m, 1H), 7.76 (ddd, *J* = 8.8, 7.4, 2.0 Hz, 1H), 7.35 (d, *J* = 8.6 Hz, 1H), 7.05 (dd, *J* = 7.8, 4.5 Hz, 1H), 6.60 (d, *J* = 8.7 Hz, 1H), 4.72 (d, *J* = 2.7 Hz, 1H), 4.27 (s, 2H), 3.31 - 3.24 (m, 1H), 3.22 - 2.89 (m, 4H), 2.80 (d, *J* = 13.3 Hz, 1H), 2.51 (s, 1H), 1.99 (t, *J* = 12.7 Hz, 2H), 1.85 (d, *J* = 11.6 Hz, 2H), 1.75 - 1.43 (m, 2H), 1.25 (s, 2H).

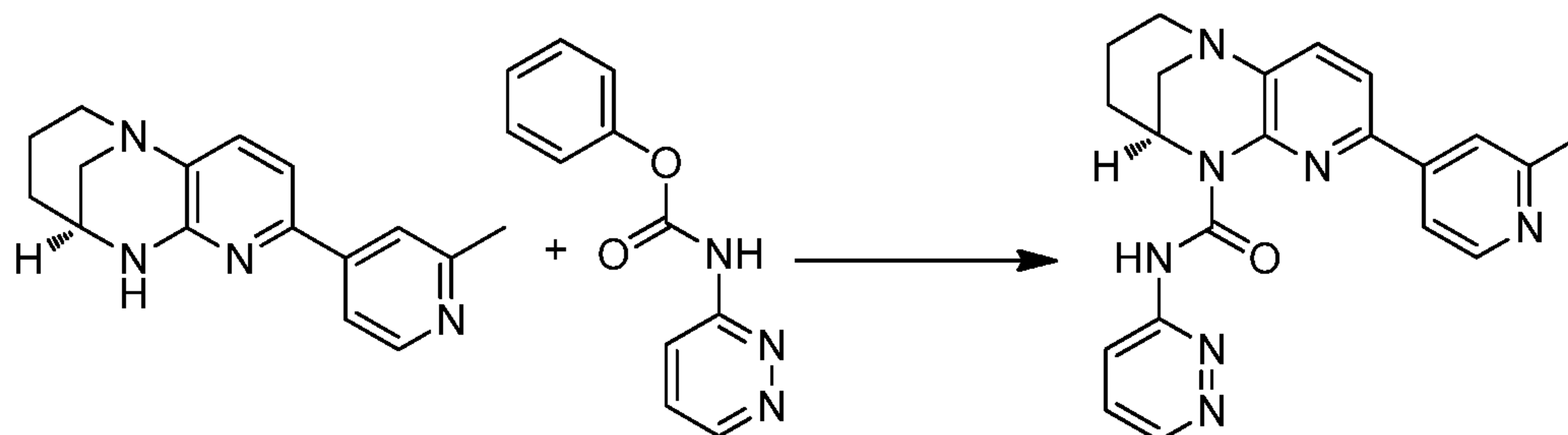
25

Example 12**Synthesis of (9*S*)-2-(2-methylpyridin-4-yl)-*N*-(pyrimidin-4-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

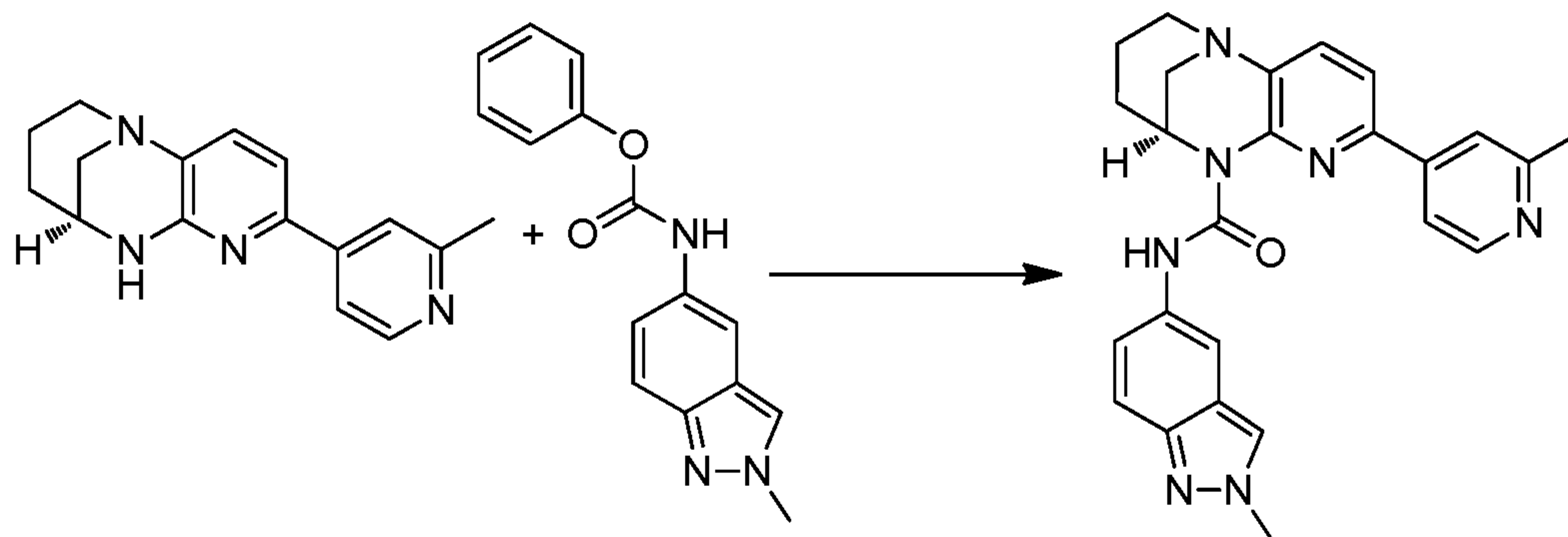
- 5 To a stirred solution of (9*S*)-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (0.350 g, 1.314 mmol), DMAP (0.482 g, 3.94 mmol) in THF (7 mL) was added phenyl pyrimidin-4-ylcarbamate (0.848 g, 3.94 mmol) at 25°C. The reaction mixture was stirred at 80 °C for 16 h. The solvent was removed under vacuo and crude was diluted with CH₂Cl₂ (20ml) and washed with water (5 mL), saturated brine
- 10 solution (5 mL) and dried over Na₂SO₄. The organic solvent was evaporated under reduced pressure to obtain the crude product. The crude mixture was purified by flash column chromatography (silica-gel: 100-200 mesh, eluted with 3% MeOH in CH₂Cl₂) to afford (9*S*)-2-(2-methylpyridin-4-yl)-*N*-(pyrimidin-4-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (207 mg, 0.529 mmol, 40.3 %
- 15 yield) as a white solid (TLC: 10% MeOH in DCM, R_f: 0.7), LCMS (*m/z*): 388.3 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆): δ 14.32 (s, 1H), 8.95 (d, *J* = 1.3 Hz, 1H), 8.69 (d, *J* = 5.8 Hz, 1H), 8.61 (d, *J* = 5.3 Hz, 1H), 8.17 (d, *J* = 1.7 Hz, 1H), 8.15 (dd, *J* = 5.8, 1.3 Hz, 1H), 7.95 (dd, *J* = 5.3, 1.8 Hz, 1H), 7.86 (d, *J* = 8.1 Hz, 1H), 7.74 (d, *J* = 8.1 Hz, 1H), 4.90 - 4.67 (m, 1H), 3.40 (dd, *J* = 13.7, 1.9 Hz, 1H), 3.28 (s, 2H), 3.00 - 2.81 (m, 1H), 2.64 (s,
- 20 3H), 2.13 - 1.71 (m, 2H), 1.41 - 1.14 (m, 2H).

Example 13**Synthesis of (9S)-2-(2-methylpyridin-4-yl)-N-(pyrazin-2-yl)-8,9-dihydro-6H-5,9-methanopyrido [2,3-b] [1,4] diazocine-10(7H)-carboxamide**

- 5 To a solution of (9S)-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (0.700 g, 2.63 mmol) in Tetrahydrofuran (THF) (30 mL) stirred under nitrogen at room temperature °C was added triethylamine (2.198 mL, 15.77 mmol) and triphosgene (0.780 g, 2.63 mmol). Then reaction mixture was stirred at room temperature for 30 minutes. pyrazin-2-amine (0.750 g, 7.88 mmol) was added at rt.
- 10 Then the reaction mixture was stirred at 65 °C for 24 hr. The reaction mixture was concentrated to dryness and then partitioned between water (20 mL) and Dichloromethane (50 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid. The crude was purified by column chromatography using (100-200 mesh) silica gel to afford pure (9S)-2-(2-methylpyridin-4-yl)-N-(pyrazin-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide as a pale yellow solid, LCMS (*m/z*): 388.3 [M+H]⁺.
- 15 ¹H NMR (400 MHz, CDCl₃): δ 14.27 (s, 1H), 9.59 (d, *J* = 0.9 Hz, 1H), 8.67 - 8.60 (m, 1H), 8.32 (d, *J* = 1.0 Hz, 2H), 8.10 (dt, *J* = 1.9, 0.7 Hz, 1H), 7.74 - 7.67 (m, 1H), 7.62 - 7.50 (m, 2H), 5.06 - 5.00 (m, 1H), 3.51 - 3.27 (m, 3H), 3.03 (d, *J* = 13.6 Hz, 1H), 2.74 (s, 3H), 2.26 (s, 1H), 2.08 - 1.84 (m, 1H), 1.43 (d, *J* = 6.2 Hz, 2H).
- 20

Example 14**Synthesis of (9*S*)-2-(2-methylpyridin-4-yl)-*N*-(pyridazin-3-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

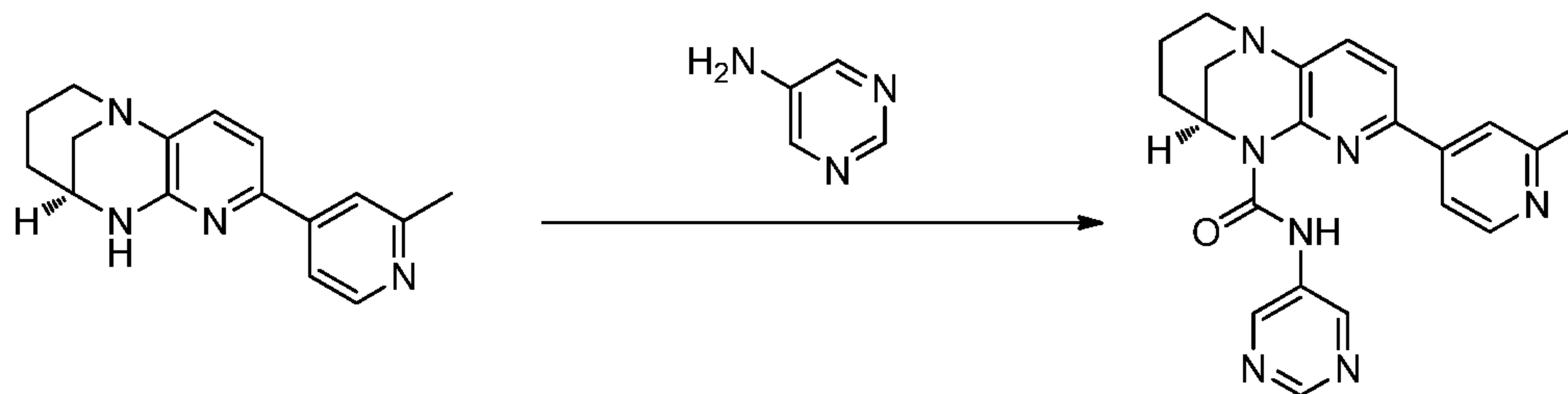
- 5 To a stirred solution of (9*S*)-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (0.150 g, 0.563 mmol), phenyl pyridazin-3-ylcarbamate (0.364 g, 1.690 mmol) in THF (3 mL) was added DMAP (0.206 g, 1.690 mmol) at 25 °C. The reaction mixture was stirred at 80 °C for 16 h. Solvent was removed under reduced pressure, crude compound was diluted with DCM (10ml), washed with
- 10 water(3ml), saturated brine solution(3ml), dried over Na₂SO₄ and concentrated under reduced pressure to obtain crude product. The crude mixture was purified by flash column chromatography (silica-gel: 100-200 mesh, eluent: 2% MeOH in CH₂Cl₂) to afford (9*S*)-2-(2-methylpyridin-4-yl)-*N*-(pyridazin-3-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (271 mg, 0.692 mmol, 123 % yield) as a white solid
- 15 (TLC: 10% MeOH in CH₂Cl₂, R_f: 0.6), LCMS (*m/z*): 388.3 [M+H]⁺.
- ¹H NMR (400 MHz, CDCl₃): δ 14.66 (s, 1H), 8.94 (dd, *J* = 4.7, 1.5 Hz, 1H), 8.66 (dd, *J* = 5.2, 0.8 Hz, 1H), 8.50 (dd, *J* = 9.1, 1.5 Hz, 1H), 8.29 (d, *J* = 1.8 Hz, 1H), 7.74 (dd, *J* = 5.4, 1.8 Hz, 1H), 7.57 (d, *J* = 4.3 Hz, 2H), 7.46 (dd, *J* = 9.0, 4.7 Hz, 1H), 5.00 (p, *J* = 2.6 Hz, 1H), 3.50 - 3.22 (m, 3H), 3.12 - 2.96 (m, 1H), 2.82 (s, 3H), 2.39 - 2.10 (m, 1H), 1.95 (tdd, *J* = 14.2, 5.5, 3.0 Hz, 1H), 1.58 - 1.33 (m, 2H).
- 20

Example 15**Synthesis of (9S)-N-(2-methyl-2H-indazol-5-yl)-2-(2-methylpyridin-4-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide**

- 5 DMAP (0.550 g, 4.51 mmol) was added to a stirred solution of (9S)-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (0.4 g, 1.502 mmol) and phenyl (2-methyl-2H-indazol-5-yl)carbamate (0.803 g, 3.00 mmol) in Tetrahydrofuran (THF) (30 mL) under nitrogen atmosphere at room temp. The reaction was stirred at 70 °C for 48 hr. Reaction was cooled to room temperature and concentrated under reduced
- 10 pressure. Added EtOAc to reaction mass and stirred for 10 minutes before being filtered and the solids washed with EtOAc. Take filtrate and concentrated to give the crude as brown solid (TLC eluent: 80% EtOAc: R_f 0.4; UV active). The crude was purified by column chromatography using neutral alumina and was eluted with 60% EtOAc in Hexane to afford pure afford (9S)-N-(2-methyl-2H-indazol-5-yl)-2-(2-methylpyridin-4-yl)-8,9-
- 15 dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (0.18 g, 0.406 mmol, 27.1 % yield) as a off-white solid, LCMS (m/z): 440.4 $[M+H]^+$.

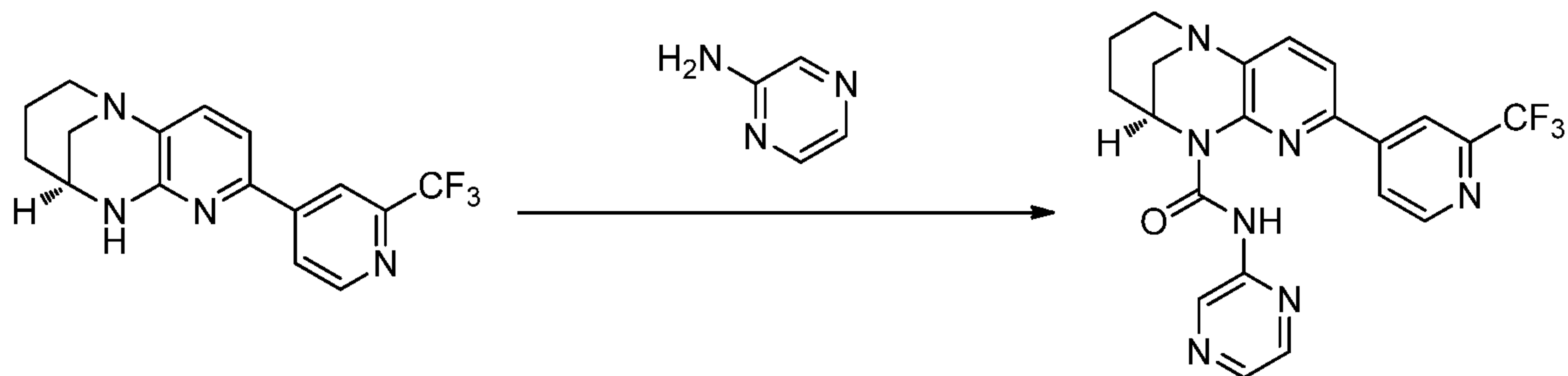
$^1\text{H NMR}$ (400 MHz, CDCl_3): δ 13.44 (s, 1H), 8.66 (d, $J = 5.2$ Hz, 1H), 8.18 (dd, $J = 1.9$, 0.8 Hz, 1H), 7.82 (d, $J = 0.9$ Hz, 1H), 7.69 (d, $J = 1.6$ Hz, 1H), 7.63 (d, $J = 9.1$ Hz, 1H), 7.60 - 7.50 (m, 2H), 7.40 (d, $J = 7.9$ Hz, 1H), 7.27 - 7.19 (m, 1H), 5.05 (t, $J = 2.7$ Hz, 1H), 4.20 (s, 3H), 3.50 - 3.23 (m, 3H), 3.01 (d, $J = 13.5$ Hz, 1H), 2.67 (s, 3H), 2.28 (s, 1H), 1.93 (s, 1H), 1.58 (s, 2H).

20

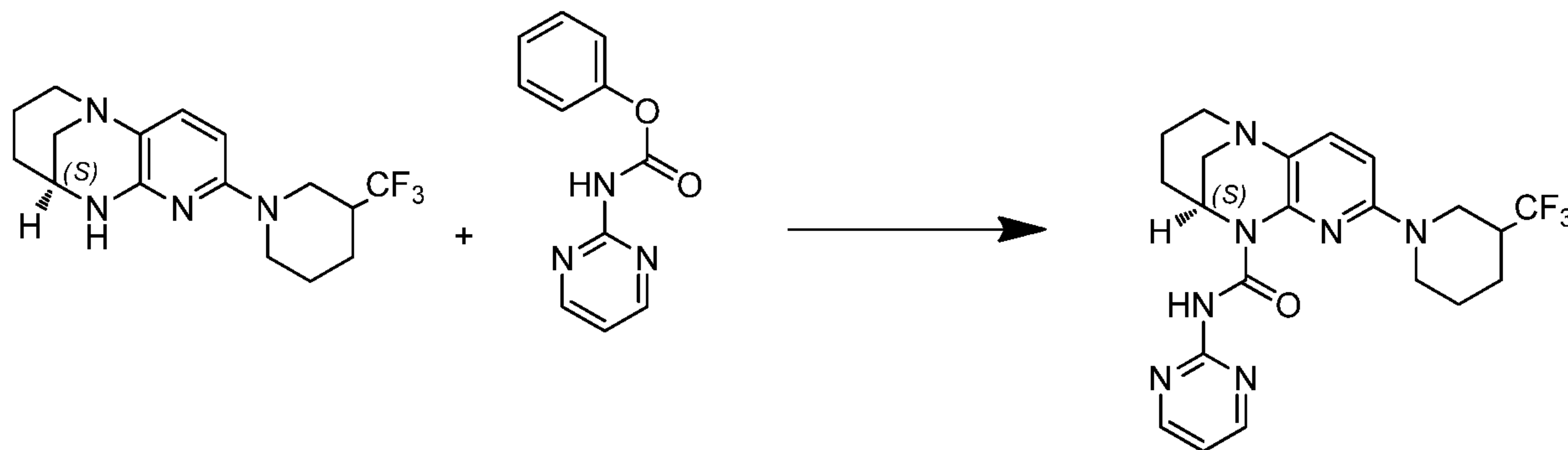
Example 16**Synthesis of (9*S*)-2-(2-methylpyridin-4-yl)-*N*-(pyrimidin-5-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

5 (9*S*)-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (0.6 g, 2.253 mmol), triethylamine (0.942 mL, 6.76 mmol) and bis(trichloromethyl) carbonate (0.334 g, 1.126 mmol) in THF (12 mL) stirred under nitrogen at room temperature for 30 min. min and then added pyrimidin-5-amine (0.643 g, 6.76 mmol) in one charge. The reaction mixture was stirred at 65 °C for 16 h. The solvent
10 was removed under reduced pressure and the obtained crude was diluted with CH₂Cl₂ (35ml), washed with water (5 mL), saturated brine solution (5 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to obtain the crude product. The crude mixture was purified by flash column chromatography (silica-gel: 100-200 mesh, eluent: 3% MeOH in CH₂Cl₂) to obtained semi pure compound. The semi pure compound was
15 again purified by Prep HPLC (30% ACN/ Water) to afford (9*S*)-2-(2-methylpyridin-4-yl)-*N*-(pyrimidin-5-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (261 mg, 0.667 mmol, 29.6 % yield) as an white solid (TLC: 10% MeOH in CH₂Cl₂, R_f: 0.4), LCMS (*m/z*): 388 [M+H]⁺.

¹H NMR (400 MHz, CDCl₃): δ 13.81 (s, 1H), 9.03 (s, 2H), 8.94 (s, 1H), 8.69 (dd, *J* = 5.2, 0.9 Hz, 1H), 7.75 - 7.55 (m, 2H), 7.55 - 7.46 (m, 1H), 7.43 (d, *J* = 8.0 Hz, 1H), 5.08 - 4.89 (m, 1H), 3.49 - 3.17 (m, 3H), 3.02 (d, *J* = 13.7 Hz, 1H), 2.69 (s, 3H), 2.36 - 2.13 (m, 1H), 2.06 - 1.79 (m, 1H), 1.59 - 1.32 (m, 2H).

Example 17**Synthesis of (9*S*)-*N*-(pyrazin-2-yl)-2-(2-(trifluoromethyl)pyridin-4-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

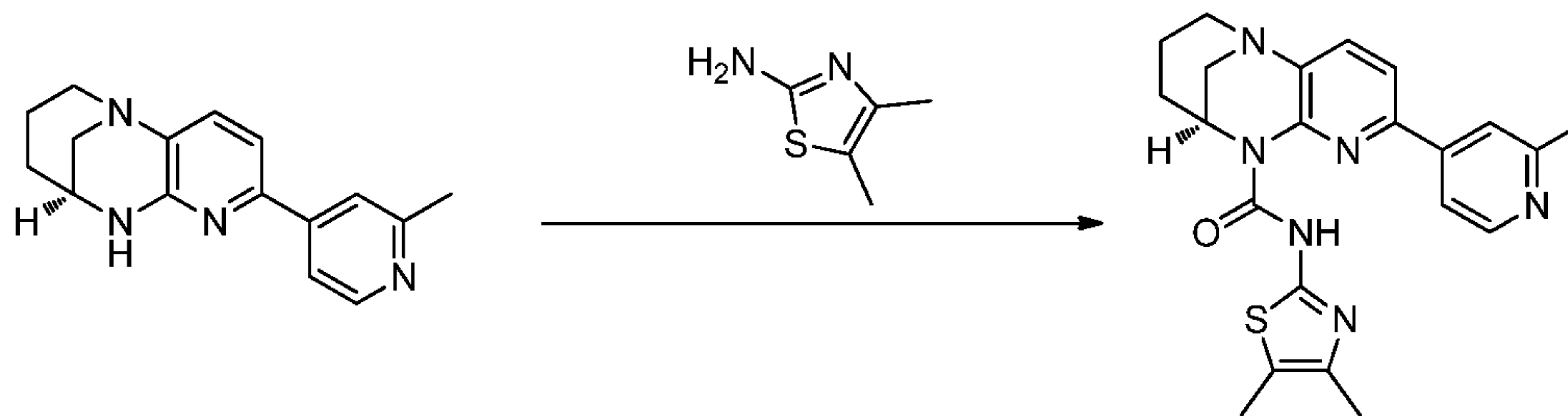
- 5 To a stirred solution of (9*S*)-2-(2-(trifluoromethyl)pyridin-4-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (0.9 g, 2.81 mmol) in THF (18 mL) was added bis(trichloromethyl) carbonate (0.417 g, 1.405 mmol) at RT and stirred for 30 min. Then Pyrazine-2-amine and Et₃N (2.94 ml, 21.8 mmol) were added. The reaction mixture was stirred at 65 °C for 16 h. The organic solvent was removed under reduced pressure and
- 10 CH₂Cl₂ (30 ml). The organic layer was washed with water (5ml), saturated brine solution (5ml), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to obtain the crude product. The crude mixture was purified by flash column chromatography (silica-gel: 100-200 mesh, eluent: 4% MeOH in DCM) to afford (9*S*)-*N*-(pyrazin-2-yl)-2-(2-(trifluoromethyl)pyridin-4-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-
- 15 10(7*H*)-carboxamide (476 mg, 1.059 mmol, 37.7% yield) as a white solid (TLC: 80% EtOAc in Hexane, R_f: 0.5), LCMS (*m/z*): 442.2 [M+H]⁺.
- ¹H NMR (400 MHz, CDCl₃): δ 14.06 (s, 1H), 9.56 (d, *J* = 1.4 Hz, 1H), 8.87 (d, *J* = 5.1 Hz, 1H), 8.46 (dd, *J* = 1.8, 0.9 Hz, 1H), 8.37 - 8.23 (m, 2H), 8.14 (dd, *J* = 5.1, 1.7 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.56 (s, 1H), 5.15 - 4.92 (m, 1H), 3.52 - 3.26 (m, 3H), 3.03 (d, *J* =
- 20 14.0 Hz, 1H), 2.26 (s, 1H), 2.05 - 1.88 (m, 1H), 1.53 - 1.29 (m, 2H).

Example 18**Synthesis of (9*S*)-N-(pyrimidin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

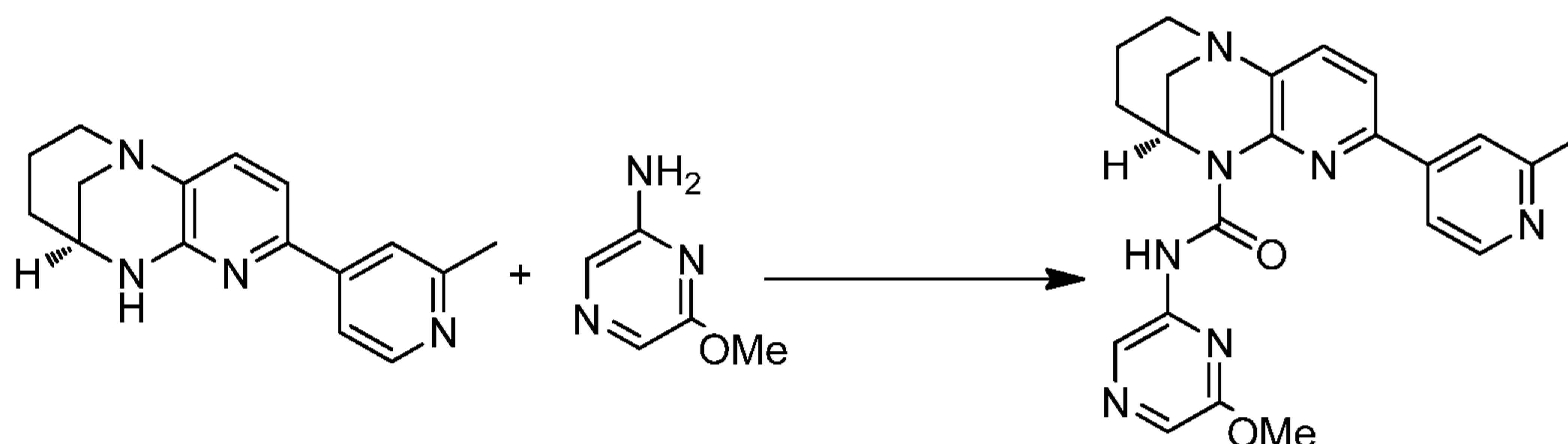
- 5 To a solution of (9*S*)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (Peak1 from the intermediate SFC separation 500 mg, 1.532 mmol) in tetrahydrofuran (500 mL), DMAP (562 mg, 4.60 mmol) and phenyl 2-(pyrimidin-2-yl)acetate (985 mg, 4.60 mmol) were added. The reaction mixture was stirred at 65 °C for 28 hr. The reaction mixture was diluted with water (50 mL) and
- 10 extracted with EtOAc (2 X 75 mL). The combined organic layer was washed with water and saturated with brine solution and dried over anhydrous Na₂SO₄, filtered and concentrated to give the crude as white solid (TLC eluent: 10% MeOH in DCM : *R_f* 0.4; UV active). The crude product was purified by column chromatography (neutral alumina) and the product was eluted with 25% ethyl acetate in hexane to afford (9*S*)-N-(pyrimidin-
- 15 2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6*H*-5,9-methano-pyrido [2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (320 mg, 0.711 mmol, 46.4 % yield) as off white solid. LCMS (*m/z*) 448.3 [M+H]⁺.

¹H NMR (400 MHz, DMSO-*d*₆): δ 13.80 (s, 1H), 8.62 (d, *J* = 4.8 Hz, 2H), 7.36 (d, *J* = 8.6 Hz, 1H), 7.15 (t, *J* = 4.8 Hz, 1H), 6.61 (d, *J* = 8.6 Hz, 1H), 4.69 (s, 1H), 4.30 (d, *J* = 12.7 Hz, 1H), 4.20 (d, *J* = 13.0 Hz, 1H), 3.26 (d, *J* = 1.9 Hz, 1H), 3.20 - 2.89 (m, 4H), 2.79 (d, *J* = 13.4 Hz, 1H), 2.55 (d, *J* = 10.8 Hz, 1H), 1.99 (d, *J* = 12.6 Hz, 2H), 1.82 (h, *J* = 6.1 Hz, 2H), 1.72 - 1.44 (m, 2H), 1.42 - 1.15 (m, 2H).

20

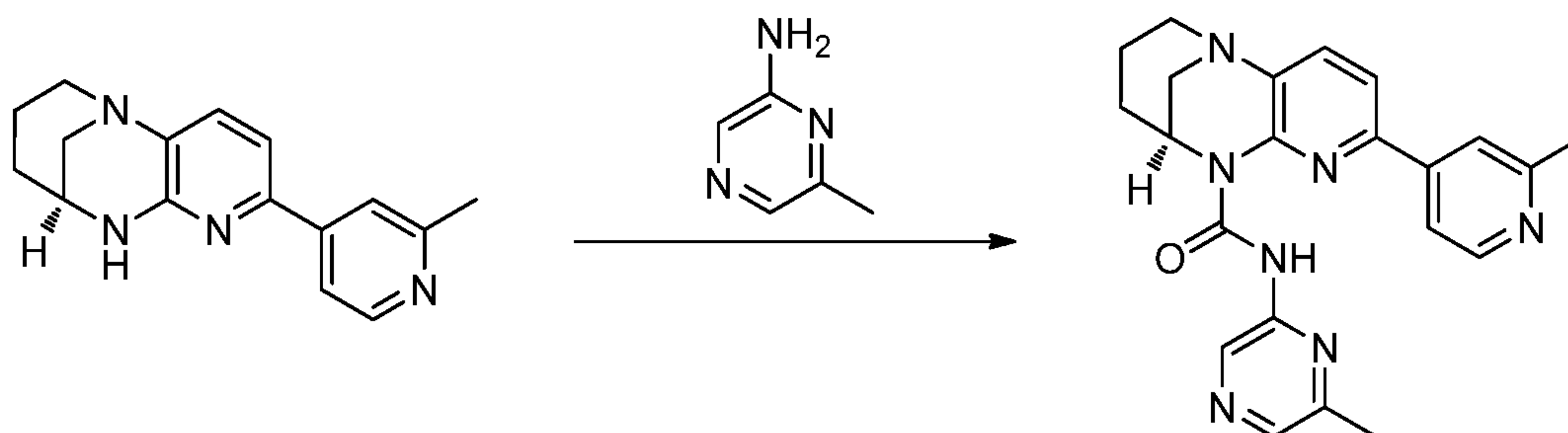
Example 19**Synthesis of (9*S*)-*N*-(4,5-dimethylthiazol-2-yl)-2-(2-methylpyridin-4-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

- 5 To a solution of (9*S*)-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (600 mg, 2.253 mmol) in THF (15 mL) were added triethylamine (0.942 mL, 6.76 mmol) and triphosgene (334 mg, 1.126 mmol) at 30 °C and stirred for 1 h. Then 4,5-dimethylthiazol-2-amine hydrochloride (556 mg, 3.38 mmol) was added at 30 °C and reaction was heated at 70 °C for 16h. The solvent evaporated under
- 10 reduced pressure, residue diluted with water (40 ml) and extracted with DCM (2x 40 ml). The combined organic layer was washed with water, brine, dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure to obtain crude compound. The crude mixture was purified by flash column chromatography and prep HPLC to afford (9*S*)-*N*-(4,5-dimethylthiazol-2-yl)-2-(2-methylpyridin-4-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (275 mg, 0.655 mmol, 42%
- 15 yield) as a pale yellow solid (TLC: 10% MeOH in EtOAc, R_f : 0.3), LCMS (m/z): 421.27 $[M+H]^+$.
- $^1\text{H NMR}$ (400 MHz, CDCl_3): δ ppm 14.79 (s, 2 H), 8.64 (d, $J=5.26$ Hz, 2 H), 8.02 (s, 1 H), 7.64 (dd, $J=5.26$, 1.53 Hz, 1 H), 7.54 (q, $J=8.11$ Hz, 1 H), 4.99 (s, 1 H), 3.42 - 3.18 (m, 3 H), 3.12 - 2.89 (m, 1 H), 2.79 (s, 3 H), 2.28 (s, 3 H), 2.22 (s, 3 H), 2.00 - 1.75 (m, 1 H), 1.52 - 1.35 (m, 2 H).
- 20

Example 20**Synthesis of (9S)-N-(6-methoxypyrazin-2-yl)-2-(2-methylpyridin-4-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide**

- 5 To a solution of (9S)-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (0.6 g, 2.253 mmol) in Tetrahydrofuran (THF) (30 mL) stirred under nitrogen at room temperature was added triethylamine (1.884 mL, 13.52 mmol) and triphosgene (0.669 g, 2.253 mmol). Then reaction mixture was stirred at room temperature for 30 minutes. 6-methoxypyrazin-2-amine (0.846 g, 6.76 mmol) was added at
- 10 rt. Then the reaction mixture was stirred at 65 °C for 16 hr. The reaction mixture was cooled to room temperature and concentrated under reduced pressure and was partitioned between water (20 mL) and EtOAc (50 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid (TLC eluent: 100% EtOAc: R_f 0.3; UV active). The crude was purified by column
- 15 chromatography using neutral alumina and was eluted with 50% EtOAc in Hexane to afford pure (9S)-N-(6-methoxypyrazin-2-yl)-2-(2-methylpyridin-4-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (0.220 g, 0.526 mmol, 23.34 % yield) as a off-white solid, LCMS (*m/z*): 418.3 [M+H]⁺.

¹H NMR (400 MHz, CDCl₃): δ 13.64 (s, 1H), 9.45 - 8.78 (m, 1H), 8.60 (dd, *J* = 5.3, 0.8 Hz, 1H), 7.96 (d, *J* = 0.5 Hz, 1H), 7.80 (ddd, *J* = 5.3, 1.7, 0.7 Hz, 1H), 7.62 (dt, *J* = 1.8, 0.7 Hz, 1H), 7.59 (s, 1H), 7.57 (s, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 5.04 (t, *J* = 2.6 Hz, 1H), 3.84 (s, 3H), 3.57 - 3.26 (m, 3H), 3.15 - 2.91 (m, 1H), 2.64 (s, 3H), 2.28 (d, *J* = 14.3 Hz, 1H), 2.12 - 1.85 (m, 1H), 1.43 (s, 2H)

Example 21**Synthesis of ((9*S*)-N-(6-methylpyrazin-2-yl)-2-(2-methylpyridin-4-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide:**

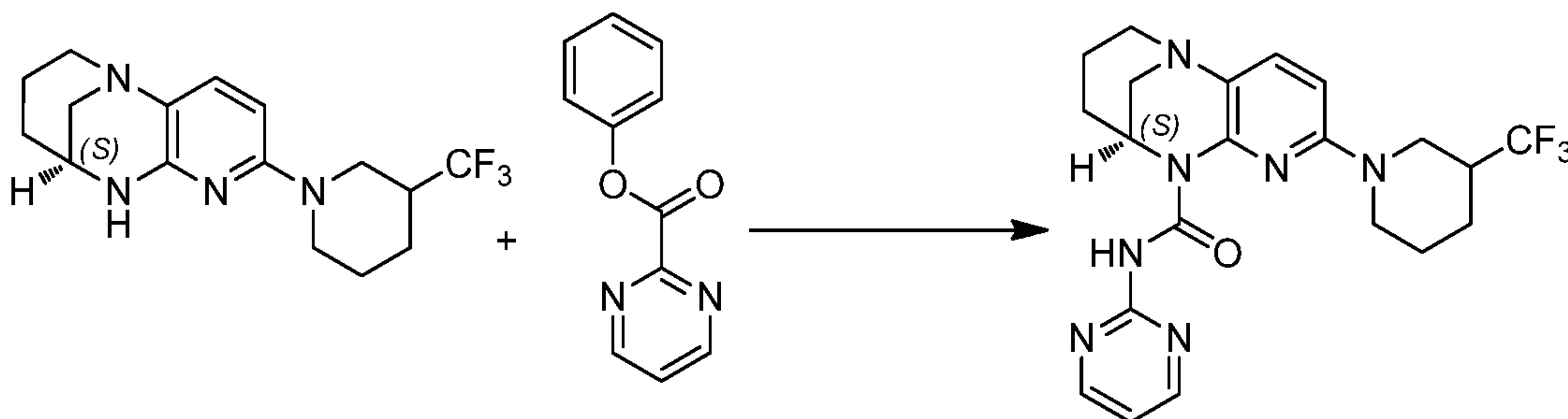
5 Triphosgene (0.279 g, 0.939 mmol) was added to a stirred solution of (9*S*)-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (0.25g, 0.939 mmol) in tetrahydrofuran (50 mL) at 25 °C. Triethylamine (0.654 mL, 4.69 mmol) was added and followed by addition of 6-methylpyrazin-2-amine (0.410 g, 3.75 mmol). The reaction mixture was stirred for 15h at 70 °C. The reaction mixture was

10 cooled to 28°C and was partitioned between water (50 mL) and dichloromethane (50 mL). The separated organic layer was washed with water and brine. The organic layer was dried over sodium sulfate filtered and filtrate was evaporated to get crude compound (TLC eluent: 10% MeOH in EtOAc; R_f = 0.4; UV active). The crude compound was purified by using neutral alumina and eluted in 100% ethyl acetate to afford (9*S*)-N-(6-methylpyrazin-

15 2-yl)-2-(2-methylpyridin-4-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (0.11g, 0.272 mmol, 29.0 % yield) as yellow solid, LCMS (m/z): 402.26 $[M+H]^+$.

¹H NMR (CDCl₃, 400 MHz): δ 14.02 (s, 1 H), 9.37 (s, 1 H), 8.68 (d, J =5.26 Hz, 1 H), 8.13 - 8.26 (m, 1 H), 7.98 (dd, J =5.26, 1.75 Hz, 1 H), 7.69 - 7.80 (m, 1 H), 7.49 - 7.63 (m, 2 H),

20 5.03 (t, J =2.19 Hz, 1 H), 3.30 - 3.47 (m, 2 H), 3.03 (br d, J =13.81 Hz, 1 H), 2.69 (s, 2 H), 2.49 - 2.59 (m, 2 H), 2.15 - 2.30 (m, 1 H), 1.84 - 1.99 (m, 1 H), 1.24 - 1.66 (m, 3 H).

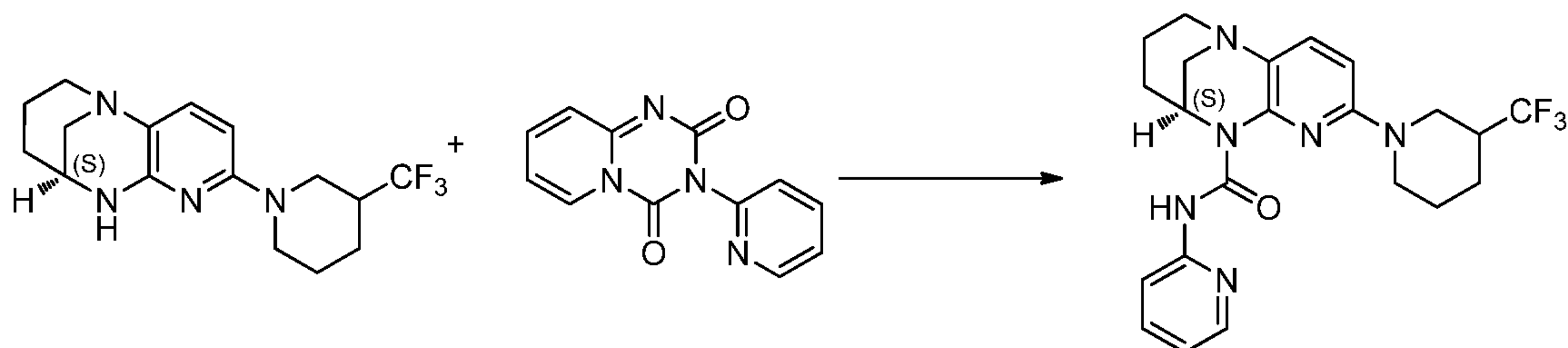
Example 22**Synthesis of (9S)-N-(pyrimidin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide**

- 5 To a solution of (9S)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (Peak II from the intermediate SFC separation 0.600 g, 1.838 mmol) in Tetrahydrofuran (THF) (30 mL) was added DMAP (0.674 g, 5.52 mmol) and phenyl pyrimidin-2-ylcarbamate (1.187 g, 5.52 mmol) stirred at 65 °C for 36 hr. Reaction was cooled to room temperature and then concentrated under reduced
- 10 pressure. The residue was partitioned between water (10 mL) and EtOAc (50 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid (TLC eluent: 80% EtOAc in Hexane: R_f 0.3; UV active). The crude was purified by column chromatography using neutral alumina and was eluted with 35-40% EtOAc in Hexane to afford pure (9S)-N-(pyrimidin-2-yl)-2-(3-
- 15 (trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (0.375 g, 0.834 mmol, 45.4 % yield) as a off-white solid, LCMS (*m/z*): 448.3 [M+H]⁺.

¹H NMR (400 MHz, CDCl₃): δ 13.97 (s, 1H), 8.59 (d, *J* = 4.8 Hz, 2H), 7.31 (d, *J* = 8.6 Hz, 1H), 6.93 (t, *J* = 4.8 Hz, 1H), 6.40 (d, *J* = 8.6 Hz, 1H), 4.93 (q, *J* = 2.6 Hz, 1H), 4.44 - 4.07 (m, 2H), 3.31 (d, *J* = 1.9 Hz, 1H), 3.19 (d, *J* = 3.4 Hz, 2H), 3.10 - 2.81 (m, 3H), 2.44 (dtt, *J* = 15.6, 7.7, 3.9 Hz, 1H), 2.29 (d, *J* = 13.4 Hz, 1H), 2.17 - 2.07 (m, 1H), 1.93 (dt, *J* = 13.2, 3.3 Hz, 1H), 1.85 (ddd, *J* = 13.8, 5.3, 3.1 Hz, 2H), 1.77 - 1.68 (m, 1H), 1.55 - 1.43 (m, 1H), 1.31 (d, *J* = 14.0 Hz, 1H).

25 Example 23**Synthesis of (9S)-N-(pyridin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide**

NaH (0.154 g, 6.43 mmol) was added to a solution of (9*S*)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]d



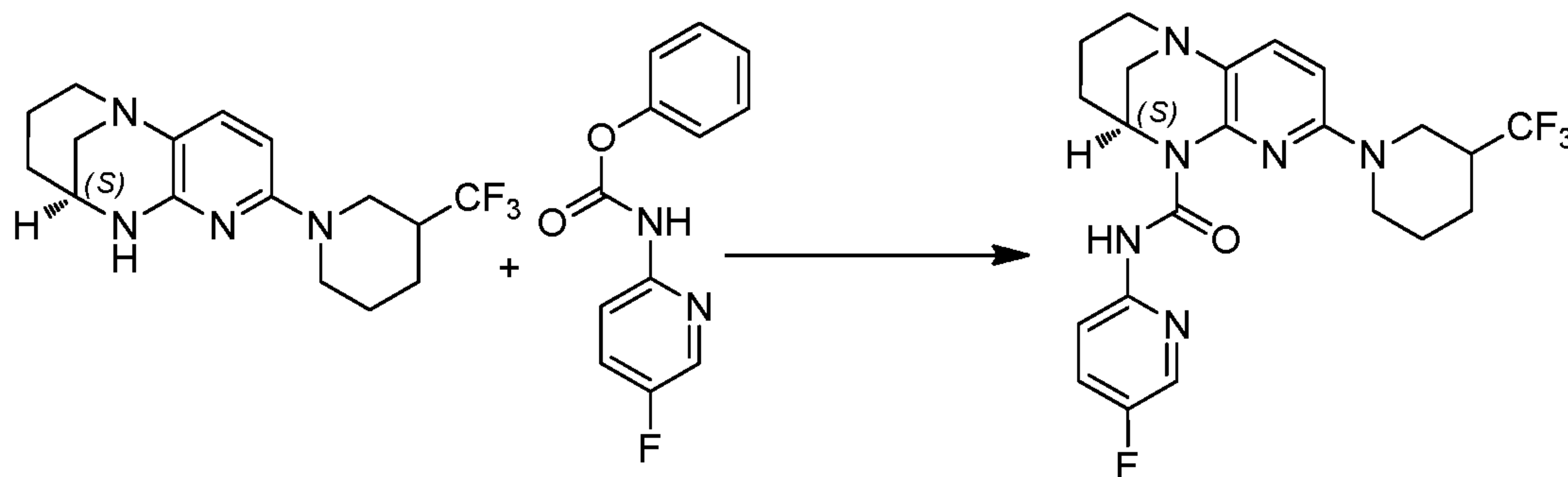
iazocine (Peak-I from intermediate SFC Separation, 0.350 g, 1.072 mmol) in

5 tetrahydrofuran (25 mL) stirred under nitrogen at 0 °C. The reaction mixture was stirred at 30 °C for 30 minutes and 3-(pyridin-2-yl)-2*H*-pyrido[1,2-*a*][1,3,5]triazine-2,4(3*H*)-dione (0.386 g, 1.609 mmol) was added. The reaction mixture was stirred at 65 °C for 24 hrs.

The reaction mixture was cooled to room temperature and quenched with methanol (5 mL). The reaction mixture was diluted with water and extracted with EtOAc (2 X

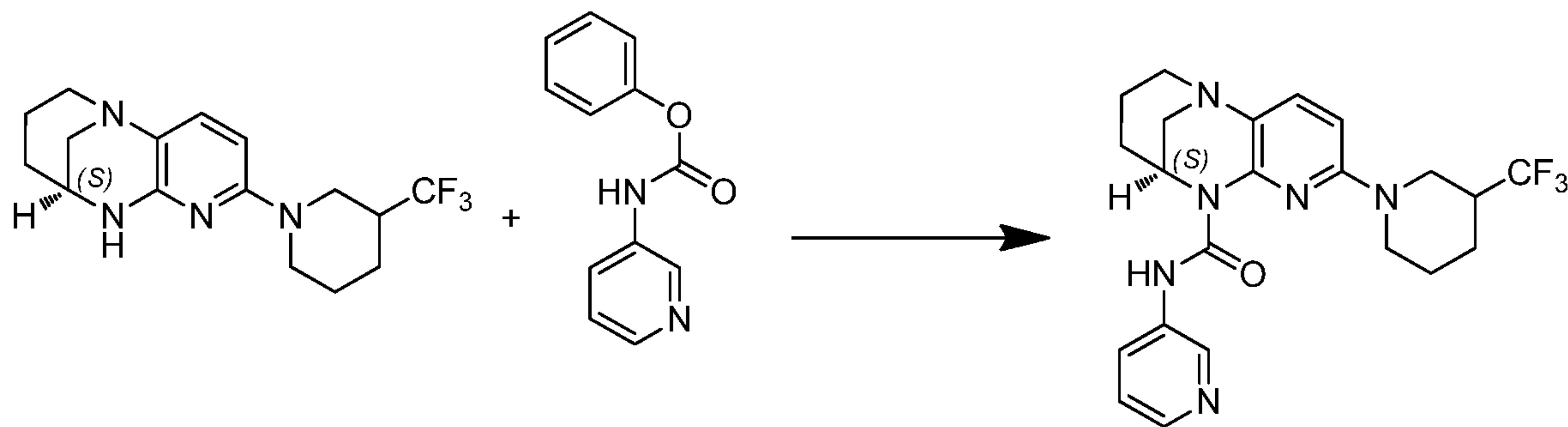
10 100mL). The organic layers were washed with water and followed by brine solution and dried over with Na₂SO₄ filtered and concentrated under reduced pressure to get crude as a brown solid. The crude was purified by column chromatography using Neutral Alumina and was eluted with 60% ethyl acetate in hexane to afford pure (9*S*)-*N*-(pyridin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (0.320 g, 0.708 mmol, 66.0 % yield) as off white solid, LCMS (*m/z*): 447.34 [M+H]⁺.

¹H NMR (400 MHz, CDCl₃): δ 13.58 (s, 1H), 8.46 - 8.04 (m, 2H), 7.65 (ddd, *J* = 8.9, 7.3, 1.9 Hz, 1H), 7.35 - 7.10 (m, 1H), 6.94 (ddd, *J* = 7.3, 4.9, 1.1 Hz, 1H), 6.38 (d, *J* = 8.6 Hz, 1H), 5.10 - 4.73 (m, 1H), 4.59 - 4.37 (m, 1H), 4.31 - 4.19 (m, 1H), 3.30 (dd, *J* = 13.4, 1.9 Hz, 1H), 3.26 - 3.13 (m, 2H), 3.06 - 2.88 (m, 3H), 2.48-2.36 (m, *J* = 16.3, 8.3, 4.1 Hz, 1H), 2.21 (d, *J* = 14.4 Hz, 1H), 2.12 (d, *J* = 12.1 Hz, 1H), 1.94 (s, 1H), 1.88 - 1.68 (m, 2H), 1.67 - 1.57 (m, 1H), 1.50 (s, 1H), 1.31 (d, *J* = 13.6 Hz, 1H).

Example 24**Synthesis of (9S)-N-(5-fluoropyridin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide**

- 5 To a solution of (9S)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (Peak-II of the intermediate SFC separation, 0.600 g, 1.838 mmol) in Tetrahydrofuran (THF) (30 mL) was added DMAP (0.674 g, 5.52 mmol) and phenyl (5-fluoropyridin-2-yl)carbamate (1.281 g, 5.52 mmol). The reaction mixture was stirred at 65 °C for 36 hr. Reaction was cooled to room temperature and concentrated under reduced pressure. The residue was partitioned between water (10 mL) and EtOAc (50 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid (TLC eluent: 80% EtOAc in Hexane: R_f 0.3; UV active). The crude was purified by column chromatography using neutral alumina and was eluted with 25-30% EtOAc in Hexane to afford pure (9S)-N-(5-fluoropyridin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (0.288 g, 0.608 mmol, 33.1 % yield) as a white solid, LCMS (*m/z*): 465.3 [M+H]⁺.

¹H NMR (400 MHz, CDCl₃): δ 13.62 (s, 1H), 8.20 (ddd, *J* = 9.2, 4.1, 0.6 Hz, 1H), 8.09 (s, 1H), 7.39 (ddd, *J* = 9.1, 7.8, 3.0 Hz, 1H), 7.30 (d, *J* = 8.5 Hz, 1H), 6.39 (d, *J* = 8.6 Hz, 1H), 4.87 (p, *J* = 2.8 Hz, 1H), 4.30 (dt, *J* = 12.5, 3.7, 1.8 Hz, 2H), 3.31 (dd, *J* = 13.4, 1.9 Hz, 1H), 3.25 - 3.13 (m, 2H), 3.07 - 2.85 (m, 3H), 2.44 (dt, *J* = 7.4, 3.6 Hz, 1H), 2.26 - 2.05 (m, 2H), 1.99 - 1.83 (m, 2H), 1.78 - 1.66 (m, 1H), 1.62 (dd, *J* = 12.4, 3.8 Hz, 1H), 1.53 - 1.44 (m, 1H), 1.37 - 1.26 (m, 1H).

Example 25**Synthesis of (9*S*)-N-(pyridin-3-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

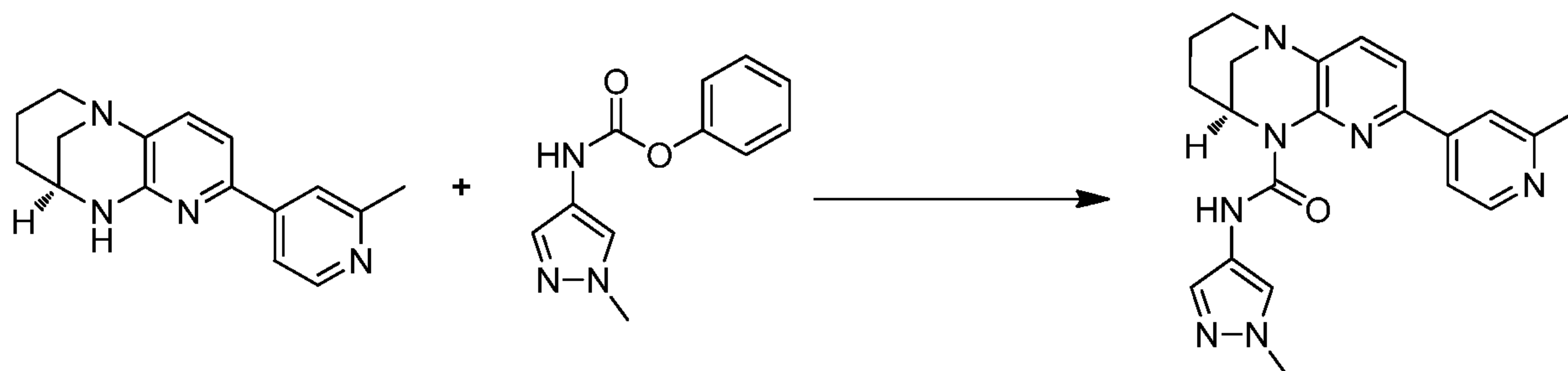
5

To a solution of (9*S*)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (Peak-I of the intermediate SFC separation, 600 mg, 1.838 mmol) in tetrahydrofuran (30 mL), DMAP (674 mg, 5.52 mmol) and phenyl pyridin-3-ylcarbamate (1182 mg, 5.52 mmol) were added. The reaction mixture was stirred at 65 °C for 16 hr. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (2x75 mL). The combined organic layers were washed with water and saturated with brine solution and dried over anhydrous sodium sulfate, filtered and concentrated to give crude as a white solid. (TLC eluent: 100% pure Ethyl Acetate; R_f value: 0.4; UV active). The crude product was purified by column chromatography (neutral alumina) product was eluted with 25% ethyl acetate in hexane to afford (9*S*)-N-(pyridin-3-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (350 mg, 0.782 mmol, 42.5 % yield) as off white solid, LCMS (m/z): 447.26 $[M+H]^+$.

^1H NMR (400 MHz, CDCl_3): δ 12.94 (s, 1H), 8.62 (dd, $J = 2.6, 0.7$ Hz, 1H), 8.32 (dd, $J = 4.7, 1.5$ Hz, 1H), 8.15 (ddd, $J = 8.4, 2.7, 1.5$ Hz, 1H), 7.33 (d, $J = 8.5$ Hz, 1H), 7.29 - 7.24 (m, 1H), 6.41 (d, $J = 8.6$ Hz, 1H), 4.90 (t, $J = 2.6$ Hz, 1H), 4.22 - 4.04 (m, 2H), 3.31 (dd, $J = 13.5, 1.9$ Hz, 1H), 3.25 - 3.14 (m, 2H), 2.99 - 2.88 (m, 3H), 2.48-2.36 (m, 1H), 2.25 - 2.18 (m, 1H), 2.13 (dd, $J = 13.2, 4.0$ Hz, 1H), 1.98 - 1.92 (m, 1H), 1.85 (tdd, $J = 13.9, 5.2, 2.9$ Hz, 1H), 1.71 (dtd, $J = 16.5, 8.3, 7.7, 4.3$ Hz, 1H), 1.63 (d, $J = 3.8$ Hz, 1H), 1.57 - 1.44 (m, 1H), 1.33 (d, $J = 14.2$ Hz, 1H).

Example 26

Synthesis of (9S)-N-(1-methyl-1H-pyrazol-4-yl)-2-(2-methylpyridin-4-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide

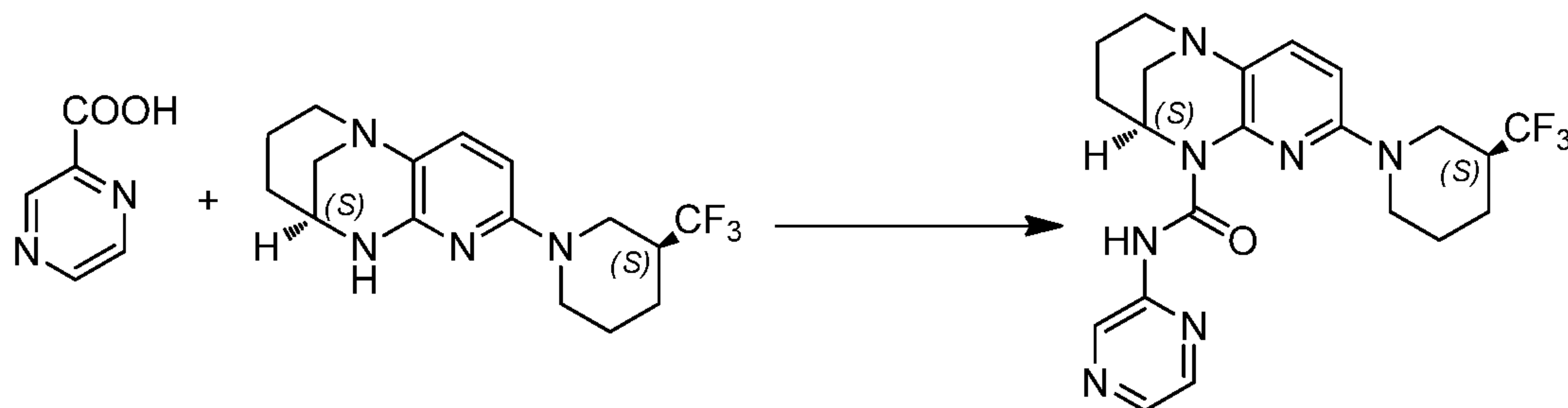


DMAP (0.573 g, 4.69 mmol) was added to a stirred solution of (9S)-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (0.5g, 1.877 mmol), and phenyl (1-methyl-1H-pyrazol-4-yl)carbamate (1.01g, 4.692 mmol) in tetrahydrofuran (10 mL) at room temperature and heated to 24h at 80 °C in sealed tube. The reaction mixture was cooled to 28 °C, and was diluted with ethyl acetate and water. The organic layer was separated and was washed with water and brine. The organic layer was dried over sodium sulfate and evaporated under reduced pressure to get crude compound (TLC eluent: 10% MeOH in EtOAc; R_f 0.4; UV active). The crude compound was purified by using neutral alumina and was eluted with 75% ethyl acetate in hexane to afford (9S)-N-(1-methyl-1H-pyrazol-4-yl)-2-(2-methylpyridin-4-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (0.17g, 0.430 mmol, 22.89 % yield) as an off-white solid, LCMS (m/z): 390.28 $[M+H]^+$.

$^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 13.27 (s, 1 H), 8.67 (d, $J=5.26$ Hz, 1 H), 7.87 (s, 1 H), 7.63 (d, $J=1.53$ Hz, 1 H), 7.53 - 7.57 (m, 2 H), 7.43 (d, $J=0.66$ Hz, 1 H), 7.40 (s, 1 H), 4.99 (t, $J=2.30$ Hz, 1 H), 3.89 (s, 3 H), 3.22 - 3.42 (m, 3 H), 2.98 (br d, $J=13.59$ Hz, 1 H), 2.70 (s, 3 H), 2.20 - 2.28 (m, 1 H), 1.92 (tdd, $J=13.67, 13.67, 5.54, 3.07$ Hz, 1 H), 1.32 - 1.50 (m, 2 H).

Example 27

Synthesis of (9S)-N-(pyrazin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide

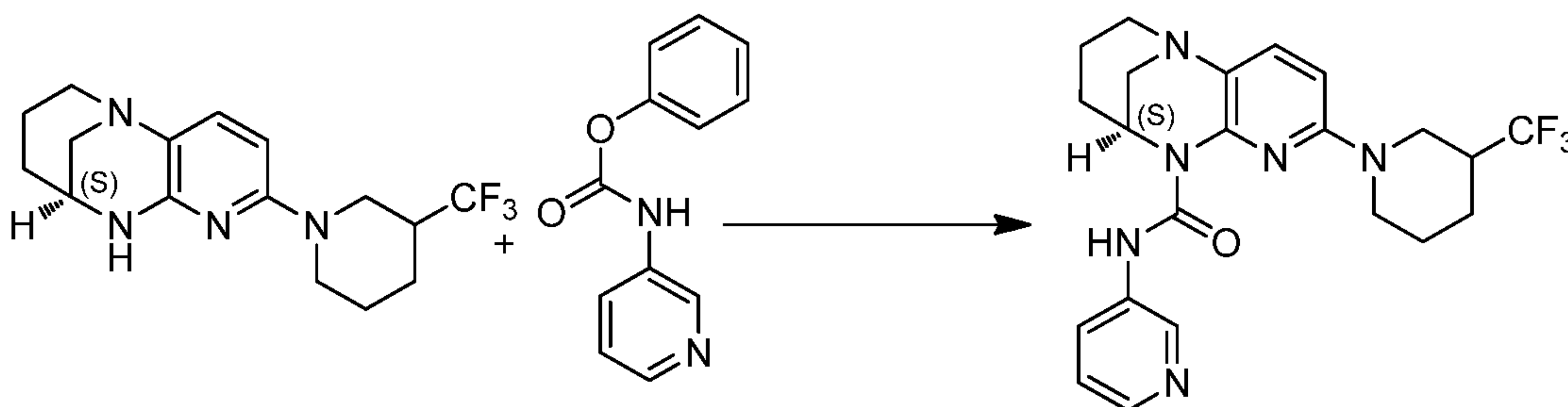


Diphenyl phosphorazidate (1.996 g, 7.25 mmol) added to a stirred solution of pyrazine-2-carboxylic acid (Peak-II of the intermediate SFC separation, 0.600 g, 4.83 mmol) and DIPEA (4.22 mL, 24.17 mmol) in Tetrahydrofuran (THF) (60 mL) stirred with argon at room temp. The reaction mixture was stirred 2 hr at room temperature and added (9S)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (1.105 g, 3.38 mmol). The reaction mixture was stirred 16 hr at 65 °C. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was partitioned between water (30 mL) and EtOAc (100 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid (TLC eluent: 100% EtOAc: R_f 0.3; UV active). The crude was purified by column chromatography using neutral alumina and was eluted with 50% EtOAc in Hexane to afford pure (9S)-N-(pyrazin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (0.367 g, 0.815 mmol, 16.86 % yield) as a pale yellow solid, LCMS (*m/z*): 448.3 [M+H]⁺.

¹H NMR (400 MHz, CDCl₃): δ 13.79 (s, 1H), 9.53 (d, *J* = 1.5 Hz, 1H), 8.25 (d, *J* = 2.5 Hz, 1H), 8.20 (d, *J* = 1.6 Hz, 1H), 7.32 (d, *J* = 8.6 Hz, 1H), 6.42 (d, *J* = 8.6 Hz, 1H), 4.90 (s, 1H), 4.28 (s, 2H), 3.32 (dd, *J* = 13.5, 1.9 Hz, 1H), 3.21 (dd, *J* = 12.5, 3.3 Hz, 2H), 3.10 - 2.86 (m, 3H), 2.44 (dt, *J* = 7.6, 3.6 Hz, 1H), 2.28 - 2.18 (m, 1H), 2.11 (d, *J* = 3.6 Hz, 1H), 1.99 - 1.80 (m, 2H), 1.78 - 1.65 (m, 1H), 1.65 - 1.60 (m, 1H), 1.49 (s, 1H), 1.35 (s, 1H).

Example 28

Synthesis of (9S)-N-(pyridin-3-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



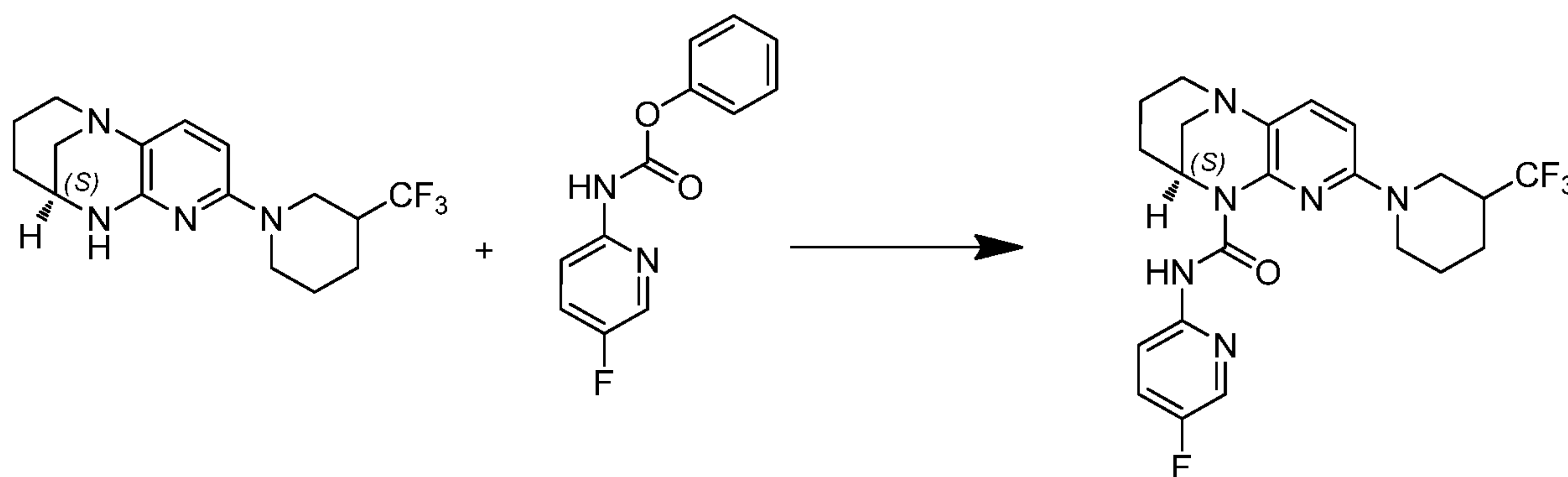
To a solution of (9S)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (Peak-II of the intermediate SFC separation, (Peak-II from intermediate SFC separation, 0.700 g, 2.145 mmol) in Tetrahydrofuran (THF) (70

mL) was added DMAP (0.786 g, 6.43 mmol) and phenyl pyridin-3-ylcarbamate (1.378 g, 6.43 mmol) stirred at 65 °C for 36 hr before the reaction was cooled to room temperature and concentrated under reduced pressure. The residue was partitioned between water (20 mL) and EtOAc (75 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid (TLC eluent: 80% EtOAc in Hexane: R_f 0.3; UV active). The crude was purified by column chromatography using neutral alumina and was eluted with 50% EtOAc in Hexane to afford pure (9*S*)-N-(pyridin-3-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (0.355 g, 0.794 mmol, 37.0 % yield) as a white solid, LCMS (*m/z*): 447.3 [M+H]⁺.

¹H-NMR (400 MHz, CDCl₃): δ ppm 12.92 (s, 1 H), 8.62 (d, *J*=2.63 Hz, 1 H), 8.32 (dd, *J*=4.71, 1.43 Hz, 1 H), 8.08 - 8.21 (m, 1 H), 7.33 (d, *J*=8.55 Hz, 1 H), 7.20 - 7.29 (m, 1 H), 6.40 (d, *J*=8.55 Hz, 1 H), 4.90 (br s, 1 H), 4.00 - 4.21 (m, 2 H), 3.31 (dd, *J*=13.48, 1.86 Hz, 1 H), 3.17 - 3.26 (m, 2 H), 2.88 - 3.03 (m, 3 H), 2.36 - 2.49 (m, 1 H), 2.19 - 2.25 (m, 1 H), 2.10 - 2.16 (m, 1 H), 1.92 - 1.99 (m, 1 H), 1.86 (tdd, *J*=13.84, 13.84, 5.10, 2.96 Hz, 1 H), 1.65 - 1.76 (m, 1 H), 1.46 - 1.58 (m, 2 H), 1.29 - 1.38 (m, 1 H)

Example 29

Synthesis of (9*S*)-N-(5-fluoropyridin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide



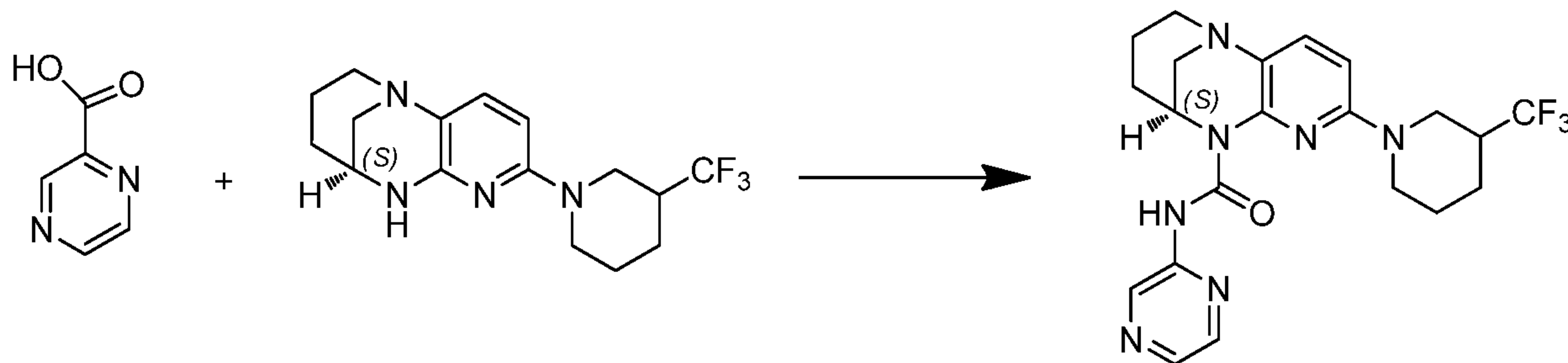
To a solution of (9*S*)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-*b*][1,4]diazocine (Peak-I from SFC separation, 450 mg, 1.379 mmol) in tetrahydrofuran (30 mL), DMAP (505 mg, 4.14 mmol) and phenyl (5-fluoropyridin-2-yl)carbamate (961 mg, 4.14 mmol) were added. The reaction mixture was stirred at 65 °C for 16 hr. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (2x75 mL). The combined organic layers were washed with water and saturated

with brine solution and dried over anhydrous sodium sulfate, filtered and concentrated to give the crude as off white solid. (TLC Eluent: 100% Ethyl Acetate; R_f value: 0.4; UV active). The crude product was purified by column chromatography (neutral alumina) product was eluted with 25% ethyl acetate in hexane to afford (9*S*)-N-(5-fluoropyridin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-
 5 *b*][1,4]diazocine-10(7*H*)-carboxamide (140 mg, 0.296 mmol, 21.46 % yield) as a white solid, LCMS (m/z): 465.21 $[M+H]^+$.

$^1\text{H NMR}$ (400 MHz, CDCl_3): δ ppm 1.15 - 1.43 (m, 1 H) 1.43 - 1.68 (m, 2 H) 1.68 - 1.89 (m, 3 H) 2.04 - 2.28 (m, 2 H) 2.42 (dtd, $J=11.67, 7.92, 7.92, 4.06$ Hz, 1 H) 2.86 - 3.09 (m,
 10 3 H) 3.10 - 3.36 (m, 3 H) 4.23 - 4.47 (m, 2 H) 4.87 (br s, 1 H) 6.39 (d, $J=8.55$ Hz, 1 H) 7.3 (d, $J=8.8$ Hz, 1 H) 7.34 - 7.59 (m, 1 H) 8.09 (d, $J=2.8$ Hz, 1 H) 8.20 (dd, $J=9.21, 4.17$ Hz, 1 H) 13.65 (s, 1 H).

Example 30

15 Synthesis of (9*S*)-N-(pyrazin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide



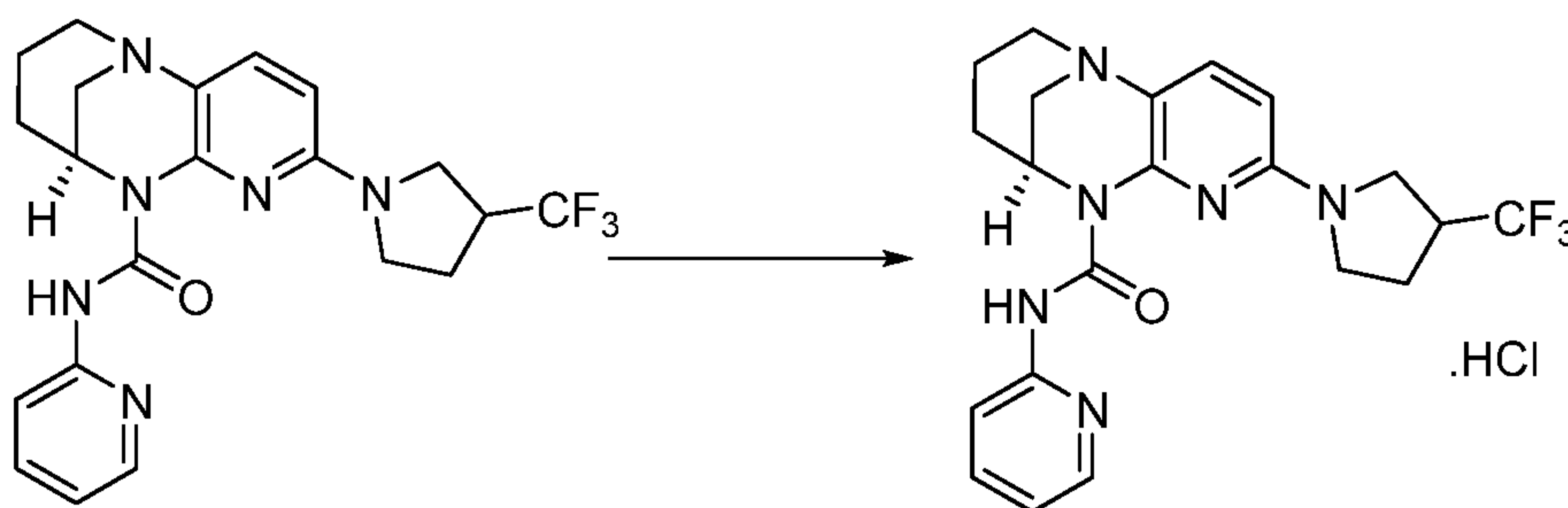
A suspension of pyrazine-2-carboxylic acid (0.6 g, 4.83 mmol) in tetrahydrofuran (50 mL) diphenylphosphoryl azide (1.996 g, 7.25 mmol) and DIPEA (4.22 mL, 24.17 mmol) added
 20 to the reaction mixture. The reaction mixture was stirred for 2 hr at 28 °C (9*S*)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diaz-ocine (Peak-I from intermediate SFC Separation 1.105 g, 3.38 mmol) was added to the reaction mixture. The reaction mixture was stirred 16 hr at 65 °C. The reaction mixture was diluted with water (50mL) and extracted with ethyl acetate (2 X 75
 25 mL). The combined organic layer was washed with water and saturated with brine solution and dried over anhydrous Na_2SO_4 , filtered and concentrated to give the crude as a off white solid. (TLC eluent: 100% ethyl acetate; R_f value: 0.3; UV active). The crude product was purified by column chromatography (neutral alumina) product was eluted with 30% ethyl acetate in hexane to afford (9*S*)-N-(pyrazin-2-yl)-2-(3-(trifluoromethyl)-

piperidin-1-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (0.330 g, 0.728 mmol, 15.05 % yield) as a white solid, LCMS (*m/z*): 448.25 [M+H]⁺.

¹H NMR (400 MHz, CDCl₃): δ ppm 1.31 – 1.34 (m, 1H) 1.45 - 1.67 (m, 2 H) 1.68 - 1.79 (m, 1 H) 1.82 - 1.99 (m, 2 H) 2.05 - 2.18 (m, 2 H) 2.42 (dddt, *J*=15.65, 11.78, 8.03, 3.97, 3.97 Hz, 1 H) 2.86 - 3.09 (m, 3 H) 3.11 - 3.35 (m, 3 H) 4.21 - 4.39 (m, 2 H) 4.89 (br s, 1 H) 6.42 (d, *J*=8.55 Hz, 1 H) 7.32 (d, *J*=8.4 Hz, 1 H) 8.14 - 8.20 (m, 1 H) 8.24 (d, *J*=2.8 Hz, 1 H) 9.53 (d, *J*=1.53 Hz, 1 H) 13.81 (s, 1 H)

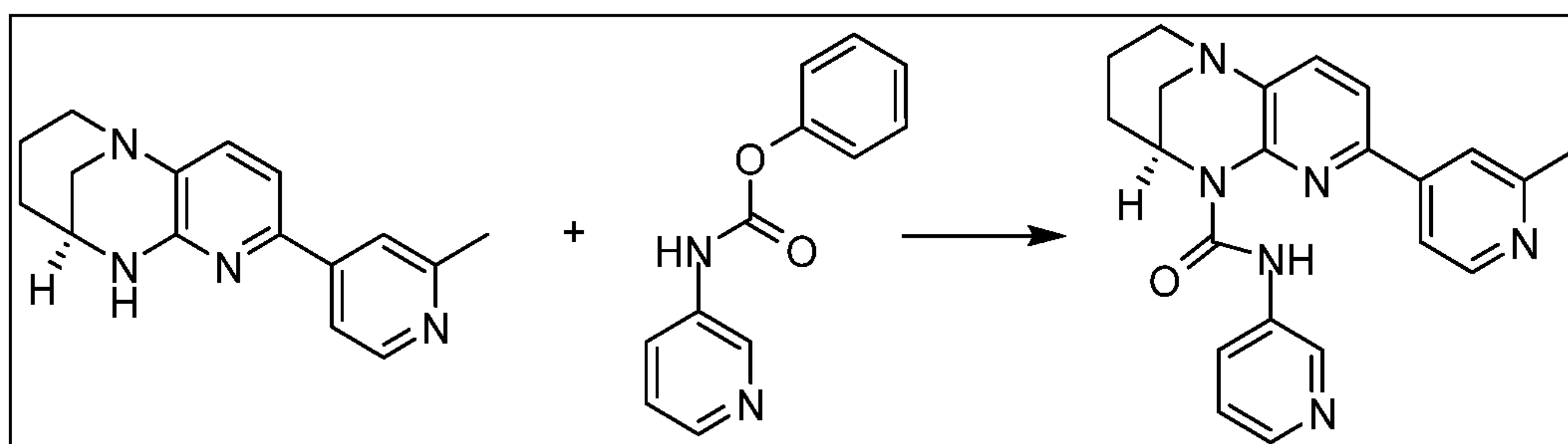
10 Example 31

Synthesis of (9*S*)-N-(pyridine-2-yl)-2-(3-(trifluoromethyl)pyrrolidin-1-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide hydrochloride

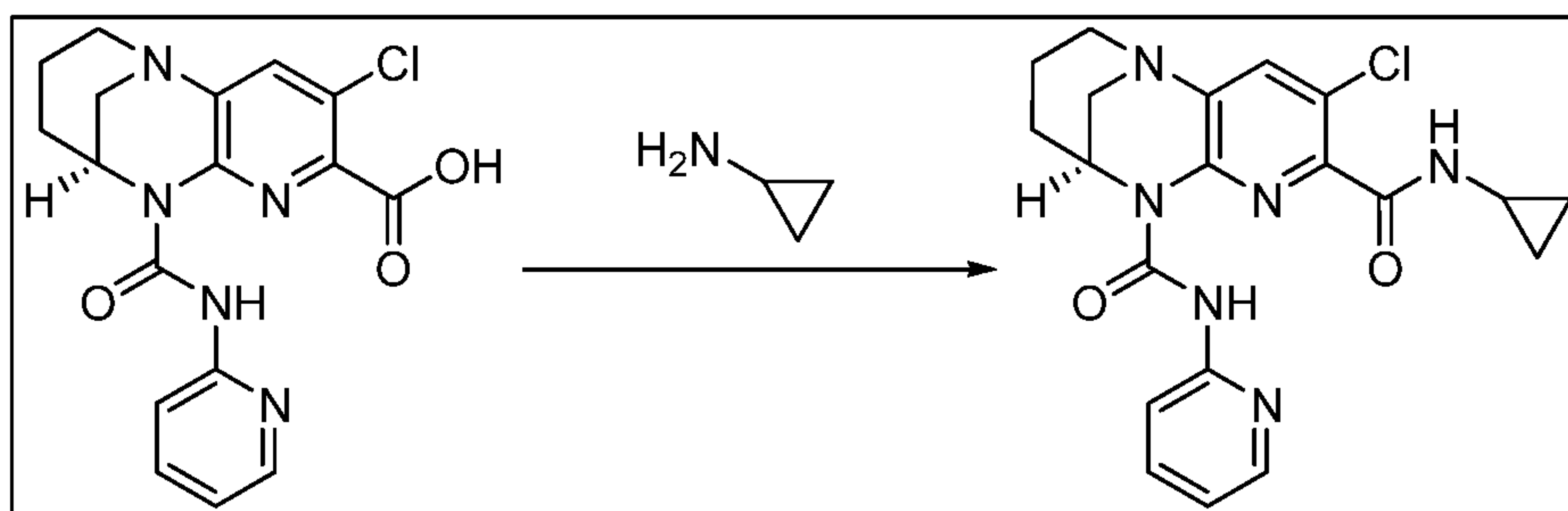


15 2.0 M Hydrochloric acid in Diethyl ether (2 mL, 4.00 mmol) was added to (9*S*)-N-(Pyridine-2-yl)-2-(3-(trifluoromethyl)pyrrolidine-1-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (180 mg, 0.416 mmol) at 0 °C. The reaction mixture was stirred at 28 °C for 4 h and concentrated to give crude compound (TLC eluent: 5% MeOH in DCM: *R_f* 0.1; UV active). The crude compound was washed
20 with Diethyl ether (2x5 mL) to afford pure (9*S*)-N-(pyridine-2-yl)-2-(3-(trifluoromethyl)pyrrolidin-1-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide hydrochloride (83mg, 0.177mmole, 42.78% yield) as pale brown solid, LCMS (*m/z*): 433.28 [M+H]⁺.

¹H NMR (400 MHz, CDCl₃): δ 15.02 (br s, 1 H), 13.34 (br s, 1 H), 8.25 (br d, *J*=3.73 Hz, 1 H), 8.13 (br d, *J*=8.33 Hz, 1 H), 8.06 (d, *J*=8.77 Hz, 1 H), 7.75- 7.68 (m, 1 H), 7.04- 6.98 (m, 1 H), 6.15 (s, 1 H), 5.30 -5.24 (b, s, 1 H), 4.12 -3.94 (m, 2 H), 3.73 -3.59 (m, 4 H), 3.49 -3.37 (m, 2 H), 3.18-3.04 (m, 1 H), 2.45 - 2.25 (m, 3 H), 1.86 (d, *J*=12.50 Hz, 2 H).

Example 32**Synthesis of (9*S*)-2-(2-methylpyridin-4-yl)-N-(pyridin-3-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

- 5 To a stirred solution of (9*S*)-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (300 mg, 1.126 mmol) in THF (30 mL) under nitrogen atmosphere at RT was added phenyl pyridin-3-ylcarbamate (724 mg, 3.38 mmol), DMAP (413 mg, 3.38 mmol) and stirred at 65 °C for 48 h. (TLC eluent: 5% MeOH in DCM: R_f 0.4; UV active). The reaction mixture was cooled to RT, concentrated and the
- 10 residue was partitioned between water (20 mL) and EtOAc (100 mL). Organic layer was separated, dried over anhydrous Na_2SO_4 , filtered and filtrate was evaporated to give crude compound. The crude was purified by column chromatography (neutral alumina, eluent: 2% MeOH in DCM) to afford the desired product (9*S*)-2-(2-methylpyridin-4-yl)-N-(pyridin-3-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-
- 15 carboxamide (200 mg, 0.516 mmol, 45.8 % yield) as a pale yellow solid. LCMS (m/z): 387.09 $[\text{M}+\text{H}]^+$, R_t = 1.21 min.
- ^1H NMR (400 MHz, CDCl_3): δ ppm 13.61 (s, 1 H), 8.84 - 8.62 (m, 2 H), 8.33 (dd, J =4.71, 1.21 Hz, 1 H), 8.17 (dd, J =8.33, 1.54 Hz, 1 H), 7.63 (s, 1 H), 7.58 (d, J =7.89 Hz, 1 H), 7.52 (br d, J =5.04 Hz, 1 H), 7.42 (d, J =8.11 Hz, 1 H), 7.28 (br d, J =4.60 Hz, 1 H), 5.02 (br
- 20 s, 1 H), 3.45 - 3.31 (m, 3 H), 3.01 (br d, J =13.59 Hz, 1 H), 2.69 (s, 3 H), 2.27 (br d, J =12.93 Hz, 1 H), 3.01 - 1.85 (m, 1 H), 1.54 - 1.32 (m, 2 H).

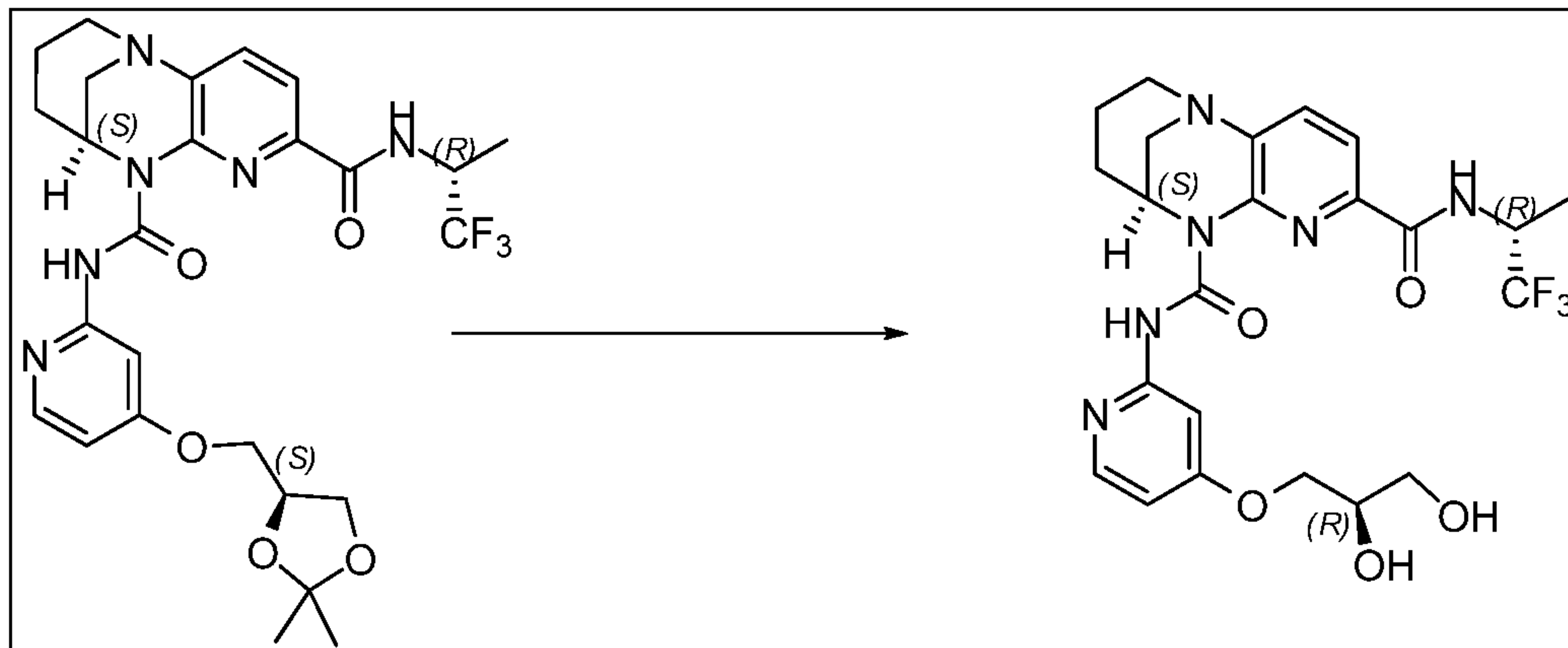
Example 33**Synthesis of (9*S*)-3-chloro-*N*2-cyclopropyl-*N*10-(pyridin-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide.**

- 5 To a stirred solution of (9*S*)-3-chloro-10-(pyridin-2-ylcarbamoyl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2-carboxylic acid (650 mg, 1.739 mmol) in DMF (6 mL), under nitrogen at RT was added DIPEA (0.911 mL, 5.22 mmol), HATU (1322 mg, 3.48 mmol) and cyclopropanamine (119 mg, 2.087 mmol), then the reaction mixture was stirred for 16 h. (TLC system: 5% Methanol in DCM. R_f value: 0.30). Reaction mass
- 10 was diluted with 50 mL of ice cold water, extracted with EtOAc (3 x 100 mL). The combined organic layer was washed with brine (100 mL), dried over Na_2SO_4 , filtered and concentrated to get crude compound. The crude material was purified by combiflash (using silica gel column, 75 % EtOAc in Hexane). Fractions containing pure compound were combined and concentrated to afford the desired compound to get (9*S*)-3-chloro-*N*2-
- 15 cyclopropyl-*N*10-(pyridin-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-*H*)-dicarboxamide (97 mg, 0.229 mmol, 13.15 % yield) as off white solid. LCMS (m/z): 413.10 $[\text{M}+\text{H}]^+$, R_t = 1.84 min.

$^1\text{H NMR}$ (400 MHz, CDCl_3): δ ppm 13.12 (br s, 1 H), 8.33 (dd, $J=4.93, 0.99$ Hz, 1 H), 8.14 (d, $J=8.33$ Hz, 1 H), 7.51 - 7.76 (m, 2 H), 7.60 (m, 1 H), 7.09 (br, 1 H), 4.97 (br s, 1 H), 3.23 - 3.44 (m, 3 H), 3.04 (ddt, $J=10.91, 7.18, 3.89, 3.89$ Hz, 1 H), 2.93 (br d, $J=13.81$ Hz, 1 H), 2.10 - 2.32 (m, 1 H), 1.80 - 2.07 (m, 1 H), 1.22 - 1.46 (m, 2 H), 0.81 - 0.98 (m, 4 H)

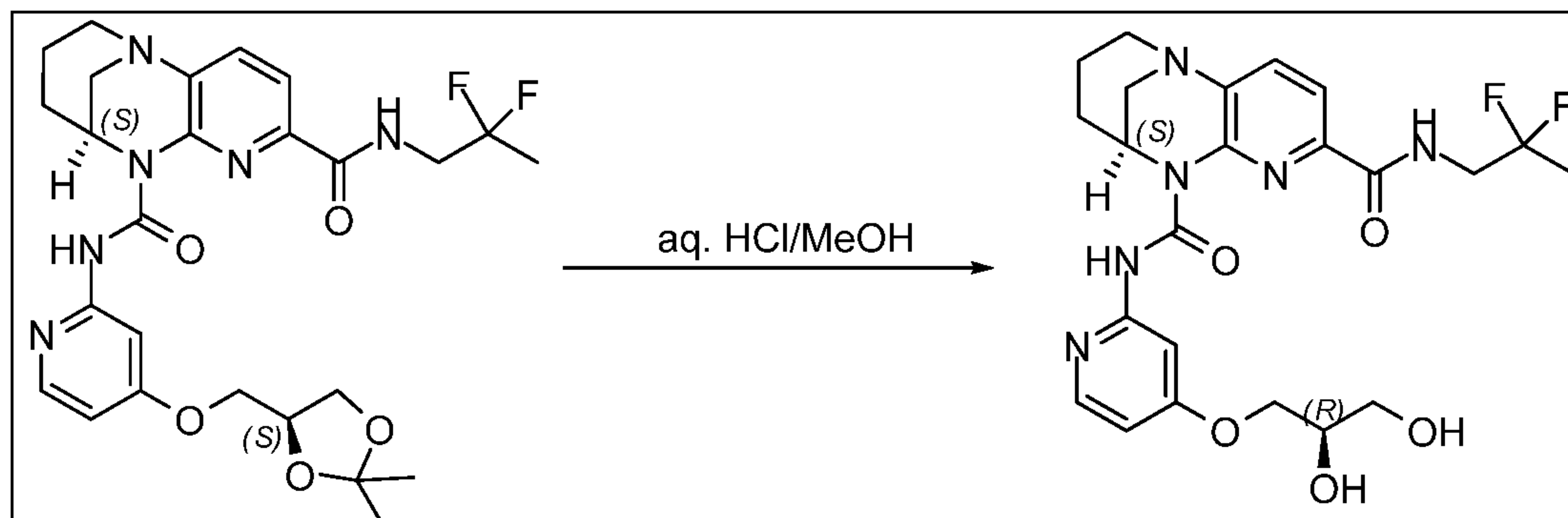
Example 34

Synthesis of (9*S*)-*N*10-(4-((*R*)-2,3-dihydroxypropoxy)pyridin-2-yl)-*N*2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide:



To a solution of (9*S*)-*N*10-(4-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-*N*2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (200 mg, 0.354 mmol) in Methanol (10 mL) was added aq.HCl (0.308 mL, 3.54 mmol), drop wise over a period of 5 min at 0 °C and stirred at room temperature for 1 h. (TLC system: 100 % Ethyl acetate. *R_f* value: 0.3). Then the reaction mixture was quenched with saturated NaHCO₃ solution (20 mL) and extracted with DCM (2x30 mL). The combined organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to obtain crude product. The crude product was triturated with pentane: diethyl ether (1:1) to afford the required purity of desired product (9*S*)-*N*10-(4-((*R*)-2,3-dihydroxypropoxy)pyridin-2-yl)-*N*2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (100 mg, 0.187 mmol, 52.7 % yield) as an off white solid. LCMS (*m/z*): 525.14 [M+H]⁺, *R_t* = 1.53 min.

¹H NMR (400 MHz, CDCl₃): δ ppm 13.84 (s, 1 H), 8.16 (br d, *J*=9.21 Hz, 1 H), 8.09 (d, *J*=5.92 Hz, 1 H), 8.00 (d, *J*=7.89 Hz, 1 H), 7.80 (d, *J*=2.19 Hz, 1 H), 7.61 (d, *J*=7.89 Hz, 1 H), 6.60 (dd, *J*=5.81, 2.30 Hz, 1 H), 5.17 - 5.04 (m, 1 H), 4.96 (br s, 1 H), 4.25 - 4.03 (m, 3 H), 3.89 - 3.71 (m, 2 H), 3.44 - 3.18 (m, 3 H), 2.96 (br d, *J*=14.03 Hz, 1 H), 2.59 (br s, 1 H), 2.22 (br d, *J*=14.47 Hz, 1 H), 2.06 - 1.76 (m, 2 H), 1.60 (d, *J*=7.02 Hz, 3 H), 1.45 - 1.33 (m, 2 H).

Example 35**Synthesis of (9*S*)-*N*2-(2,2-difluoropropyl)-*N*10-(4-((*R*)-2,3-dihydroxypropoxy)pyridin-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide**

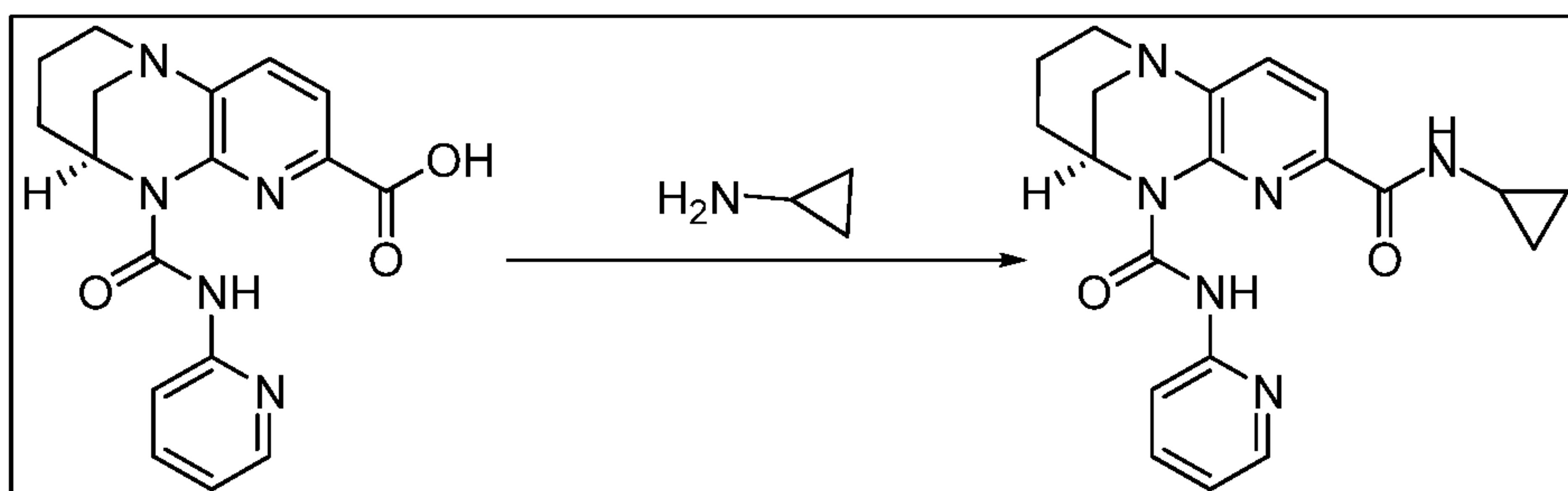
5

To a solution of (9*S*)-*N*2-(2,2-difluoropropyl)-*N*10-(4-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (210 mg, 0.384 mmol) in Methanol (10 mL) was added aq.HCl (0.667 mL, 7.68 mmol) drop wise over a period of 5 min at 0 °C and stirred at room temperature for 1 h. (TLC system: 100 % Ethyl acetate. *R_f* value: 0.3). Then, the reaction mixture was quenched with saturated NaHCO₃ solution (20 mL) and extracted with DCM (2x30 mL). The combined organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure and was triturated with pentane: diethyl ether (1:1) to afford the desired product (9*S*)-*N*2-(2,2-difluoropropyl)-*N*10-(4-((*R*)-2,3-

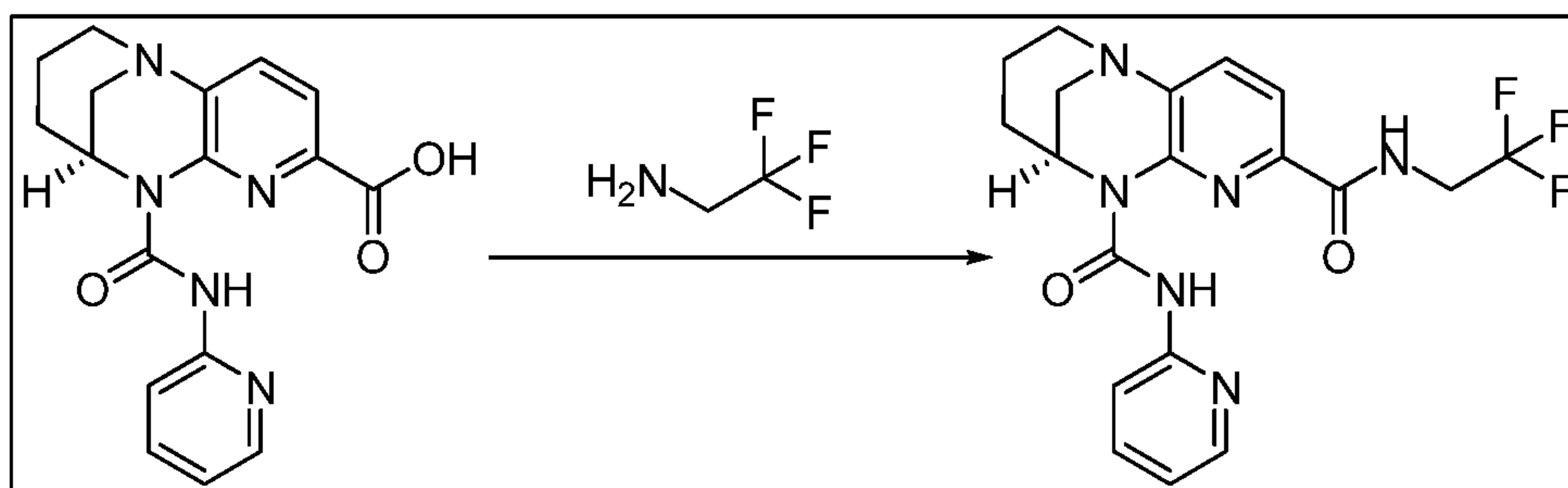
10 dihydroxypropoxy)pyridin-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (95 mg, 0.186 mmol, 48.5 % yield) as an off white solid. LCMS (*m/z*): 507.14 [M+H]⁺, *R_t* = 1.40 min.

¹H NMR (400 MHz, CDCl₃): δ ppm 14.25 (s, 1 H), 8.69 (br t, *J*=6.47 Hz, 1 H), 8.12 (d, *J*=5.92 Hz, 1 H), 7.95 (d, *J*=8.11 Hz, 1 H), 7.71 (d, *J*=2.19 Hz, 1 H), 7.60 (d, *J*=7.89 Hz, 1 H), 6.58 (dd, *J*=5.92, 2.41 Hz, 1 H), 4.94 (br s, 1 H), 4.24 - 4.08 (m, 3 H), 4.04 - 3.90 (m, 2 H), 3.87 - 3.73 (m, 2 H), 3.43 - 3.24 (m, 3 H), 2.97 (br d, *J*=14.03 Hz, 1 H), 2.62 - 2.50 (m, 1 H), 2.27 - 2.16 (m, 1 H), 2.08 - 1.89 (m, 2 H), 1.69 (t, *J*=18.64 Hz, 3 H), 1.465 - 1.35 (m, 2 H).

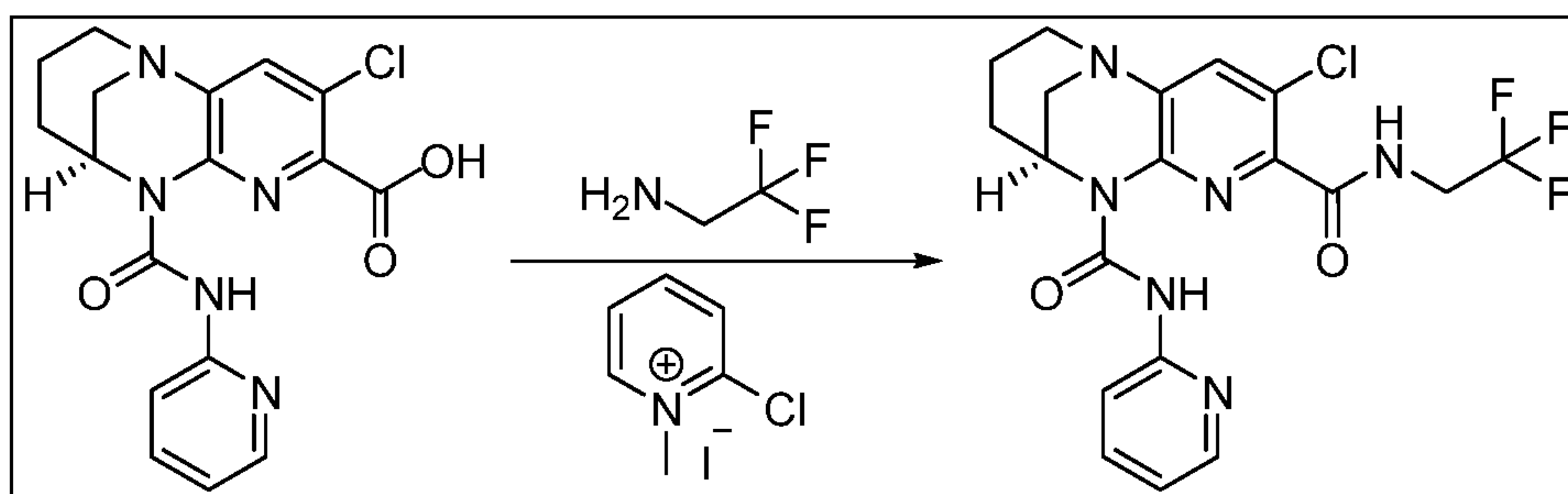
20

Example 36**Synthesis of (9S)-N2-cyclopropyl-N10-(pyridin-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide**

- 5 To a stirred solution of (9S)-10-(pyridin-2-ylcarbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2-carboxylic acid (6g, 17.68 mmol) in DMF (60 mL), under nitrogen at 0 °C was added DIPEA (9.26 mL, 53.0 mmol), HATU (13.45 g, 35.4 mmol) and cyclopropanamine (1.514 g, 26.5 mmol) and the reaction mixture was stirred at RT for 16 h. (TLC system: 70 % Ethylacetate in Hexane, R_f value: 0.3). To the reaction
- 10 mixture was added ice cold water (100 mL) and stirred for 15 min. The resultant solid was filtered, dried and purified by combiflash chromatography (using silicagel column, eluted with 3 % Methanol in DCM) to afford (9S)-N2-cyclopropyl-N10-(pyridin-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide (4.05 g, 10.70 mmol, 60.5 % yield) as an off-white solid. LCMS (m/z): 379.16 [M+H]⁺, R_t=1.97
- 15 min.
- ¹H NMR (400 MHz, CDCl₃): δ ppm 13.70 - 14.14 (m, 1 H), 8.29 - 8.42 (m, 1 H), 8.16 (dt, J=8.33, 0.88 Hz, 2 H), 7.97 (d, J=7.89 Hz, 1 H), 7.72 (td, J=7.84, 1.64 Hz, 1 H), 7.58 (d, J=7.89 Hz, 1 H), 7.04 (ddd, J=7.29, 4.99, 1.10 Hz, 1 H), 4.97 (t, J=2.19 Hz, 1 H), 3.29 - 3.42 (m, 3 H), 3.10 (ddt, J=10.93, 7.32, 3.92, 3.92 Hz, 1 H), 2.96 (br d, J=13.59 Hz, 1 H),
- 20 2.16 - 2.29 (m, 1 H), 1.86 - 1.99 (m, 1 H), 1.59 (s, 1 H), 1.32 - 1.45 (m, 2 H), 0.81 - 1.02 (m, 4 H).

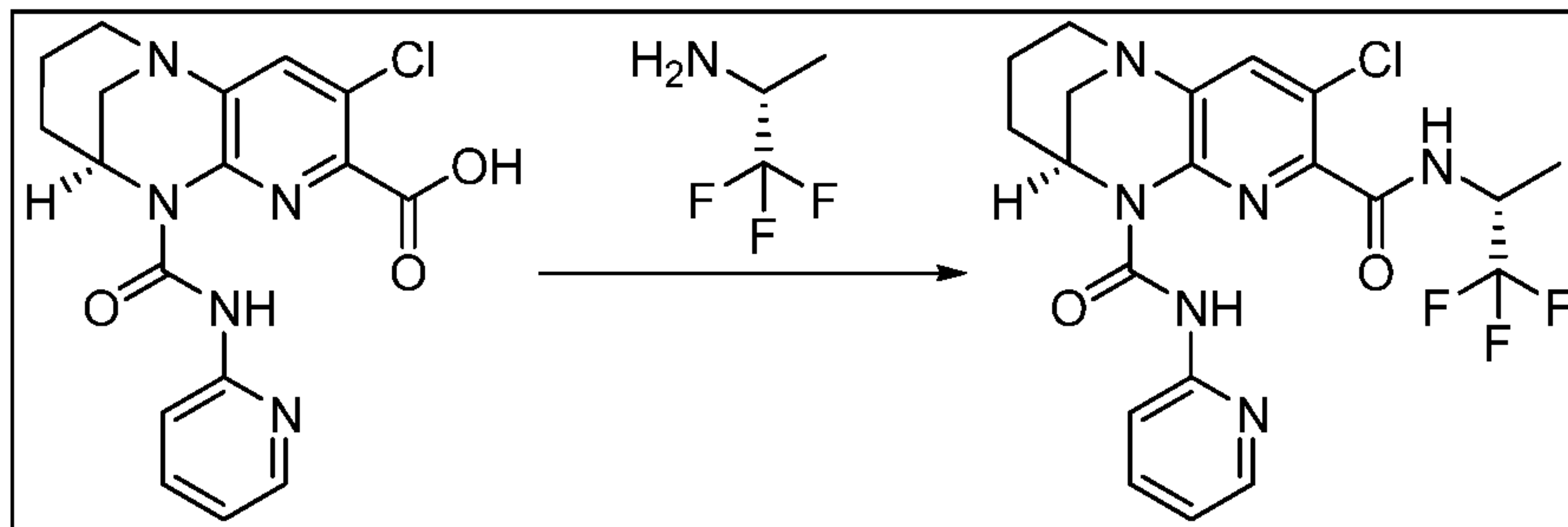
Example 37**Synthesis of (9*S*)-N10-(pyridin-2-yl)-N2-(2,2,2-trifluoroethyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide**

- 5 To a stirred solution of (9*S*)-10-(pyridin-2-ylcarbamoyl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2-carboxylic acid (7g, 20.63 mmol) in DMF (50 mL), under nitrogen at 0 °C was added DIPEA (10.81 mL, 61.9 mmol), HATU (15.69 g, 41.3 mmol) and 2,2,2-trifluoroethanamine (2.248 g, 22.69 mmol) then the reaction was stirred at RT for 16 h. (TLC system: 70 % Ethylacetate in Hexane, *R_f* value: 0.3). To the reaction
- 10 mixture was added ice cold water (200 mL) and stirred for 30 min. The resultant solid was filtered, dried and purified by combiflash chromatography (using silicagel column, eluted with 3 % Methanol in DCM) to afford the product as an off-white solid. The solid product was dissolved in Ethanol (500 mL) at 80 °C and added palladium scavenger (Silicycle Brand, 8 g) and continued heating for 5 h. The reaction mixture was filtered through celite,
- 15 filtrate was concentrated to afford (9*S*)-N10-(pyridin-2-yl)-N2-(2,2,2-trifluoroethyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (8.1 g, 19.17 mmol, 93 % yield) as an off-white solid. LCMS (*m/z*): 421.1 [*M*+*H*]⁺, *R_t*=2.23 min. ¹**H NMR** (400 MHz, CDCl₃): δ ppm 14.13 - 14.37 (m, 1 H), 8.71 (br t, *J*=5.92 Hz, 1 H), 8.25 (dd, *J*=4.93, 0.99 Hz, 1 H), 8.11 (d, *J*=8.33 Hz, 1 H), 7.96 (d, *J*=7.89 Hz, 1 H), 7.70 - 7.77 (m, 1 H), 7.61 (d, *J*=7.89 Hz, 1 H), 7.00 - 7.05 (m, 1 H), 4.97 (t, *J*=2.30 Hz, 1 H), 4.13 - 4.37 (m, 2 H), 3.27 - 3.45 (m, 3 H), 2.97 (br d, *J*=13.59 Hz, 1 H), 2.18 - 2.27 (m, 1 H), 1.87 - 2.00 (m, 1 H), 1.34 - 1.46 (m, 2 H).
- 20

Example 38**Synthesis of (9*S*)-3-chloro-*N*10-(pyridin-2-yl)-*N*2-(2,2,2-trifluoroethyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide.**

- 5 To a suspension of (9*S*)-3-chloro-10-(pyridin-2-ylcarbamoyl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2-carboxylic acid (900 mg, 2.408 mmol) in Acetonitrile (15 mL), under nitrogen at RT was added DIPEA (1.262 mL, 7.22 mmol), 2-chloro-1-methylpyridin-1-ium iodide (615 mg, 2.408 mmol) and 2,2,2-trifluoroethanamine (238 mg, 2.408 mmol), then the resulting reaction mixture was stirred
- 10 for 16 h. (TLC system: 75% EtOAc in Hexane, R_f value: 0.30). Reaction mass was diluted with 100 mL of water and extracted with EtOAc (2 x 150 mL). The combined organic layer was washed with brine (80 mL), dried over Na_2SO_4 , filtered and concentrated to get crude. The crude material was purified by combiflash (using silica gel column, 70% EtOAc in Hexane). Fractions containing compound were combined and
- 15 concentrated to afford the desired compound (9*S*)-3-chloro-*N*10-(pyridin-2-yl)-*N*2-(2,2,2-trifluoroethyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (52 mg, 0.114 mmol, 4.75 % yield) as an off white solid. LCMS (m/z): 455.05 $[\text{M}+\text{H}]^+$, $R_t = 2.17$ min.

- ^1H NMR** (400 MHz, CDCl_3): δ ppm 13.43 (s, 1 H), 8.29 - 8.49 (m, 1 H), 8.25 (d, $J=4.17$ Hz, 1 H), 8.11 (d, $J=8.33$ Hz, 1 H), 7.67 - 7.84 (m, 1 H), 7.59 (s, 1 H), 6.95 - 7.14 (m, 1 H), 4.97 (br s, 1 H), 4.12 - 4.37 (m, 2 H), 3.28 - 3.44 (m, 3 H), 2.95 (br d, $J=13.81$ Hz, 1 H), 2.10 - 2.33 (m, 1 H), 1.86 - 2.07 (m, 1 H), 1.25 - 1.50 (m, 2 H).
- 20

Example 39**Synthesis of (9*S*)-3-chloro-*N*10-(pyridin-2-yl)-*N*2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide.**

5

To a stirred solution of (9*S*)-3-chloro-10-(pyridin-2-ylcarbamoyl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2-carboxylic acid (500 mg, 1.338 mmol) in DMF (6 mL), under nitrogen at RT was added DIPEA (0.701 mL, 4.01 mmol), HATU (1017 mg, 2.68 mmol) and (*R*)-1,1,1-trifluoropropan-2-amine (182 mg, 1.605 mmol), then the

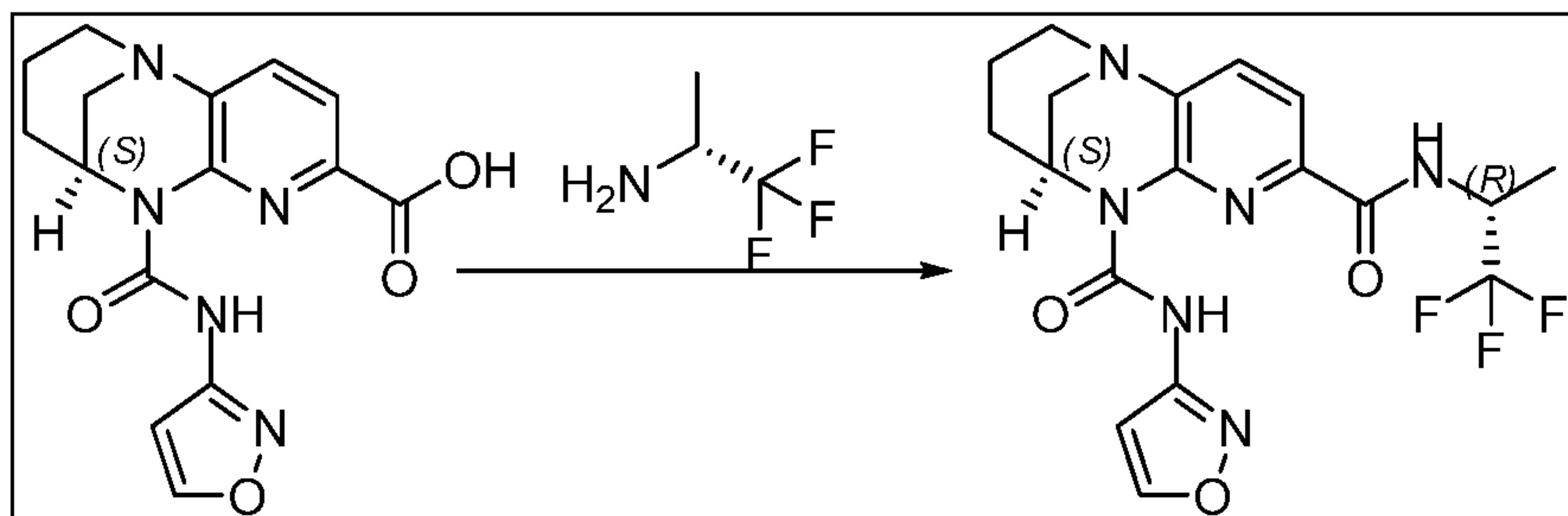
10 resulting reaction mixture was stirred at RT for 16 h. (TLC system: 5% MeOH in DCM, *R_f* value: 0.30). Reaction mass was diluted with 50 mL of ice cold water, extracted with EtOAc (2x100 mL). The combined organic layer was washed with brine (80 mL), dried over Na₂SO₄, filtered and concentrated to get crude compound. The crude material was purified by combiflash (using silica gel column, 80% EtOAc in Hexane). Fractions

15 containing compound were combined and concentrated to give the desired compound with 60 % purity by LCMS. This was further purified by prep HPLC (Column: Kromsil phenyl (150x25) mm 10μ; MP-A: 10mM Ammonium Bicarbonate (aq), MP-B: Acetonitrile; Method: 50:50; Flow: 20 ml/min Solubility: ACN+THF) to afford the desired product (9*S*)-3-chloro-*N*10-(pyridin-2-yl)-*N*2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (50 mg, 0.106 mmol, 7.93

20 % yield) as an off white solid. LCMS (*m/z*): 469.16 [M+H]⁺, *R_t* = 2.22 min.

¹H NMR (400 MHz, CDCl₃): δ ppm 12.89 (s, 1 H), 8.27 (d, *J*=4.17 Hz, 1 H), 8.15 (d, *J*=8.33 Hz, 1 H), 7.64 - 7.80 (m, 2 H), 7.59 (s, 1 H), 6.90 - 7.15 (m, 1 H), 5.09 (dq, *J*=16.83, 7.55 Hz, 1 H), 4.89 - 5.01 (m, 1 H), 3.26 - 3.44 (m, 3 H), 2.94 (br d, *J*=13.81 Hz, 1 H), 2.14 - 2.35 (m, 1 H), 1.85 - 2.09 (m, 1 H), 1.39 - 1.63 (m, 3 H), 1.40-1.32 (s, 2 H).

25

Example 40**Synthesis of (9*S*)-*N*10-(isoxazol-3-yl)-*N*2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide**

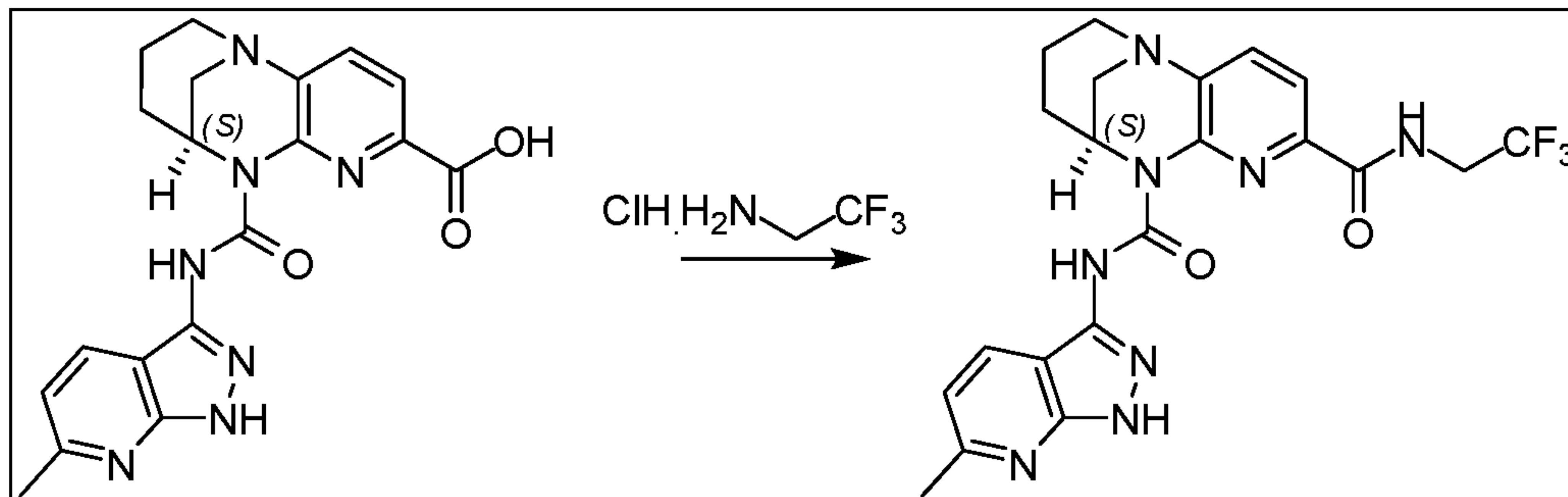
- 5 To a stirred solution of (9*S*)-10-(isoxazol-3-ylcarbamoyl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2-carboxylic acid (400 mg, 1.215 mmol) and (*R*)-1,1,1-trifluoropropan-2-amine (206 mg, 1.822 mmol) in DMF (15 mL) under nitrogen at RT was added HATU (554 mg, 1.458 mmol), DIPEA (0.424 mL, 2.429 mmol) and stirred for 16 h. (TLC system: Ethylacetate. *R_f* value: 0.6). The reaction mixture was quenched
- 10 with ice cold water (30 mL) and extracted with ethyl acetate (3x50 mL). The combined organic layer was washed with brine solution (30 mL), dried over anhydrous sodium sulphate and concentrated under reduced pressure to obtain crude compound. The crude product was purified by flash column chromatography (Silica gel, using 60% Ethylacetate in petether) to afford the desired product (9*S*)-*N*10-(isoxazol-3-yl)-*N*2-((*R*)-1,1,1-
- 15 trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (160 mg, 0.373 mmol, 30.7 % yield) as an off white solid. LCMS (*m/z*): 425.09 [*M*+*H*]⁺, *R_t* = 2.14 min.

¹**H NMR** (400 MHz, CDCl₃): δ ppm 13.55 (s, 1 H), 8.30 (d, *J*=1.32 Hz, 1 H), 7.96 (d, *J*=7.89 Hz, 1 H), 7.62 (d, *J*=7.89 Hz, 1 H), 7.23 (br s, 1 H), 6.99 (d, *J*=1.75 Hz, 1 H), 4.89 - 5.05 (m, 2 H), 3.32 - 3.47 (m, 3 H), 2.96 (br d, *J*=13.81 Hz, 1 H), 2.22 (dt, *J*=14.69, 2.96 Hz, 1 H), 1.90 - 2.02 (m, 1 H), 1.64 (d, *J*=7.02 Hz, 3 H), 1.33 - 1.45 (m, 2 H).

20

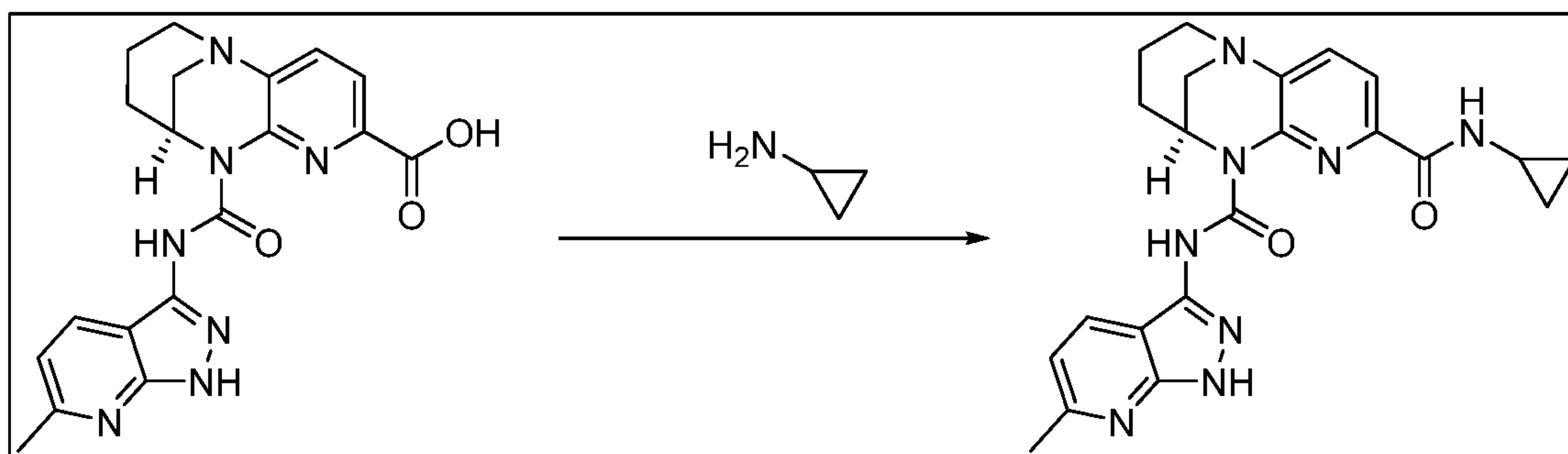
Example 41

Synthesis of (9*S*)-N10-(6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-N2-(2,2,2-trifluoroethyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide.



To a stirred solution of (9*S*)-10-((6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)carbamoyl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2-carboxylic acid (200 mg, 0.508 mmol) in N,N-Dimethylformamide (5 mL), HATU (193 mg, 0.508 mmol) and DIPEA (0.089 mL, 0.508 mmol) were added under nitrogen atmosphere at 28 °C and stirred for 30 min. at room temperature and followed by 2,2,2-trifluoroethanamine hydrochloride (103 mg, 0.763 mmol) was added and the reaction mixture was stirred at 28 °C for 16 h. (TLC: 10 % MeOH/CH₂Cl₂, R_f value: 0.4). The reaction mixture was diluted with water (40 mL) and extracted with EtOAc (2x40 mL). The combined organic layer was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to get crude compound. The crude product was purified by flash column chromatography (Silicagel:100-200 Mesh, Eluent: 5% Methanol /DCM) to afford the desired product (9*S*)-N10-(6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-N2-(2,2,2-trifluoroethyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (100 mg, 0.211 mmol, 41.5 % yield) as an off white solid. LCMS (*m/z*): 475.10 [M+H]⁺, R_t = 1.96 min.

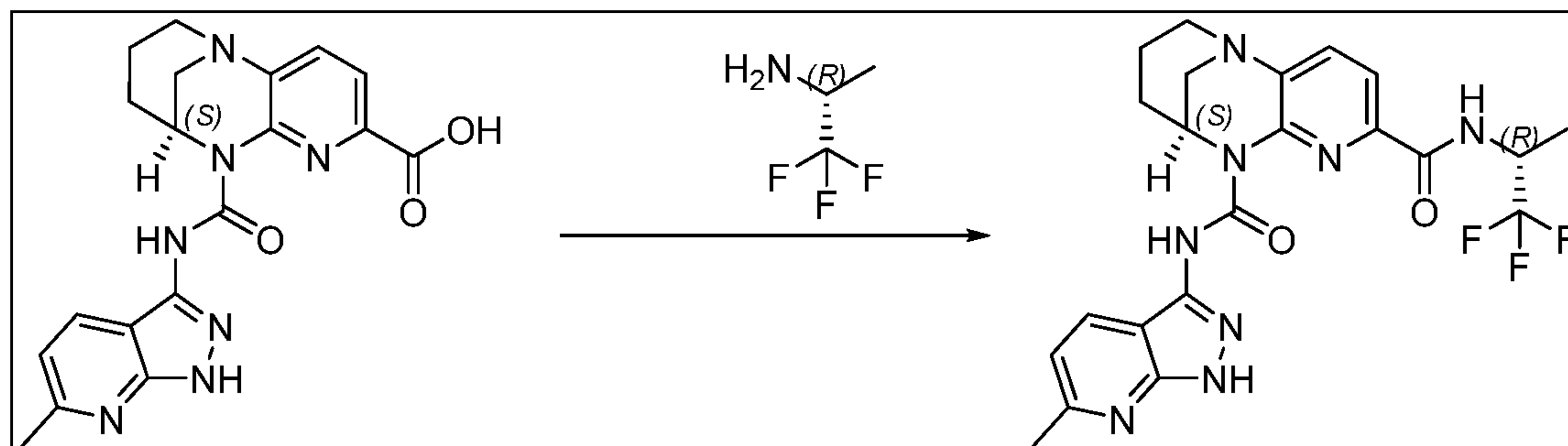
¹H NMR (400 MHz, CDCl₃-*d*): δ ppm 13.85 (s, 1 H), 10.39 (s, 1 H), 8.75 (d, *J*=8.33 Hz, 1 H), 8.04 -7.90 (m, 2 H), 7.63 (d, *J*=7.89 Hz, 1 H), 7.03 (d, *J*=8.55 Hz, 1 H), 5.00 (d, *J*=2.19 Hz, 1 H), 4.37- 4.12 (m, 2 H), 3.48 -3.30 (m, 3 H), 3.00 (d, *J*=13.59 Hz, 1 H), 2.69 (s, 3 H), 2.37 -2.25 (m, 1 H), 2.07 -1.88 (m, 1 H), 1.48 -1.39 (m, 2 H).

Example 42**Synthesis of (9*S*)-N2-cyclopropyl-N10-(6-methyl-1H-pyrazolo[3,4-*b*]pyridin-3-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide**

- 5 To a stirred solution of (9*S*)-10-((6-methyl-1H-pyrazolo[3,4-*b*]pyridin-3-yl)carbamoyl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2-carboxylic acid (300 mg, 0.763 mmol), HATU (435 mg, 1.144 mmol) and DIPEA (0.400 mL, 2.288 mmol) in DMF (15 mL) was added cyclopropanamine (65.3 mg, 1.144 mmol) at room temperature and stirred the reaction mixture at RT for 16 h. (TLC: neat ethyl acetate, *R_f* value: 0.3, UV
- 10 active). The reaction mixture was diluted with water (100 mL) and extracted with EtOAc (2x100 mL). The combined organic layer was washed with saturated brine solution (50 mL) and dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to obtain crude compound. The crude product was purified by flash column chromatography (Silica gel: 100-200 Mesh, Eluent: 80% ethyl acetate in n-hexane) to afford the desired product (9*S*)-
- 15 N2-cyclopropyl-N10-(6-methyl-1H-pyrazolo[3,4-*b*]pyridin-3-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (90 mg, 0.202 mmol, 26.5 % yield) as an off white solid. LCMS (*m/z*): 433.16 [M+H]⁺, *R_t*=1.76 min.
- ¹H NMR (400 MHz, CDCl₃): δ ppm 13.67 -13.63 (m, 1 H), 13.65 (s, 1 H), 11.16 (s, 1 H), 8.71 (d, *J*=8.33 Hz, 1 H), 7.93 (d, *J*=7.89 Hz, 1 H), 7.60 (d, *J*=8.11 Hz, 1 H), 7.52 (d, *J*=2.85 Hz, 1 H), 7.03 (d, *J*=8.55 Hz, 1 H), 5.01 (s, 1 H), 3.42- 3.31 (m, 3 H), 3.12 -3.05 (m, 1 H), 3.00 (d, *J*=13.81 Hz, 1 H), 2.73 (s, 3 H), 2.27 (d, *J*=14.47 Hz, 1 H), 2.01- 1.88 (m, 1 H), 1.45- 1.36 (m, 2 H), 0.91- 0.85 (m, 3 H).
- 20

Example 43**Synthesis of (9*S*)-N10-(6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-N2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide**

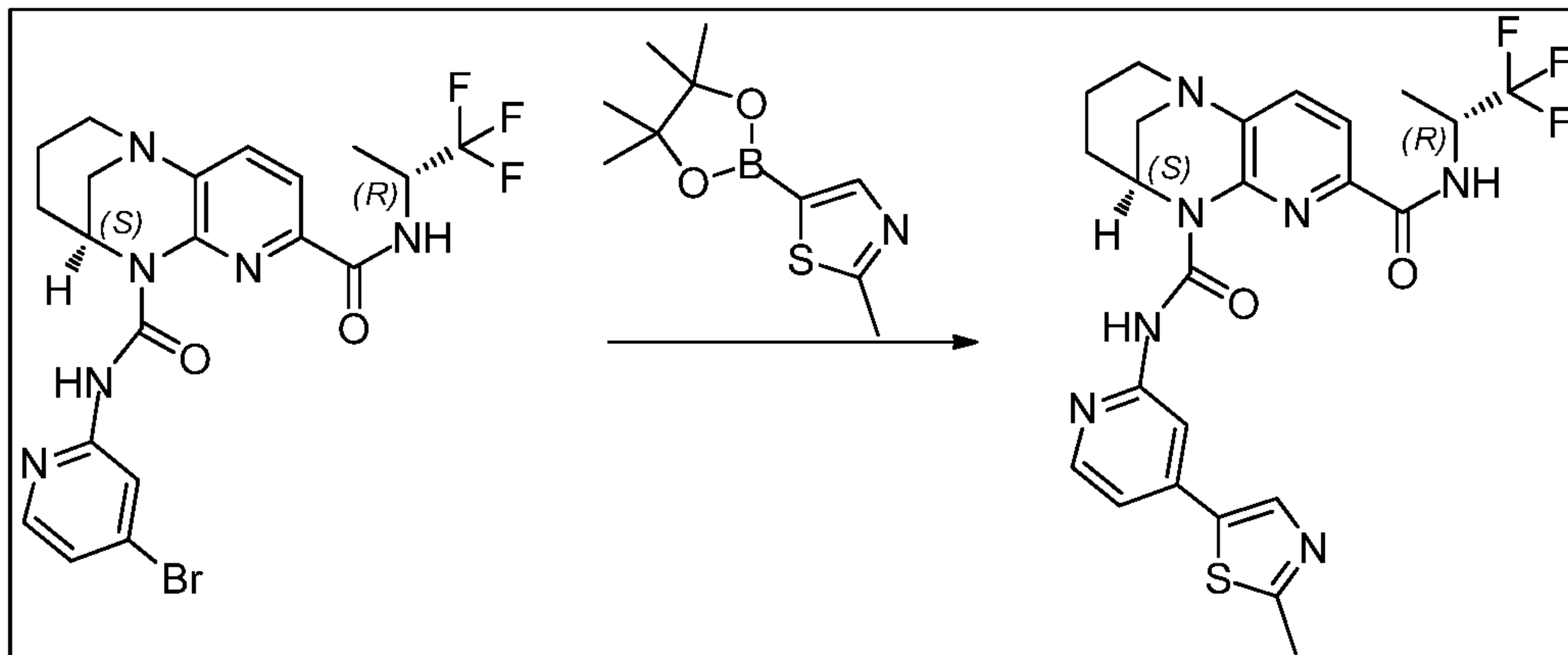
5



To a stirred solution of (9*S*)-10-((6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)carbamoyl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2-carboxylic acid (200 mg, 0.508 mmol), HOBt (97 mg, 0.635 mmol), EDC (122 mg, 0.635 mmol) and DIPEA (0.266 mL, 1.525 mmol) in DMF (15 mL) was added (*R*)-1,1,1-trifluoropropan-2-amine (69.0 mg, 0.610 mmol) at room temperature and stirred the reaction mixture at RT for 16 h. (TLC: neat ethyl acetate, *R_f* value: 0.3, UV active). The reaction mixture was diluted with water (100 mL) and extracted with EtOAc (2x100 mL). The combined organic layer was washed with brine solution (50 mL) and dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to obtain crude compound. The crude product was purified by flash

column chromatography (using 100-200 silica gel, compound eluted at 80% ethyl acetate in n-hexane) to afford the desired product (9*S*)-N10-(6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-N2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (119 mg, 0.237 mmol, 46.6 % yield) as an off white solid. LCMS: (*m/z*): 489.2 [M+H]⁺, *R_t* = 2.06 min.

¹H NMR (400 MHz, CDCl₃): δ ppm 13.63 (s, 1 H), 10.14 (s, 1 H), 8.69 (d, *J*=8.33 Hz, 1 H), 7.96 (d, *J*=7.89 Hz, 1 H), 7.69 (d, *J*=9.21 Hz, 1 H), 7.62 (d, *J*=7.89 Hz, 1 H), 7.03 (d, *J*=8.55 Hz, 1 H), 5.12- 4.95 (m, 2 H), 3.51-3.18 (m, 3 H), 2.93 - 3.05 (m, 1 H), 2.68 (s, 3 H), 2.29 (d, *J*=14.47 Hz, 1 H), 2.04- 1.87 (m, 1 H), 1.57 (s, 3 H), 1.49 - 1.25 (m, 2 H).

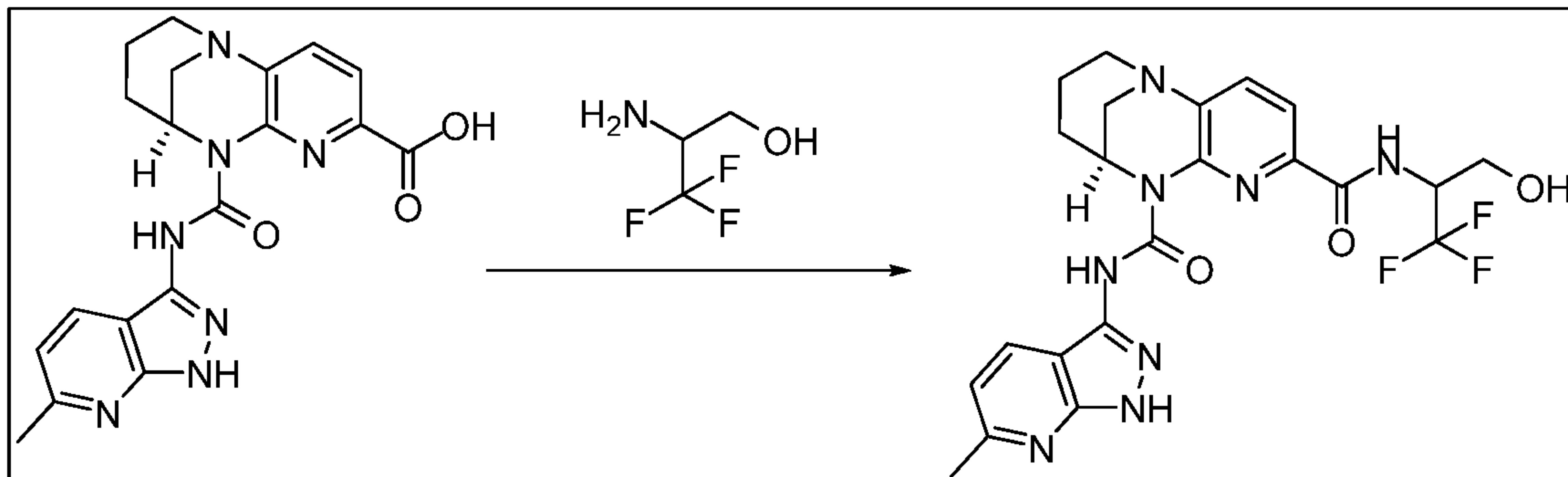
Example 44**Synthesis of (9*S*)-*N*10-(4-(2-methylthiazol-5-yl)pyridin-2-yl)-*N*2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide**

To a solution of (9*S*)-*N*10-(4-bromopyridin-2-yl)-*N*2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (350 mg, 0.682 mmol) and 2-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole (230 mg, 1.023 mmol) in 1,4-Dioxane (20 mL) and water (1.0 mL) under nitrogen at RT was added K_3PO_4 (434 mg, 2.046 mmol) and degassed by purging argon for 20 min. Then added $PdCl_2(dppf) \cdot CH_2Cl_2$ adduct (55.7 mg, 0.068 mmol) and the mixture was at 110 °C for 3.5 h. (TLC system: 100% Ethyl acetate. R_f value: 0.3). The reaction mixture was cooled to RT and diluted with water (30 mL), extracted with ethyl acetate (2x50 mL). The combined organic layer was washed with brine solution (50 mL) and dried over anhydrous sodium sulphate and concentrated under reduced pressure to obtain crude compound. The crude product was purified by flash column chromatography (100-200 silicagel eluted with 50% of EtOAc in Pet ether) to afford the desired product (9*S*)-*N*10-(4-(2-methylthiazol-5-yl)pyridin-2-yl)-*N*2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (320 mg, 0.594 mmol, 87 % yield) as an off white solid. LCMS (m/z): 532.20 $[M+H]^+$, R_t = 2.60 min.

^1H NMR (400 MHz, $CDCl_3$): δ ppm 13.88 (s, 1 H), 8.39 (s, 1 H), 8.26 (d, J =5.26 Hz, 1 H), 8.13 - 7.89 (m, 3 H), 7.62 (d, J =8.11 Hz, 1 H), 7.15 (dd, J =5.26, 1.53 Hz, 1 H), 5.20 - 5.06 (m, 1 H), 5.00 (br s, 1 H), 3.44 - 3.21 (m, 3 H), 3.03 - 2.90 (m, 1 H), 2.76 (s, 3 H), 2.25 (br d, J =14.47 Hz, 1 H), 2.05 - 1.89 (m, 1 H), 1.62 (d, J =7.02 Hz, 3 H), 1.49 - 1.36 (m, 2 H).

Example 45

Synthesis of (9*S*)-*N*10-(6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-*N*2-(1,1,1-trifluoro-3-hydroxypropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide.

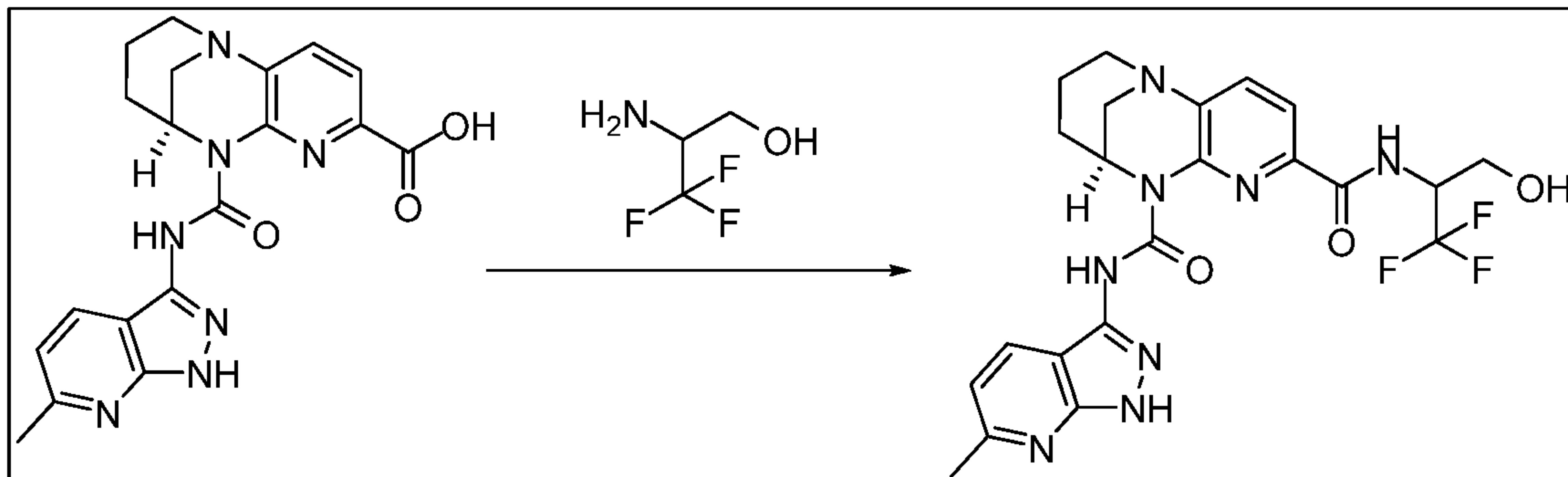


To a stirred suspension of (9*S*)-10-((6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)carbamoyl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2-carboxylic acid (4.5 g, 11.44 mmol) in pyridine (40 mL) under nitrogen at 0 °C was added EDC (4.39 g, 22.88 mmol) followed by 2-amino-3,3,3-trifluoropropan-1-ol (2.215 g, 17.16 mmol) and the reaction mixture was stirred at RT for 16 h. (TLC system 5% Methanol in DCM. R_f value 0.3). The reaction mixture was concentrated and the residue was dissolved in EtOAc (200mL) and washed with water (2 x 100 mL). Combined organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated to get crude as brown solid. The solid was triturated with diethylether (50mL), filtered and dried to get the desired compound as diastereomeric mixture. The diastereomers were separated by preparative chiral SFC (Column/dimensions: Chiralpak AD-H (250 x 30)mm, 5μ; % CO₂: 50.0; % Co-solvent: 50.0% (MeOH); Total Flow: 100.0 g/min, Back Pressure: 100.0 bar; UV: 213 nm, Stack time: 6.7 min, Load/inj: 95.0mg, Solubility: MeOH, Total No of injections: 60, Instrument details: Make/Model: Thar SFC-200 NEW-1).

Peak-1 Collected fraction from SFC was concentrated and triturated with diethylether (20 mL), dried and grinded in mortar to afford (9*S*)-*N*10-(6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-*N*2-(1,1,1-trifluoro-3-hydroxypropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (920 mg, 1.821 mmol, 15.92 % yield) as an off- white solid. LCMS (*m/z*): 505.23 [M+H]⁺. R_t = 1.85 min.

Example 46

Synthesis of (9*S*)-N10-(6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-N2-(1,1,1-trifluoro-3-hydroxypropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide.



5

To a stirred suspension of (9*S*)-10-((6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)carbamoyl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2-carboxylic acid (4.5 g, 11.44 mmol) in pyridine (40 mL) under nitrogen at 0 °C was added EDC (4.39 g, 22.88 mmol) followed by 2-amino-3,3,3-trifluoropropan-1-ol (2.215 g, 17.16 mmol) and the reaction mixture was stirred at RT for 16 h. (TLC system 5% Methanol in DCM. R_f value 0.3). The reaction mixture was concentrated and the residue was dissolved in EtOAc (200mL) and washed with water (2 x 100 mL). Combined organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated to get crude as brown solid. The solid was triturated with diethylether (50mL), filtered and dried to get the desired compound as diastereomeric mixture. The diastereomers were separated by preparative chiral SFC (Column/dimensions: Chiralpak AD-H (250 x 30)mm, 5μ; % CO₂: 50.0; % Co-solvent: 50.0% (MeOH); Total Flow: 100.0 g/min, Back Pressure: 100.0 bar; UV: 213 nm, Stack time: 6.7 min, Load/inj: 95.0mg, Solubility: MeOH, Total No of injections: 60, Instrument details: Make/Model: Thar SFC-200 NEW-1).

10

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Peak-2 Collected fraction from SFC was concentrated and washed with diethylether (20 mL), dried and grinded in mortar to afford (9*S*)-N10-(6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-N2-(1,1,1-trifluoro-3-hydroxypropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (1 g, 1.966 mmol, 17.19 % yield) as an off-white solid. LCMS (*m/z*): 505.2323 [M+H]⁺. R_t=1.85 min

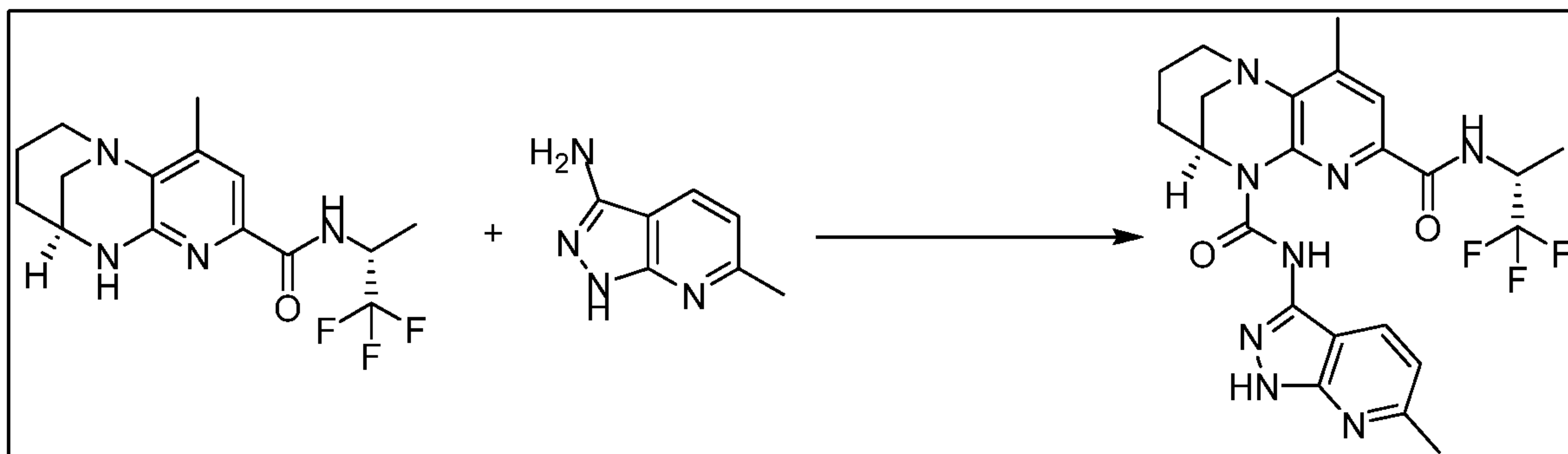
25

¹H NMR (400 MHz, DMSO-*d*₆): δ ppm 13.15 (s, 1 H), 12.92 - 13.08 (m, 1 H), 8.56 (d, *J*=8.99 Hz, 1 H), 8.39 (d, *J*=8.33 Hz, 1 H), 7.60 - 7.85 (m, 2 H), 7.03 (d, *J*=8.55 Hz, 1 H), 5.24 (t, *J*=6.36 Hz, 1 H), 4.74 - 4.91 (m, 2 H), 3.85 (t, *J*=6.25 Hz, 2 H), 3.43 (dd, *J*=13.48,

1.64 Hz, 3 H), 2.81 - 3.04 (m, 1 H), 2.57 (s, 3 H), 2.05 (br d, $J=18.42$ Hz, 2 H), 1.33 (br d, $J=8.33$ Hz, 2 H).

Example 47

5 Synthesis of (9*S*)-4-methyl-N10-(6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-N2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide:

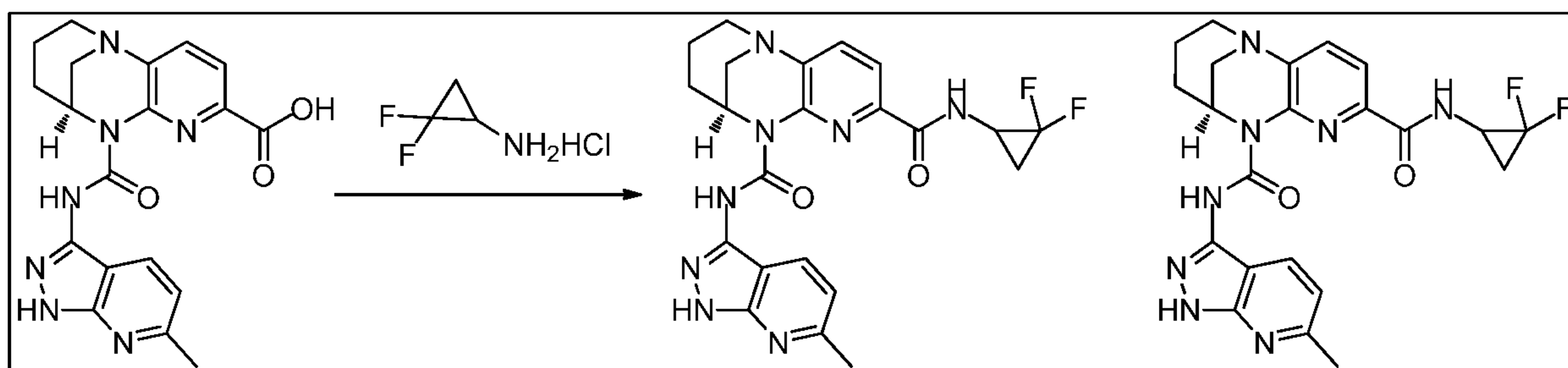


- 10 To a stirred solution of (9*S*)-4-methyl-N-((*R*)-1,1,1-trifluoropropan-2-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2-carboxamide (300 mg, 0.914 mmol) in THF (30 mL) at RT was added TEA (0.764 mL, 5.48 mmol), triphosgene (271 mg, 0.914 mmol) and stirred for 30 min, then added 6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (271 mg, 1.827 mmol) and the reaction mixture was heated at 65 °C for 16 h.
- 15 (TLC eluting system: 100% EtOAc: R_f 0.4; UV active). The reaction mixture was cooled to RT, quenched with water (20 mL) and extracted into EtOAc (2x35 mL). Organic layer was separated, dried over anhydrous sodium sulphate, filtered and filtrate was evaporated to get the crude product. The crude was purified by chromatography (GRACE using C-18 reserval column, Mobile phase A: 0.1% Formic Acid in water; B: ACN, eluent 44% B in
- 20 A) and combined fractions were concentrated and basified with saturated NaHCO_3 . The aqueous layer was extracted with DCM, combined DCM layer was dried over anhydrous Na_2SO_4 , filtered and filtrate was evaporated to afford (9*S*)-4-methyl-N10-(6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-N2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (105 mg, 0.204 mmol, 22.34
- 25 % yield) as yellow solid. LCMS (m/z): 503.25 $[\text{M}+\text{H}]^+$, R_t = 2.38 min.
- ¹**H NMR** (400MHz, CDCl_3): δ ppm 13.87 (s, 1 H), 9.81 (br s, 1 H), 8.70 (d, $J=8.1$ Hz, 1 H), 7.84 (s, 1 H), 7.68 (br d, $J=8.8$ Hz, 1 H), 7.02 (d, $J=8.3$ Hz, 1 H), 5.11 - 4.90 (m, 2 H),

3.39 (br d, $J=13.4$ Hz, 1 H), 3.21 (br d, $J=6.8$ Hz, 2 H), 2.97 (br d, $J=14.0$ Hz, 1 H), 2.66 (s, 3 H), 2.43 (s, 3 H), 2.38 - 2.13 (m, 1 H), 2.13 - 1.86 (m, 1 H), 1.63 - 1.49 (m, 3 H), 1.42 (br d, $J=6.1$ Hz, 2 H).

5 Example 48

Synthesis of (9*S*)-N2-(2,2-difluorocyclopropyl)-N10-(6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide



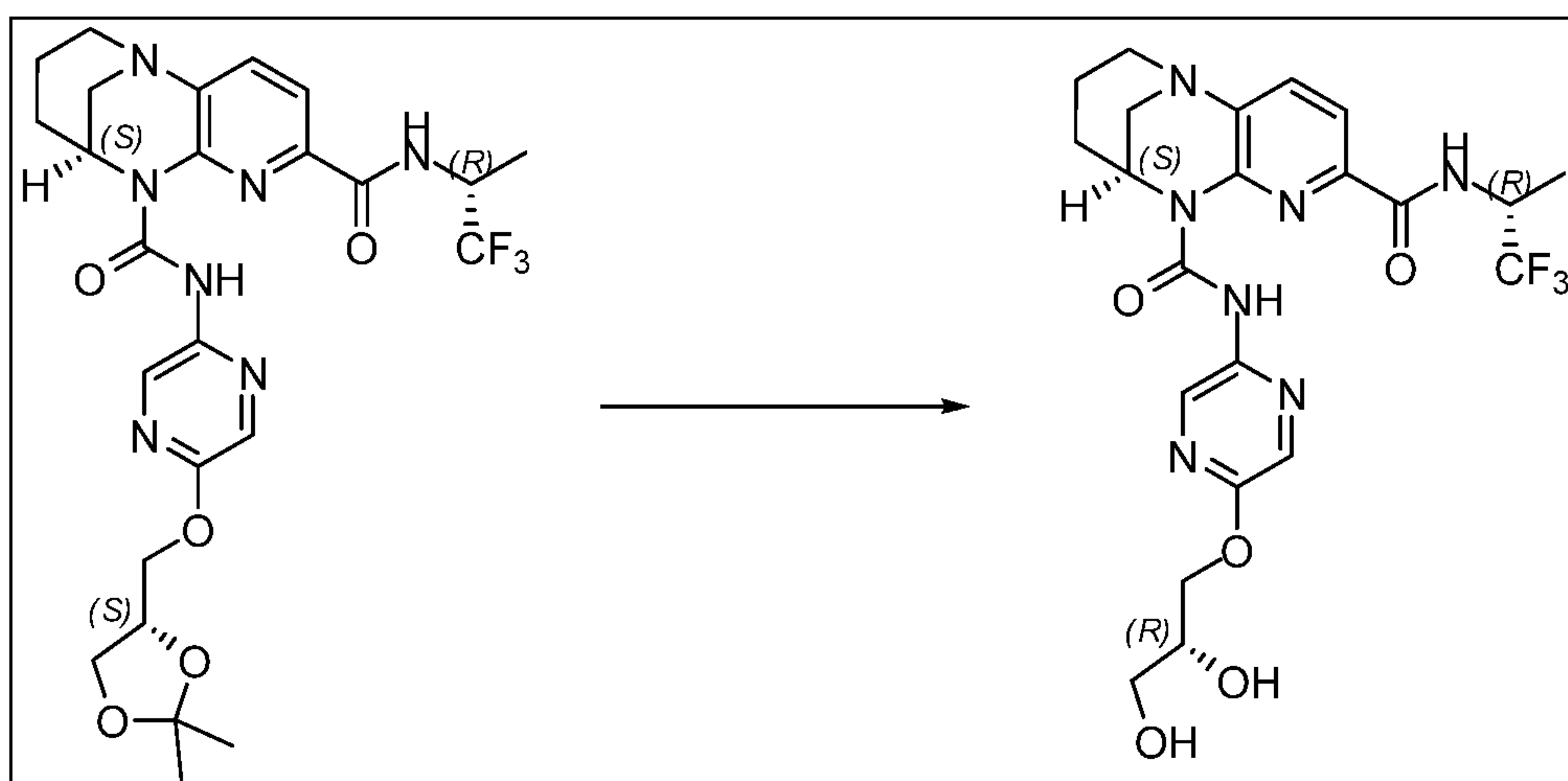
To a stirred solution of (9*S*)-10-((6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)carbamoyl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2-carboxylic acid (500 mg, 2.54 mmol) in DMF (10 mL) stirred under nitrogen at room temp were added HATU (2.9 g, 7.63 mmol) and DMAP (0.621 g, 5.08 mmol). To this 2,2-difluorocyclopropanamine, Hydrochloride (0.823 g, 6.35 mmol) was added and the reaction mixture was stirred at 50 °C for 16 h (TLC eluent: 10% MeOH in DCM: R_f 0.4; UV active). Reaction mixture was allowed to cool to room temperature and diluted with ice water, extracted with ethylacetate (2x50 mL). The combined organic layer was washed with brine solution and dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford crude compound. The crude product was purified by column chromatography (silicagel: 100-200 Mesh, Eluent: 2% Methanol in DCM) to afford the diastereomeric mixture and submitted for SFC separation (Conditions: (Column/dimensions): Chiralpak IC (250 X30)mm, 5 μ , % CO₂: 50.0% Co solvent: 50.0% (100% M ETHANOL), Total Flow: 100.0 g/min, Back Pressure: 100.0 bar, UV: 217 nm, Stack time: 16.5 min, Load/inj: 46.0 mg, Solubility: Methanol, Total No of injections: 7, Instrument details: Make/Model: Thar SFC- 200 (OLD)) to afford two peaks as peak-I and peak-II.

Peak-II: (9*S*)-N2-(2,2-difluorocyclopropyl)-N10-(6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (73 mg, 0.155 mmol, 6.08 % yield) as a pale brown solid. LCMS (*m/z*): 469.2 [*M*+*H*]⁺, *R*_t = 1.94 min.

- 5 ¹**H NMR** (400 MHz, DMSO-*d*₆): δ ppm 13.2 (s, 1 H), 12.8 (s, 1 H), 8.64 (br s, 1 H), 8.41 (d, *J*=8.55 Hz, 1 H), 7.74 - 7.6 (m, 1 H), 7.64 - 7.62 (m, 1 H), 7.05 (d, *J*=8.55 Hz, 1 H), 4.87 (s, 1 H), 3.56 - 3.39 (m, 2 H), 3.35 (br s, 2 H), 2.87 (d, *J*=13.37 Hz, 1 H), 2.55 (s, 3 H), 2.07 - 1.9 (m, 4 H), 1.35 - 1.22 (m, 2 H).

10 Example 49

Synthesis of (9*S*)-N10-(5-((*R*)-2,3-dihydroxypropoxy)pyrazin-2-yl)-N2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide



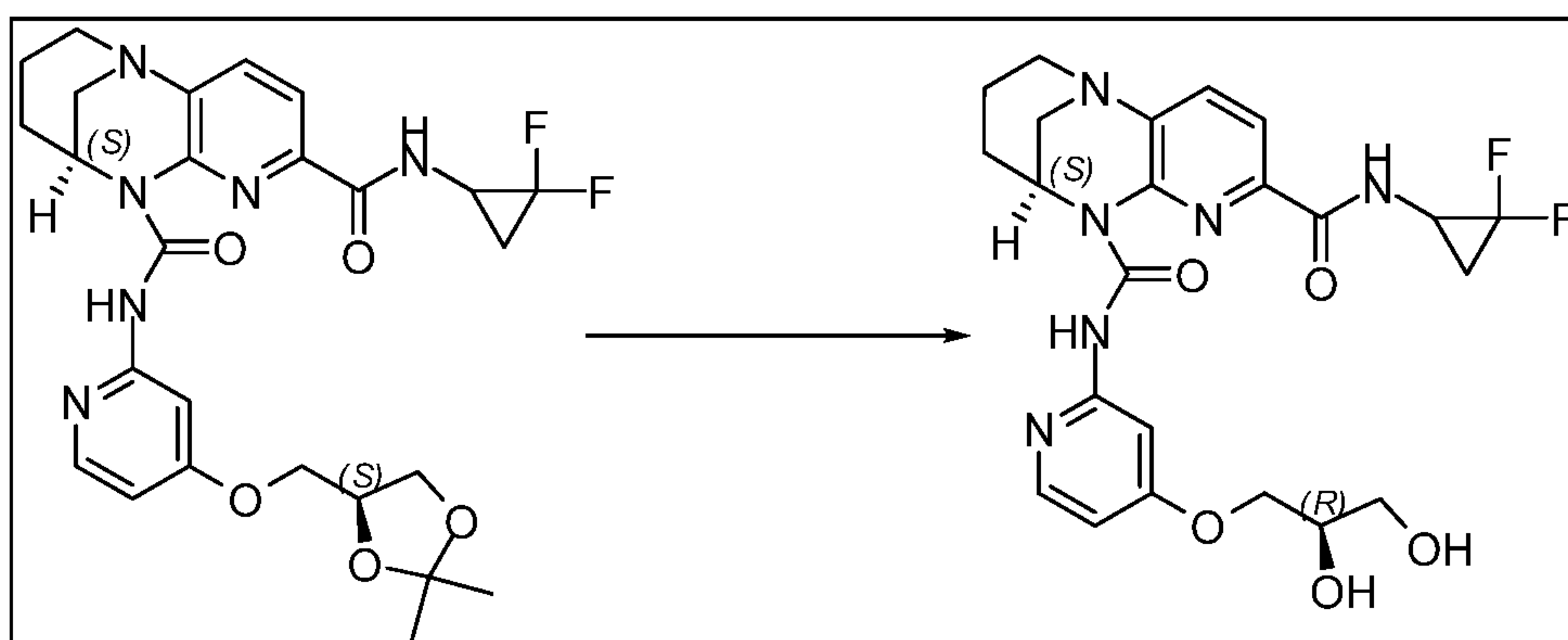
- 15 To a stirred solution of (9*S*)-N10-(5-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-yl)-N2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (200 mg, 0.354 mmol) in Methanol (10 mL) under nitrogen atmosphere was added aq HCl (4 mL, 16.00 mmol) at 0 °C. The resulted reaction mixture was stirred at 28 °C for 1 h. (TLC System: 5% Methanol in DCM, *R*_f: 0.3, UV active).
- 20 The solvent was evaporated under reduced pressure, basified with saturated NaHCO₃ solution (till pH:8-9) and the precipitated solid was filtered to afford the desired product (9*S*)-N10-(5-((*R*)-2,3-dihydroxypropoxy)pyrazin-2-yl)-N2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (110 mg, 0.207 mmol, 58.4 % yield) as an off-white solid. LCMS

(*m/z*): 526.26 [M+H]⁺, *R_t* = 1.87 min.

¹H NMR (400 MHz, CDCl₃): δ 13.64 (s, 1 H), 8.99 (s, 1 H), 8.96-7.96 (m, 2 H), 7.71 (d, *J*=9.43 Hz, 1 H), 7.62 (d, *J*=7.89 Hz, 1 H), 5.15-4.93 (m, 2 H), 4.54-4.39 (m, 2 H), 4.16-4.03 (m, 1 H), 3.85-3.66 (m, 2 H), 3.46-3.27 (m, 3 H), 3.08 (d, *J*=3.95 Hz, 1 H), 2.96 (d, *J*=14.03 Hz, 1 H), 2.33-2.18 (m, 2 H), 2.02-1.87 (m, 1 H), 1.57 (d, *J*=7.02 Hz, 3 H), 1.42 (s, 2 H).

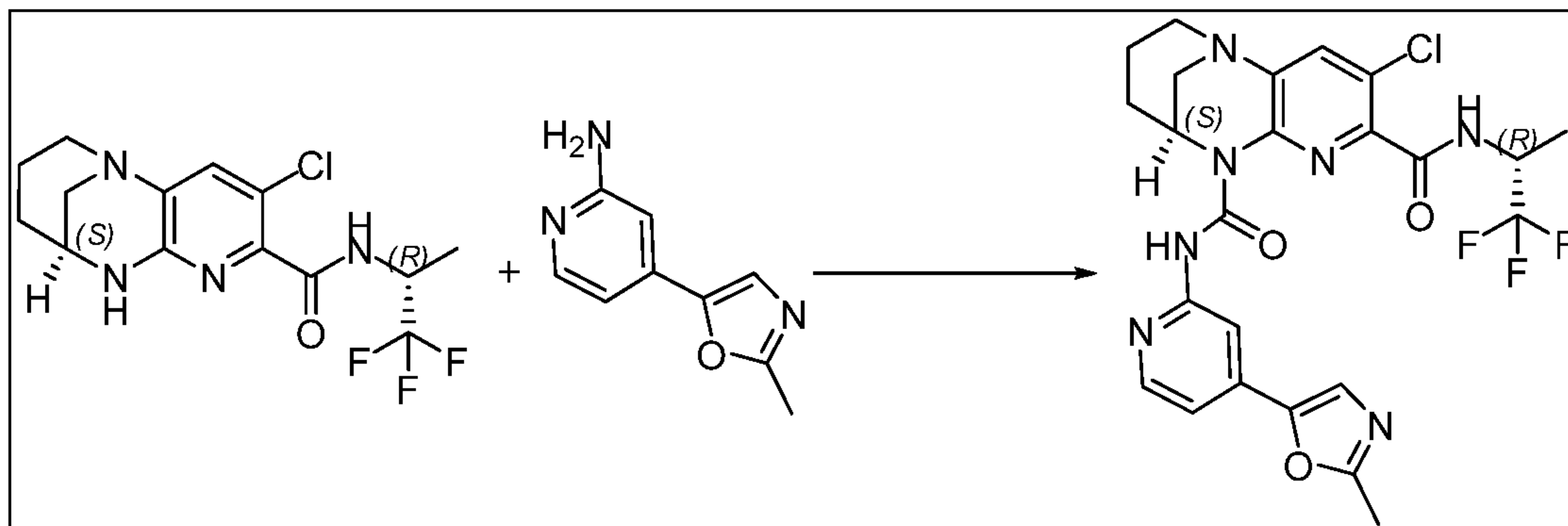
Example 50

Synthesis of (9*S*)-N2-(2,2-difluorocyclopropyl)-N10-(4-((*R*)-2,3-dihydroxypropoxy)pyridin-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide



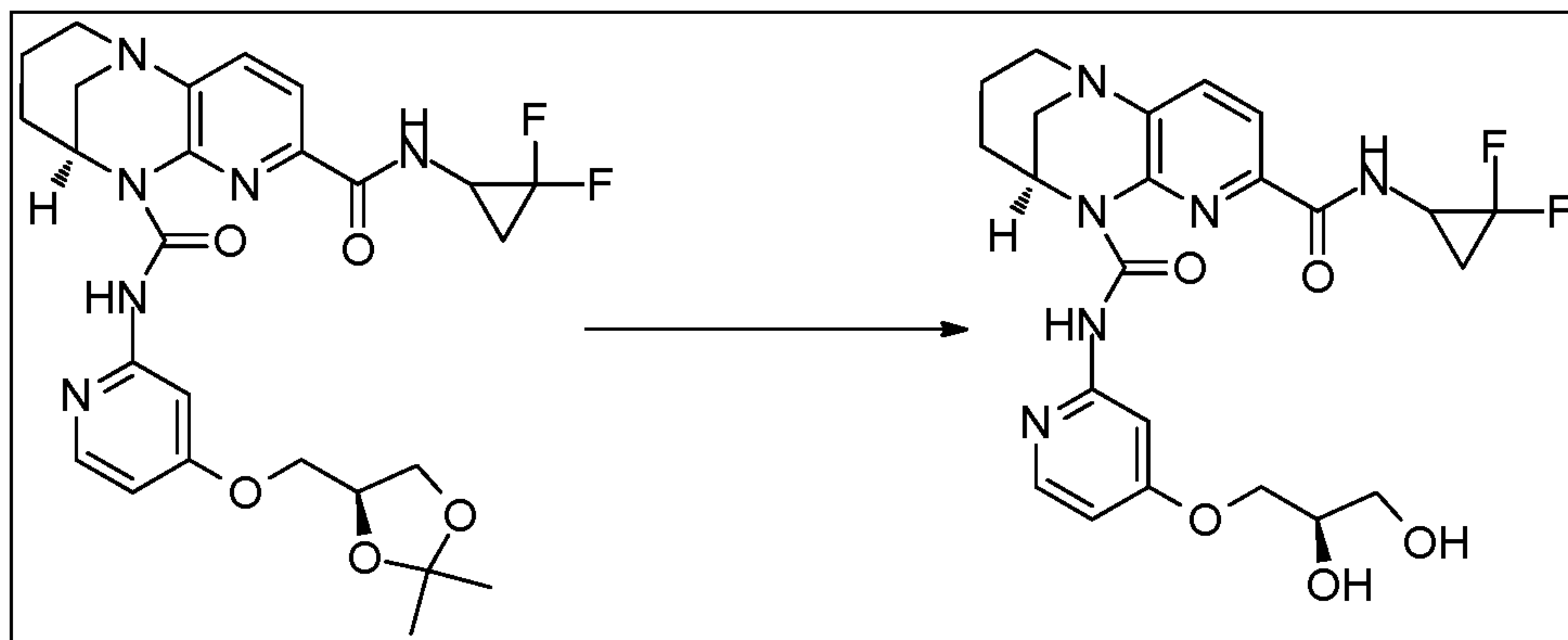
To a stirred solution of (9*S*)-N2-(2,2-difluorocyclopropyl)-N10-(4-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (270 mg, 0.496 mmol) in Methanol (5 mL) at 0 °C was added hydrochloric acid (0.030 mL, 0.992 mmol) drop wise over a period of 5 min. Then the reaction mixture was stirred at RT for 2 h. (TLC eluent: 5% MeOH in DCM: *R_f* 0.1; UV active). The reaction mixture was neutralization with sodium bicarbonate solution and filtered the obtain solid, washed with diethyl ether (2x 30 ml), *n*-pentane (2x 30 ml) to afford the desired product (9*S*)-N2-(2,2-difluorocyclopropyl)-N10-(4-((*R*)-2,3-dihydroxypropoxy)pyridin-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (150 mg, 0.297 mmol, 59.8 % yield) as an off white solid. LCMS (*m/z*): 505.23 [M+H]⁺, *R_t* = 1.50 min.

¹H NMR (400 MHz, TFA-*d*): δ ppm 8.58- 8.49 (m, 1 H), 8.28 (d, *J*=7.7 Hz, 1 H), 8.01-7.98 (m, 1 H), 7.39 - 7.20 (m, 2 H), 5.40-5.36 (m, 1 H), 4.68- 4.39 (m, 3 H), 4.45 - 3.80 (m, 6 H), 3.62 - 3.49 (m, 1 H), 2.39 - 2.29 (m, 1 H), 2.30 - 1.80 (m, 5 H).

Example 51**Synthesis of (9*S*)-3-chloro-*N*10-(4-(2-methyloxazol-5-yl)pyridin-2-yl)-*N*2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide**

To a solution of (9*S*)-3-chloro-*N*10-((*R*)-1,1,1-trifluoropropan-2-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2-carboxamide (700mg, 2.007mmol) in THF (20 mL) at RT, was added TEA (1.399 mL, 10.04 mmol) followed by triphosgene (357 mg, 1.204 mmol) and stirred for 30 min. then added 4-(2-methyloxazol-5-yl)pyridin-2-amine (527 mg, 3.01 mmol) and the reaction mixture was heated to 80 °C for 16 h. (TLC system:100% Ethylacetate, *R_f* value: 0.4). The reaction mixture diluted with cold water (50 mL) and extracted with EtOAc (2x50 mL). The combined organic layer was washed with brine solution (30 mL), dried over anhydrous sodiumsulphate and concentrated under reduced pressure to obtain crude compound. The crude product was purified by prep HPLC (Column: Kromosil Phenyl C18 (150*25)mm 10u, Mobile Phase-A: 10 mM Ammonium Bicarbonate (Aq), Mobile Phase-B: Acetonitrile, Gradient: Time T/%B = 0/45, 12/45,12.5/100, 15/100, 15.5/45, Column Temp: Ambient, Flow Rate: 30 ml/min, Diluent: Acetonitrile+THF+Water) to afford (9*S*)-3-chloro-*N*10-(4-(2-methyloxazol-5-yl)pyridin-2-yl)-*N*2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]-diazocine-2,10(7*H*)-dicarboxamide (95 mg, 0.171 mmol, 8.53 % yield) as an off white solid. LCMS (*m/z*): 550.20 [*M*+*H*]⁺, *R_t* = 2.36 min.

¹H NMR (400 MHz, DMSO-*d*₆): δ ppm 13.04 (s, 1 H), 9.22 (d, *J*=8.77 Hz, 1 H), 8.36 (s, 1 H), 8.30 (d, *J*=5.26 Hz, 1 H), 7.81 (d, *J*=12.50 Hz, 2 H), 7.39 (dd, *J*=5.26, 1.53 Hz, 1 H), 4.93 - 4.71 (m, 2 H), 3.48 - 3.39 (m, 1 H), 3.32 - 3.30 (m, 2 H), 2.88 (br d, *J*=13.81 Hz, 1 H), 2.53 (s, 3 H), 2.02 - 1.88 (m, 2 H), 1.46 (d, *J*=7.02 Hz, 3 H), 1.38 - 1.22 (m, 2 H).

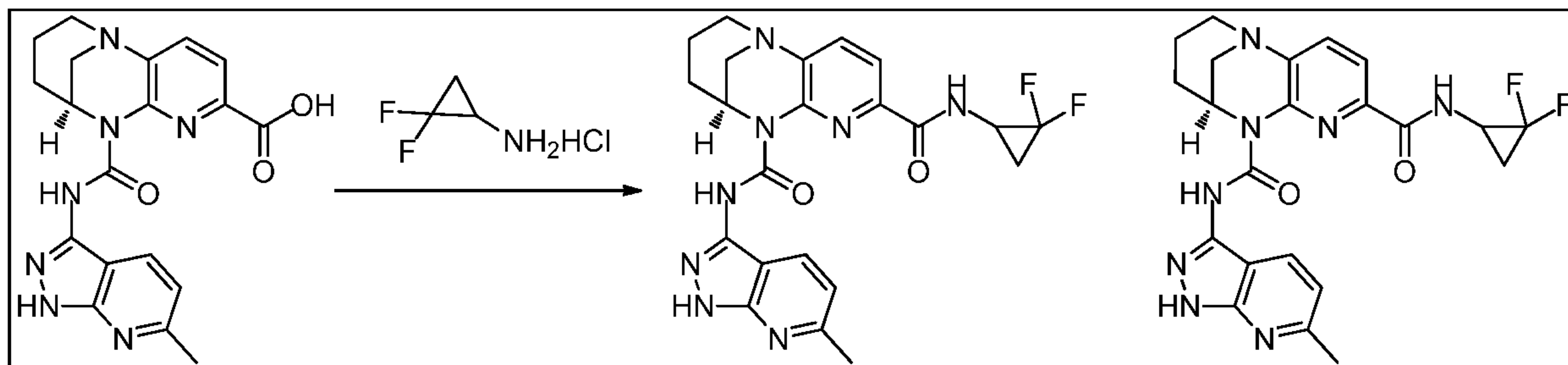
Example 52**Synthesis of (9S)-N2-(2,2-difluorocyclopropyl)-N10-(4-((R)-2,3-dihydroxypropoxy)pyridin-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide**

To a stirred solution of (9S)-N2-(2,2-difluorocyclopropyl)-N10-(4-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide (230 mg, 0.422 mmol) in Methanol (5 mL) at 0 °C was added hydrochloric acid (0.026 mL, 0.845 mmol), drop wise over a period of 5 min. Then the reaction mixture was stirred at RT for 2 h. (TLC eluent: 5% MeOH in DCM: R_f 0.1; UV active). Then reaction mixture was neutralized with sodium bicarbonate solution and obtained solid was filtered and washed with ether (2x 30 ml), pentane (2x 30 ml) to afford the desired product (9S)-N2-(2,2-difluorocyclopropyl)-N10-(4-((R)-2,3-dihydroxypropoxy)pyridin-2-yl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-2,10(7H)-dicarboxamide (175 mg, 0.346 mmol, 82 % yield) as an off white solid. LCMS (m/z): 505.23 $[M+H]^+$, R_t = 1.50 min.

¹H NMR (400 MHz, TFA-d): δ ppm 8.56 - 8.50 (m, 1 H), 8.39 (d, J =7.7 Hz, 1 H), 8.02 (m, 1 H), 7.24 - 7.10 (m, 2 H), 5.41-5.39 (m, 1 H), 4.64- 4.41 (m, 3 H), 4.39 - 3.89 (m, 6 H), 3.68 - 3.51-3.39 (m, 1 H), 2.41 - 2.29 (m, 1 H), 2.22 - 1.76 (m, 5 H).

Example 53**Synthesis of (9*S*)-N2-(2,2-difluorocyclopropyl)-N10-(6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide**

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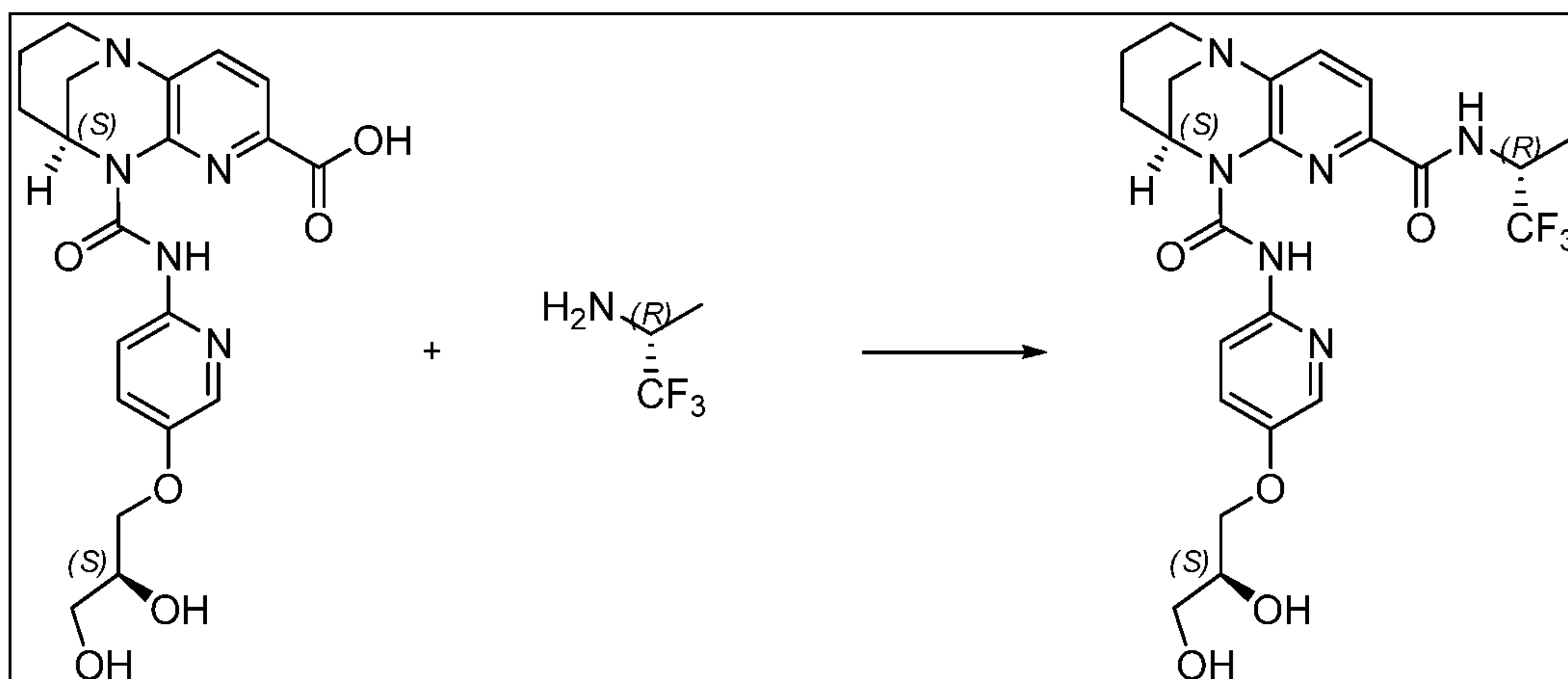
To a stirred solution of (9*S*)-10-((6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)carbamoyl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2-carboxylic acid (500 mg, 2.54 mmol) in DMF (10 mL) stirred under nitrogen at room temp were added HATU (2.9 g, 7.63 mmol) and DMAP (0.621 g, 5.08 mmol). To this 2,2-difluorocyclopropanamine, Hydrochloride (0.823 g, 6.35 mmol) was added and the reaction mixture was stirred at 50 °C for 16 h (TLC eluent: 10% MeOH in DCM: *R_f* 0.4; UV active). Reaction mixture was allowed to cool to room temperature and diluted with ice water, extracted with ethylacetate (2x50 mL). The combined organic layer was washed with brine solution and dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford crude compound. The crude product was purified by column chromatography (silicagel: 100-200 Mesh, Eluent: 2% Methanol in DCM) to afford the diastereomeric mixture and submitted for SFC separation (Conditions: (Column/dimensions): Chiralpak IC (250 X30)mm, 5μ, % CO₂: 50.0% Co solvent: 50.0% (100% M ETHANOL), Total Flow: 100.0 g/min, Back Pressure: 100.0 bar, UV: 217 nm, Stack time: 16.5 min, Load/inj: 46.0 mg, Solubility: Methanol, Total No of injections: 7, Instrument details: Make/Model: Thar SFC- 200 (OLD)) to afford two peaks as peak-I and peak-II.

Peak-I: (9*S*)-N2-(2,2-difluorocyclopropyl)-N10-(6-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (70 mg, 0.147 mmol, 5.80 % yield) as an off white solid. LCMS (*m/z*): 469.2 [M+H]⁺, *R_t* = 1.92 min.

¹H NMR (400 MHz, DMSO-*d*₆): δ ppm 13.26 (s, 1 H), 12.99 (s, 1 H), 8.64 (br s, 1 H), 8.41 (d, *J*=8.55 Hz, 1 H), 7.74 - 7.69 (m, 1 H), 7.66 - 7.62 (m, 1 H), 7.03 (d, *J*=8.55 Hz, 1 H), 4.87 (br s, 1 H), 3.54 - 3.39 (m, 2 H), 3.32 (br s, 2 H), 2.88 (d, *J*=13.37 Hz, 1 H), 2.57 (s, 3 H), 2.07 - 1.88 (m, 4 H), 1.35 - 1.24 (m, 2 H).

5

Example 54 Synthesis of (9*S*)-N10-(5-((*S*)-2,3-dihydroxypropoxy)pyridin-2-yl)-N2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide



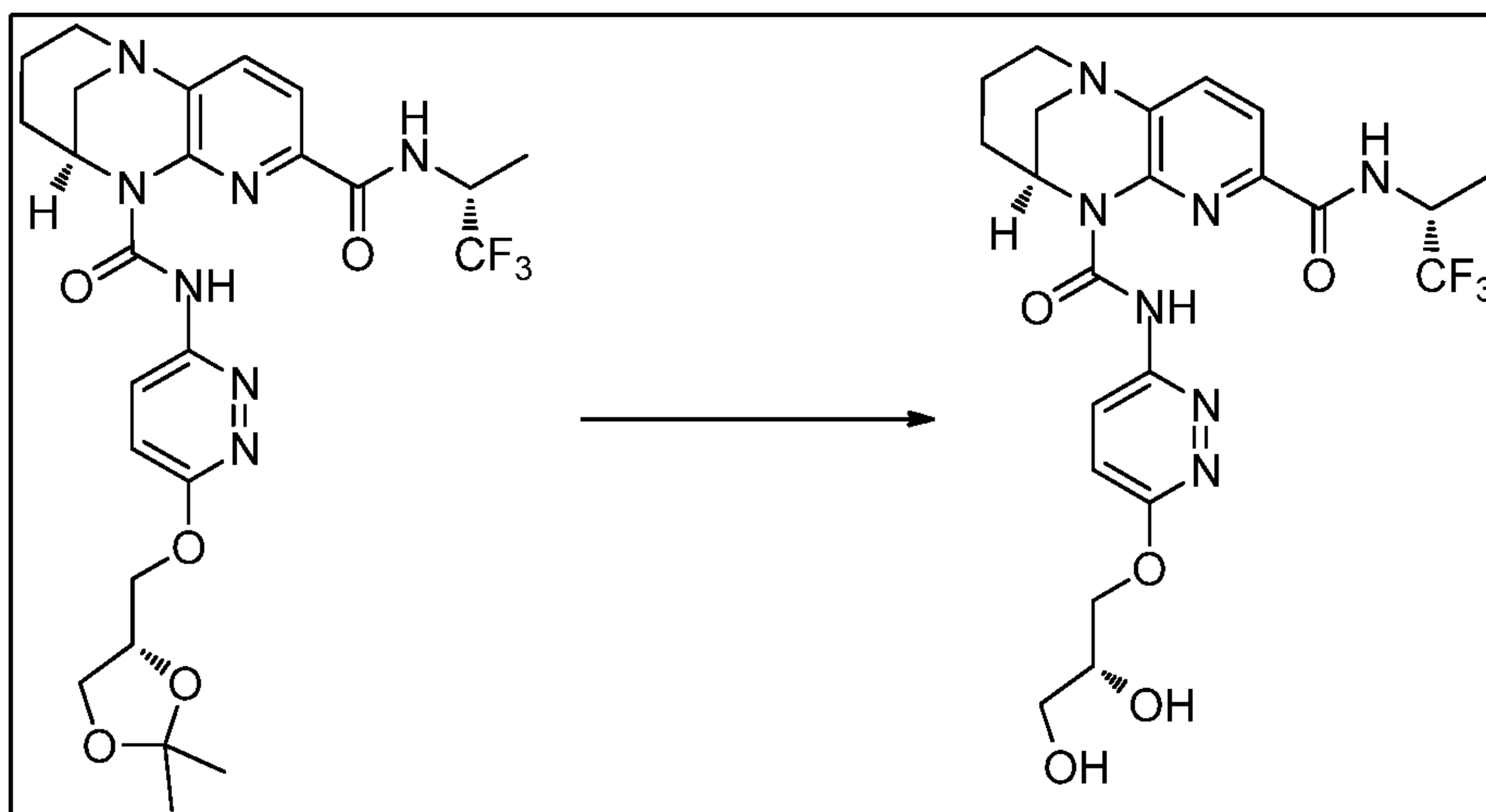
- 10 To a stirred solution of (9*S*)-10-((5-((*S*)-2,3-dihydroxypropoxy)pyridin-2-yl)carbamoyl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2-carboxylic acid (300 mg, 0.699 mmol) and HATU (398 mg, 1.048 mmol) in N,N-Dimethylformamide (10 mL) was added DIPEA (0.488 mL, 2.79 mmol) and followed by (*R*)-1,1,1-trifluoropropan-2-amine Hydrochloride (104 mg, 0.699 mmol) at room temperature to the reaction mixture.
- 15 The resulted reaction mixture was stirred for 16 h. at 28 °C (TLC eluent: 10% MeOH in DCM: *R_f*-0.3; UV active). The reaction mixture was poured in to water (30 mL) and extracted with EtOAc (2X20 mL). The combined organic layer was washed with brine solution dried over Na₂SO₄, filtered and evaporated to get crude compound. The crude compound was purified by GRACE (C-18 reservel column, Eluent: 60% of MeOH and
- 20 0.1% of Formic Acid in water) to afford the desired product (9*S*)-N10-(5-((*S*)-2,3-dihydroxypropoxy)pyridin-2-yl)-N2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (169 mg, 0.310 mmol, 44.4 % yield) as a pale yellow solid. LCMS (*m/z*): 525.25 [*M*+*H*]⁺, *R_t* = 1.86 min.

25 ¹H NMR (400 MHz, CDCl₃): δ ppm 13.62 (s, 1 H), 8.14 (d, *J*=8.99 Hz, 1 H), 8.06 - 7.90 (m, 3 H), 7.71 - 7.51 (m, 1 H), 7.41 7.24 (m, 1 H), 5.02 - 5.21 (m, 1 H), 4.98 (br s, 1 H),

4.24 - 4.03 (m, 3 H), 3.95 - 3.83 (m, 1 H), 3.76 - 3.82 (m, 1 H), 3.41 - 3.27 (m, 3 H), 2.95 (d, $J=14.03$ Hz, 1 H), 2.55 (br s, 1 H), 2.23 (d, $J=15.13$ Hz, 1 H), 1.87 - 2.01 (m, 2 H), 1.61 (d, $J=7.02$ Hz, 3 H), 1.48 - 1.34 (m, 2 H).

5 Example 55

Synthesis of (9*S*)-N10-(6-((*R*)-2,3-dihydroxypropoxy)pyridazin-3-yl)-N2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide

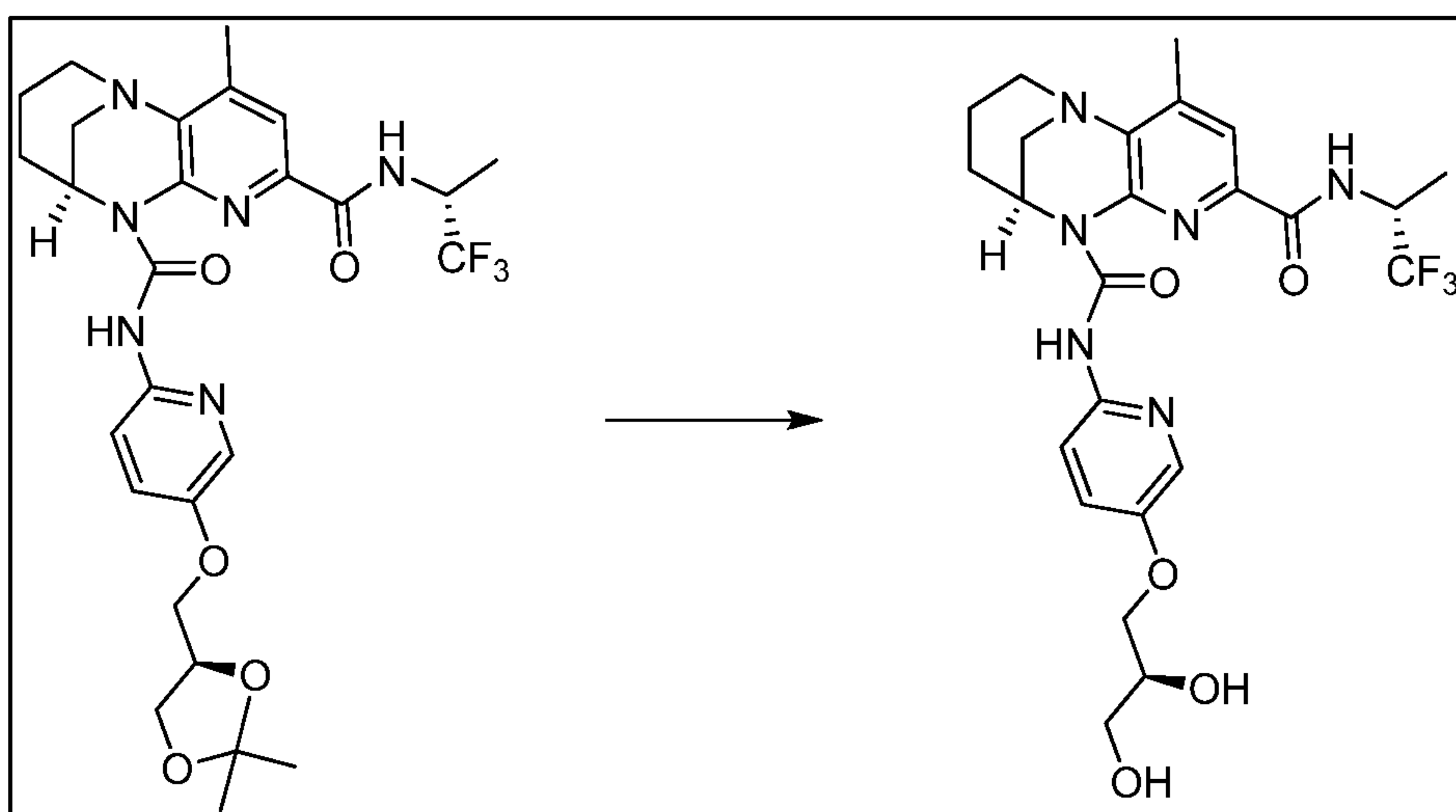


- 10 To a stirred solution of (9*S*)-N10-(6-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridazin-3-yl)-N2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (220 mg, 0.389 mmol) in Methanol (10 mL) under nitrogen was added Aq HCl (0.8 ml, 3.20 mmol) at 0 °C. The reaction mixture was stirred at 28 °C for 1 h. (TLC System: 5% Methanol in DCM, R_f : 0.3, UV active). The solvent was evaporated under reduced pressure, basified with saturated NaHCO₃ solution (till pH: 8-9) and extracted with DCM (3X30 mL). The combined organic layer was washed with water, brine solution, filtered and evaporated to get crude compound. The crude compound was purified by Prep HPLC (conditions: MP-A: 10 Mm Ammonium Acetate(Aq) MP-B: Acetonitrile Column: Kromasil C18(250*21.2) mm, 10 μ Method (T/%B): 65:35 Flow: 20 ml/min Solubility: Acetonitrile + MeOH + THF) to afford the desired product (9*S*)-N10-(6-((*R*)-2,3-dihydroxypropoxy)pyridazin-3-yl)-N2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (130 mg, 0.234 mmol, 60.1 % yield) as an off-white solid. LCMS (m/z): 524.2 $[M+H]^+$, R_t = 5.98 min.

¹H NMR (400 MHz, CDCl₃): δ ppm 14.34 (s, 1 H), 8.42 (d, *J*=9.43 Hz, 1 H), 8.07 (d, *J*=9.21 Hz, 1 H), 8.01 (d, *J*=7.89 Hz, 1 H), 7.63 (d, *J*=7.89 Hz, 1 H), 7.10 (d, *J*=9.43 Hz, 1 H), 5.12 - 5.00 (m, 1 H), 4.96 (br s, 1 H), 4.65 - 4.54 (m, 2 H), 4.16 - 4.08 (m, 1 H), 3.82 - 3.66 (m, 2 H), 3.49 (br s, 1 H), 3.43 - 3.30 (m, 3 H), 2.97 (d, *J*=13.81 Hz, 1 H), 2.34 (br s, 1 H), 2.26 - 2.17 (m, 1 H), 2.01 - 1.89 (m, 1 H), 1.71 (d, *J*=7.02 Hz, 3 H), 1.47 - 1.36 (m, 2 H).

Example 56

Synthesis of (9*S*)-N10-(5-((*S*)-2,3-dihydroxypropoxy)pyridin-2-yl)-4-methyl-N2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide:



To a stirred solution of (9*S*)-N10-(5-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-4-methyl-N2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (300 mg, 0.519 mmol) in methanol (15 mL) at 0 °C was added aq.HCl (1.5 ml, 49.4 mmol) and stirred under nitrogen at RT for 1 h. (TLC eluent: 5% MeOH in DCM *R_f* 0.2; UV active). The reaction mixture at 0 °C was basified with saturated sodiumbicarbonate solution (till pH-8-9) and solvent was evaporated under reduced pressure. The residue was diluted with water and resultant solid was filtered through Buchner funnel, dried under reduced pressure to afford crude compound. The crude was purified by chromatography (GRACE using C-18 reserval column, Mobile phase A: 0.1% Formic Acid in water; B: ACN, eluent 45-50% B in A) and combined fractions were concentrated then basified with saturated NaHCO₃. The precipitated solid was filtered and dried to afford (9*S*)-N10-(5-((*S*)-2,3-

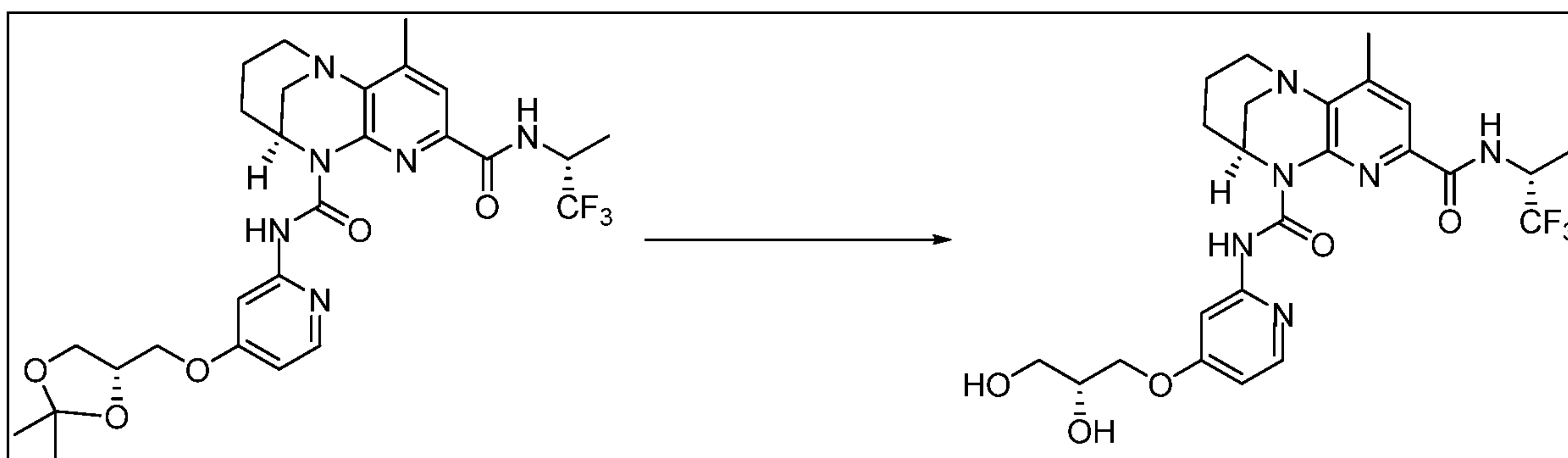
dihydroxypropoxy)pyridin-2-yl)-4-methyl-N2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (214 mg, 0.395 mmol, 76 % yield) as an off white solid. LCMS (*m/z*): 539.29 [M+H]⁺, *R*_t = 2.14 min.

- 5 ¹H NMR (400 MHz, CDCl₃): δ ppm 13.83 (s, 1 H), 8.14 (d, *J*=8.99 Hz, 1 H), 7.93 - 8.01 (m, 2 H), 7.87 (s, 1 H), 7.32 (dd, *J*=9.10, 2.96 Hz, 1 H), 5.04 - 5.17 (m, 1 H), 4.95 (br s, 1 H), 4.04 - 4.19 (m, 3 H), 3.72 - 3.92 (m, 2 H), 3.37 (dd, *J*=13.59, 1.75 Hz, 1 H), 3.14 - 3.25 (m, 2 H), 2.92 (br d, *J*=13.37 Hz, 1 H), 2.58 (d, *J*=4.60 Hz, 1 H), 2.41 (s, 3 H), 2.26 (br d, *J*=14.47 Hz, 1 H), 1.86 - 2.02 (m, 2 H), 1.53 - 1.64 (m, 3 H), 1.33 - 1.47 (m, 2 H).

10

Example 57

Synthesis of (9*S*)-N10-(4-((*R*)-2,3-dihydroxypropoxy)pyridin-2-yl)-4-methyl-N2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide:



15

To a stirred solution of (9*S*)-N10-(4-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-4-methyl-N2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (300mg, 0.519 mmol) in methanol (15 mL) at 0 °C was added aq.HCl (1.5 mL, 18.00 mmol) and stirred under nitrogen at RT for 1 h. (TLC eluent:5% MeOH in DCM *R*_f0.3; UV active). The reaction mixture at 0 °C was basified with saturated sodiumbicarbonate solution (till pH-8-9) and the solvent was evaporated under reduced pressure. The residue was diluted with water and the precipitated solid was filtered, dried under reduced pressure to afford crude. The crude was purified by chromatography (GRACE using C-18 reserval column, Mobile phase A: 0.1% Formic Acid in water; B: ACN, eluent 50-55% B in A) and combined fractions were concentrated then basified with saturated NaHCO₃ solution. The

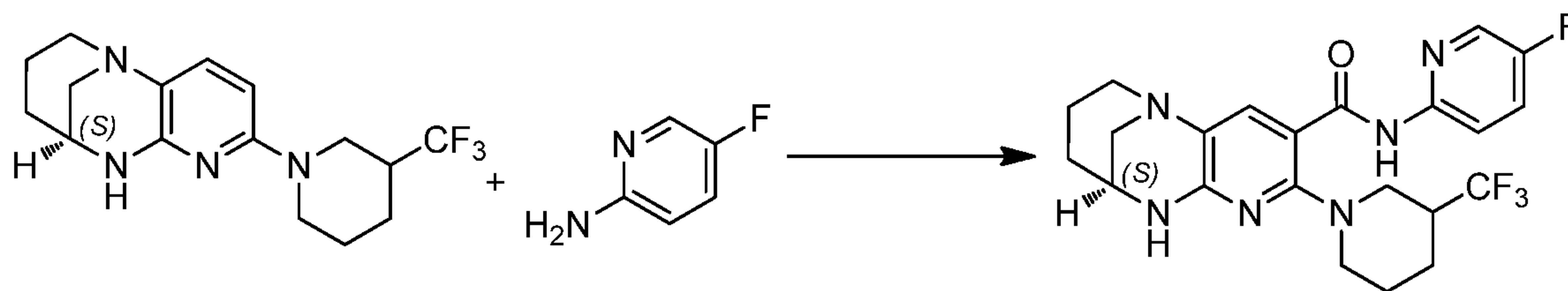
25

precipitated solid was filtered and dried to afford (9*S*)-N10-(4-((*R*)-2,3-dihydroxypropoxy)pyridin-2-yl)-4-methyl-N2-((*R*)-1,1,1-trifluoropropan-2-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-2,10(7*H*)-dicarboxamide (230 mg, 0.424 mmol, 82 % yield) as off white solid. LCMS (*m/z*): 539.32 [*M*+*H*]⁺, *R*_t = 1.78 min.

5 ¹H NMR (400 MHz, CDCl₃): δ ppm 14.06 (s, 1 H), 8.15 (br d, *J*=9.21 Hz, 1 H), 8.08 (d, *J*=5.92 Hz, 1 H), 7.89 (s, 1 H), 7.81 (d, *J*=2.41 Hz, 1 H), 6.59 (dd, *J*=5.70, 2.41 Hz, 1 H), 5.00 - 5.15 (m, 1 H), 4.93 (br s, 1 H), 4.10 - 4.22 (m, 3 H), 3.69 - 3.90 (m, 2 H), 3.37 (dd, *J*=13.48, 1.64 Hz, 1 H), 3.13 - 3.23 (m, 2 H), 2.93 (br d, *J*=13.37 Hz, 1 H), 2.57 (d, *J*=3.73 Hz, 1 H), 2.41 (s, 3 H), 2.26 (br d, *J*=14.25 Hz, 1 H), 1.86 - 2.07 (m, 2 H), 1.50 - 1.63 (m, 10 3 H), 1.32 - 1.45 (m, 2 H).

Example 58

Synthesis of (9*S*)-N-(5-fluoropyridin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-3-carboxamide



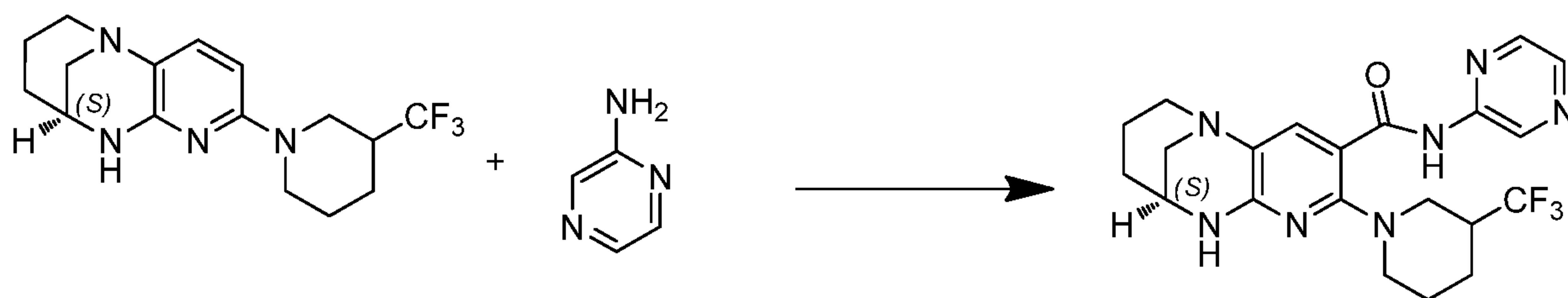
To a solution of (9*S*)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (Peak-II from the intermediate SFC Separation, 0.700 g, 2.145 mmol) in tetrahydrofuran (THF) (40 mL) stirred under nitrogen at room temperature was added triethylamine (1.794 mL, 12.87 mmol) and triphosgene (0.636 g, 2.145 mmol). And then the reaction mixture was stirred at room temperature for 30 minutes. 5-fluoropyridin-2-amine (0.721 g, 6.43 mmol) was added at room temperature and then the reaction mixture was stirred at 65 °C for 16 hr. The reaction mixture was cooled to 28 °C and concentrated to dryness. The residue was partitioned between water (10 mL) and Dichloromethane (50 mL). Organic layer was separated and was dried over 25 anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid (TLC eluent: 100% EtOAc: *R*_f 0.3; UV active). The crude was purified by column chromatography using neutral alumina and was eluted with 35-40% EtOAc in Hexane to afford pure (9*S*)-N-(5-fluoropyridin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-3-carboxamide (0.220 g, 0.468

mmol, 21.82 % yield) as a off- white solid, LCMS (m/z): 4653 $[M+H]^+$.

¹H NMR (400 MHz, CDCl₃): δ 12.14 (s, 1H), 8.41 (dd, $J = 9.2, 4.2$ Hz, 1H), 8.15 (d, $J = 3.0$ Hz, 1H), 8.02 (s, 1H), 7.42 (ddd, $J = 9.2, 7.7, 3.0$ Hz, 1H), 5.51 (d, $J = 4.6$ Hz, 1H), 3.76 (s, 1H), 3.58 - 3.46 (m, 1H), 3.41 - 3.17 (m, 4H), 3.03 (s, 1H), 2.88 - 2.72 (m, 3H), 2.14 (d, $J = 13.7$ Hz, 1H), 2.08 (s, 1H), 2.00 - 1.77 (m, 3H), 1.53 - 1.42 (m, 2H), 1.37 - 1.21 (m, 2H).

Example 59

Synthesis of (9S)-N-(pyrazin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-*b*][1,4]diazocine-3-carboxamide



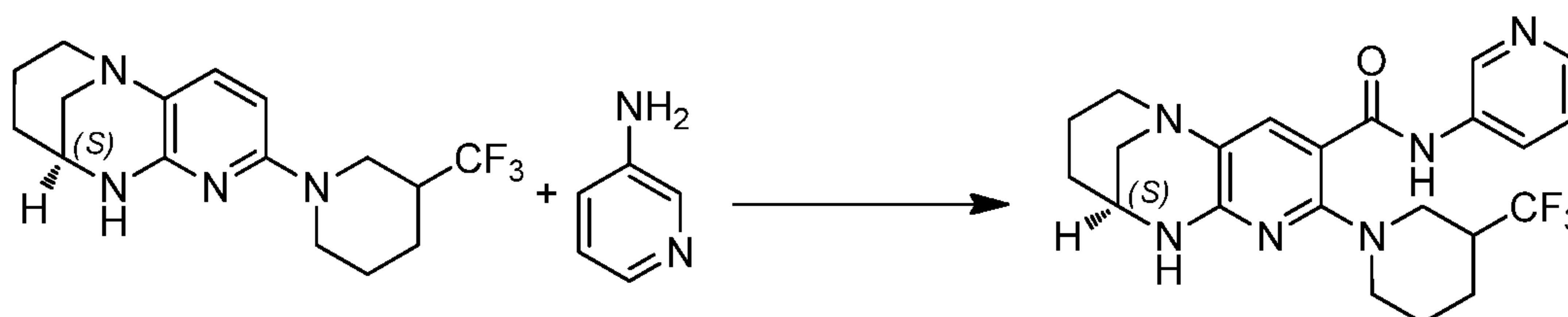
To a suspension of (9S)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-*b*][1,4]diazocine (Peak-I from the intermediate SFC Separation, 500 mg, 1.532 mmol) in tetrahydrofuran (50mL) stirred at room temp for 10 min, triethylamine (0.641 mL, 4.60 mmol) and triphosgene (227 mg, 0.766 mmol) added. The reaction mixture was stirred for 30 min pyrazin-2-amine (437 mg, 4.60 mmol) was added. The reaction mixture was stirred for 16 hr at 65°C. The reaction mass concentrated and the residue was diluted with water (25 mL) and extracted with EtOAc (2x75 mL). The combined organic layer was washed with water and saturated brine solution and dried over anhydrous Na₂SO₄, filtered and concentrated to give the crude as white solid (TLC eluent: 100%Ethyl Acetate: R_f 0.4; UV active). The crude product was purified by column chromatography (neutral alumina) product was eluted with 60% ethyl acetate in hexane to afford (9S)-N-(pyrazin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-*b*][1,4]diazocine-3-carboxamide (150 mg, 0.319 mmol, 20.82 % yield) as a off white solid, LCMS (m/z): 448.35 $[M+H]^+$.

¹H NMR (400 MHz, CDCl₃): δ 12.31 (s, 1H), 9.70 (d, $J = 1.5$ Hz, 1H), 8.30 (dd, $J = 2.5, 0.4$ Hz, 1H), 8.26 (dd, $J = 2.6, 1.5$ Hz, 1H), 8.03 (d, $J = 0.7$ Hz, 1H), 5.55 (d, $J = 4.6$ Hz, 1H), 3.76 (s, 1H), 3.56 - 3.39 (m, 1H), 3.29 - 3.20 (m, 4H), 3.02 (t, $J = 11.7$ Hz, 1H), 2.93 -

2.78 (m, 3H), 2.15 (d, $J = 13.1$ Hz, 1H), 2.06 - 1.82 (m, 4H), 1.54 - 1.42 (m, 2H), 1.36 (s, 1H).

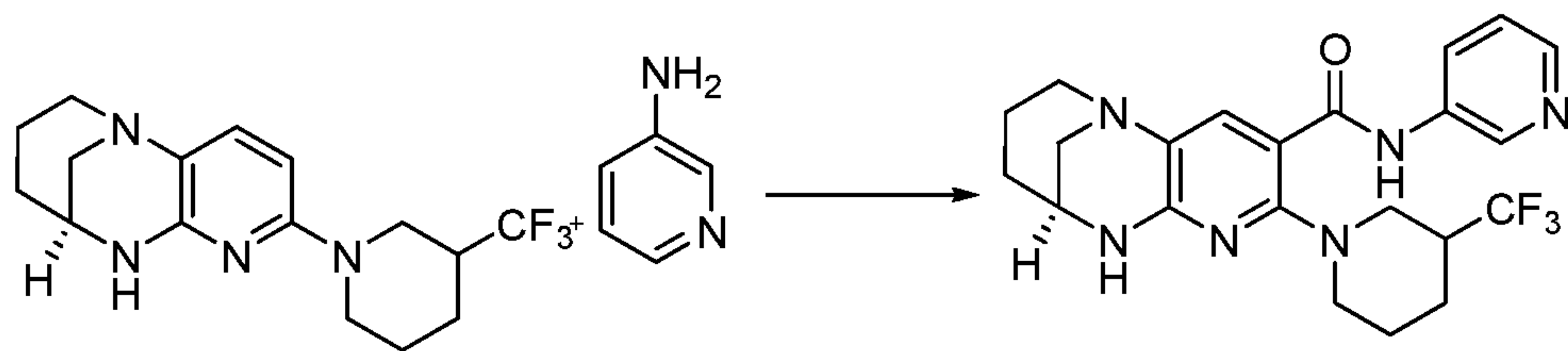
Example 60

5 Synthesis of (9S)-N-(pyridin-3-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-3-carboxamide



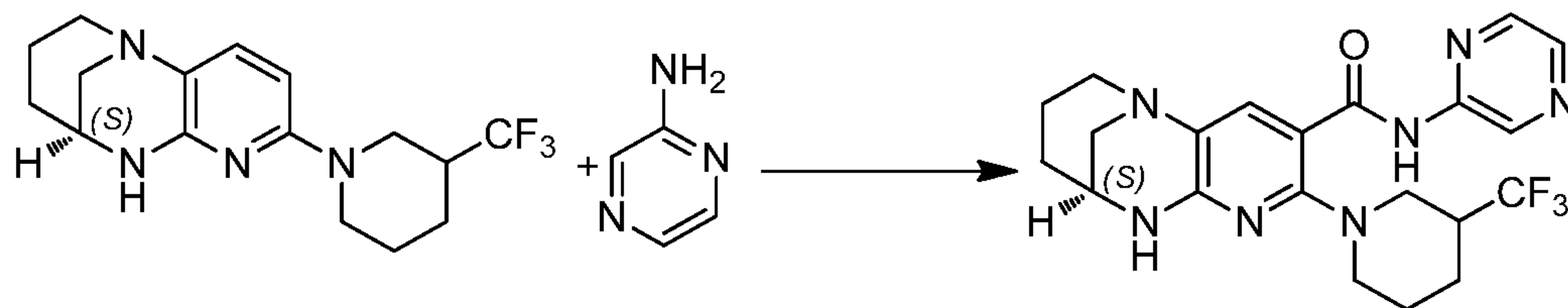
To a solution of (9S)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (Peak-II from intermediate SFC separation, 0.550 g, 1.685 mmol) in Tetrahydrofuran (THF) (30 mL) stirred under nitrogen at room temperature was added Triethylamine (1.409 mL, 10.11 mmol) and Triphosgene (0.500 g, 1.685 mmol). Then reaction mixture was stirred at room temperature for 30 minute and then pyridin-3-amine (0.476 g, 5.06 mmol) was added at room temperature. Then the reaction mixture was stirred at 65 °C for 16 hr before being cooled to 28 °C and concentrated under reduced pressure. The residue was partitioned between water (10 mL) and Dichloromethane (50 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid (TLC eluent: 100% EtOAc: R_f 0.3; UV active). The crude was purified by column chromatography using neutral alumina and was eluted with 50-60% EtOAc in Hexane to afford pure (9S)-N-(pyridin-3-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-3-carboxamide (0.264 g, 0.587 mmol, 34.8 % yield) as a off - white solid, LCMS (m/z): 447.3 [M+H]⁺.

¹H NMR (400 MHz, CDCl₃): δ 11.74 (s, 1H), 8.59 (d, $J = 2.6$ Hz, 1H), 8.44 (ddd, $J = 8.4, 2.7, 1.5$ Hz, 1H), 8.32 (dd, $J = 4.7, 1.6$ Hz, 1H), 8.02 (d, $J = 0.8$ Hz, 1H), 7.30 (dd, $J = 8.4, 4.7$ Hz, 1H), 5.53 (d, $J = 5.0$ Hz, 1H), 3.76 (s, 1H), 3.58 - 3.41 (m, 1H), 3.24 (ddt, $J = 10.1, 7.3, 2.2$ Hz, 4H), 3.12 (t, $J = 11.7$ Hz, 1H), 2.90 - 2.70 (m, 2H), 2.55 (dtt, $J = 15.5, 7.5, 3.9$ Hz, 1H), 2.12 (d, $J = 13.0$ Hz, 1H), 2.01 - 1.84 (m, 3H), 1.81 - 1.66 (m, 1H), 1.53 (td, $J = 12.7, 4.3$ Hz, 2H), 1.35 (s, 1H), 1.26 (s, 1H).

Example 61**Synthesis of (9*S*)-N-(pyridin-3-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-3-carboxamide**

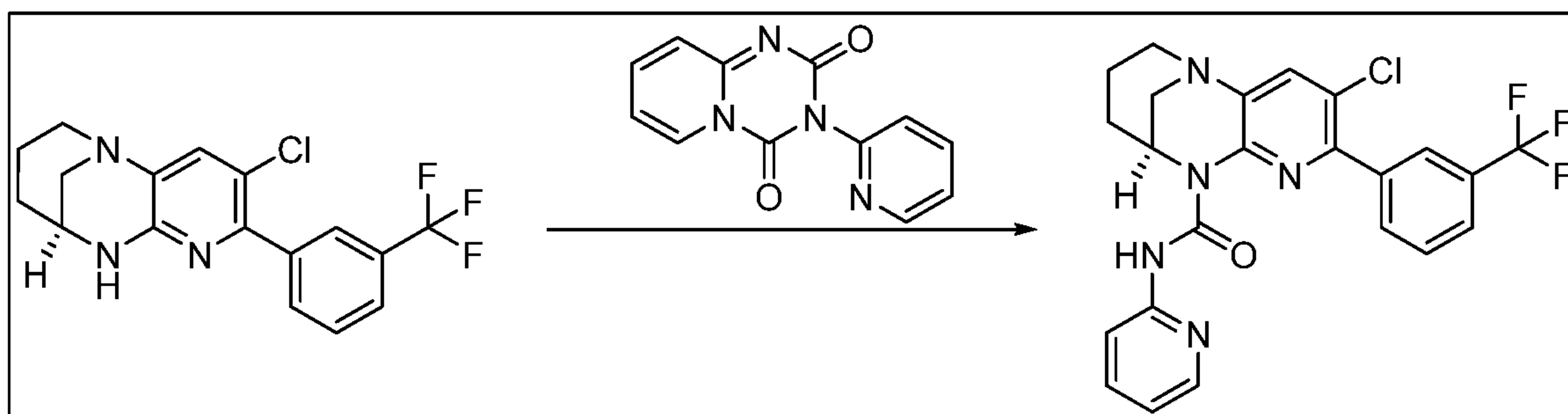
5 To a suspension of (9*S*)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (Peak-I of the intermediate SFC separation, 600 mg, 1.840 mmol) in tetrahydrofuran (50mL) stirred at room temp for 10 min, triethylamine (0.796 mL, 5.521 mmol) and triphosgene (279 mg, 0.9202 mmol) added. The reaction mixture was stirred for 30 min pyridin-2-amine (519 mg, 5.521 mmol) was added. The
 10 reaction mixture was stirred for 16 hr at 65°C. The reaction mass concentrated and the residue was diluted with water (25 mL) and extracted with EtOAc (2x75 mL). The combined organic layer was washed with water and saturated brine solution and dried over anhydrous Na₂SO₄, filtered and concentrated to give the crude as white solid (TLC eluent: 100%Ethyl Acetate: R_f0.4; UV active). The crude product was purified by column
 15 chromatography (neutral alumina) product was eluted with 60% ethyl acetate in hexane to afford (9*S*)-N-(pyradin-3-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-3-carboxamide (354 mg, 0.791mmol, 43 % yield) as a off white solid, LCMS (*m/z*): 447.28 [M+H]⁺.

20 ¹H NMR (400 MHz, CDCl₃): δ 11.66 (s, 1 H), 8.60 (s, 1 H), 8.47 - 8.40 (m, 1 H), 8.33 (s, 1 H), 8.01 (s, *J*=0.66 Hz, 1 H), 7.34 - 7.28 (m, 1 H), 5.53 (br d, *J*=4.17 Hz, 1 H), 3.76 (s, 1 H), 3.47 (dt, *J*=12.17, 1.70 Hz, 1 H), 3.30 - 3.19 (m, 4 H), 3.09 (t, *J*=11.62 Hz, 1 H), 2.95 - 2.76 (m, 2 H), 2.65 - 2.48 (m, 1 H), 2.18 - 2.07 (m, 1 H), 1.98 - 1.85 (m, 3 H), 1.78 - 1.68 (m, 2 H), 1.62 - 1.44 (m, 2 H), 1.39 - 1.30 (m, 1 H).

Example 62**Synthesis of (9S)-N-(pyrazin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-3-carboxamide**

- 5 To a solution of (9S)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine (Peak-II from intermediate SFC separation, 0.700 g, 2.145 mmol) in Tetrahydrofuran (THF) (30 mL) stirred under nitrogen at room temperature was added Triethylamine (1.794 mL, 12.87 mmol) and Triphosgene (0.636 g, 2.145 mmol). Then the reaction mixture was stirred at room temperature for 30 minutes
- 10 before pyrazin-2-amine (0.612 g, 6.43 mmol) was added. Then the reaction mixture was stirred at 65 °C for 16 hr. The reaction mixture was cooled to 28 °C and concentrated under reduced pressure. The resulting residue was partitioned between water (10 mL) and Dichloromethane (50 mL). Organic layer was separated and was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude as brown solid (TLC eluent:
- 15 100% EtOAc: R_f 0.3; UV active). The crude was purified by column chromatography using neutral alumina and was eluted with 50-60% EtOAc in Hexane to afford pure (9S)-N-(pyrazin-2-yl)-2-(3-(trifluoromethyl)piperidin-1-yl)-7,8,9,10-tetrahydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-3-carboxamide (105 mg, 0.227 mmol, 10.58 % yield) as a pale yellow solid, LCMS (*m/z*): 448.3 [M+H]⁺.
- 20 ¹H-NMR (400 MHz, CDCl₃): δ ppm 12.36 (s, 1 H), 9.69 (d, *J*=1.53 Hz, 1 H), 8.28 (d, *J*=2.41 Hz, 1 H), 8.23 - 8.25 (m, 1 H), 8.03 (s, 1 H), 5.54 (br d, *J*=4.17 Hz, 1 H), 3.75 (br s, 1 H), 3.48 (br d, *J*=10.30 Hz, 1 H), 3.18 - 3.27 (m, 4 H), 3.05 (t, *J*=11.84 Hz, 1 H), 2.74 - 2.85 (m, 3 H), 2.14 (br d, *J*=12.93 Hz, 1 H), 2.02 (dt, *J*=13.21, 4.03 Hz, 1 H), 1.81 - 1.92 (m, 3 H), 1.39 - 1.52 (m, 2 H), 1.27 - 1.36 (m, 1 H).

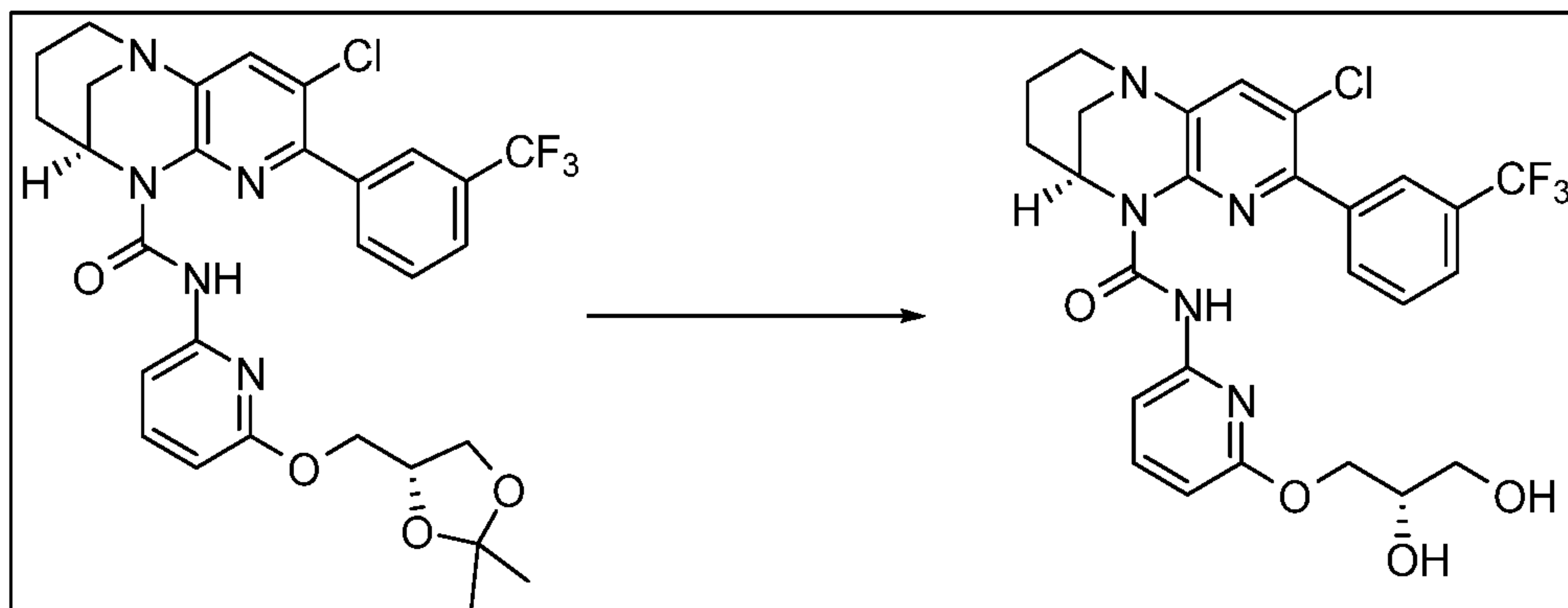
25

Example 63**Synthesis of (9*S*)-3-chloro-N-(pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

- 5 To a stirred solution of (9*S*)-3-chloro-2-(3-(trifluoromethyl)phenyl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (500 mg, 1.413 mmol) in THF (10 mL) was added NaH (33.9 mg, 1.413 mmol) at 0 °C and stirred under nitrogen atmosphere for 30 min. Then 3-(pyridin-2-yl)-2*H*-pyrido[1,2-*a*][1,3,5]triazine-2,4(3*H*)-dione (340 mg, 1.413 mmol) was added to this reaction mixture and stirred at 80 °C for 16 h. (TLC eluent: Neat ethyl acetate; R_f 0.25). Reaction mixture was quenched with ice water and extracted with Ethyl acetate (2x15 mL). The combined organic layer was washed with brine solution (20 mL) and dried over anhydrous sodium sulphate and concentrated under reduced pressure to obtain crude compound. The crude product was purified by flash column chromatography (silica-gel: 100-200 mesh, eluent: 70% Ethyl acetate in petether) to afford the desired product (9*S*)-3-chloro-N-(pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (260 mg, 0.548 mmol, 38.7 % yield) as an off white solid. LCMS (m/z): 474.05 $[M+H]^+$, R_t = 3.0 min. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ ppm 13.40 (s, 1 H), 8.30 - 8.23 (m, 2 H), 8.20 - 8.26 (m, 1 H), 8.20 - 8.13 (m, 1 H), 7.78 - 7.69 (m, 1 H), 7.59 - 7.57 (m, 2 H), 7.52 - 7.48 (m, 1 H), 6.97 - 6.91 (m, 1 H), 5.02 - 4.97 (m, 1 H), 3.41 - 3.37 (m, 3 H), 2.96 - 2.95 (m, 1 H), 2.92 (d, J =14.03 Hz, 1 H), 2.41 - 2.06 (m, 1 H), 1.63 - 1.21 (m, 2 H).
- 10
- 15
- 20

Example 64

Synthesis of (9*S*)-3-chloro-N-(6-((*S*)-2,3-dihydroxypropoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide:

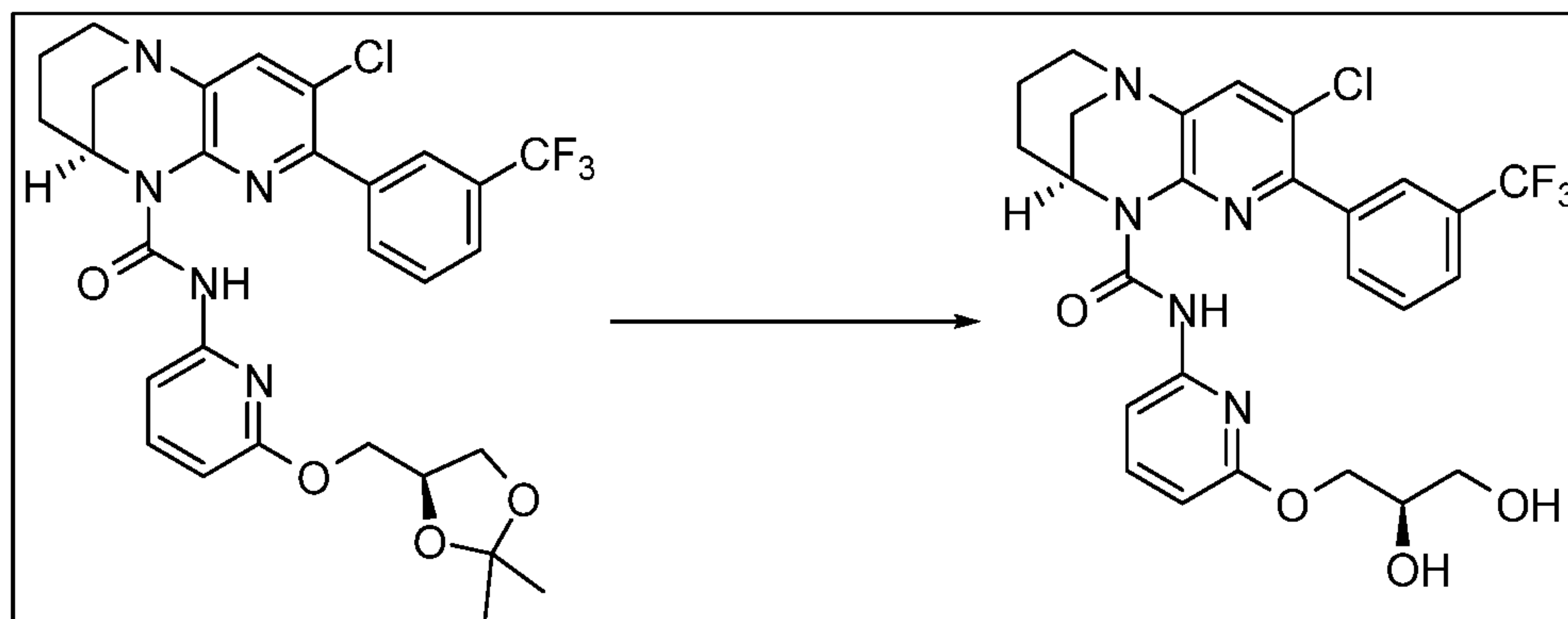


To a stirred solution of (9*S*)-3-chloro-N-(6-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (250 mg, 0.414 mmol) in methanol (10 mL) was added aq.HCl (0.345 mL, 4.14 mmol, 36 %) 0 °C and stirred at RT for 4 h. After completion of the reaction by TLC, the volatiles were evaporated under reduced pressure to get the crude (TLC eluent system: 100% EtOAc, *R_f*-0.5, UV active). The crude was diluted with water (5 ml) and basified with the 10% sodium bicarbonate solution (up to pH 8), the precipitated solid was filtered, washed with the water and dried under vacuum to afford (9*S*)-3-chloro-N-(6-((*S*)-2,3-dihydroxypropoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (162 mg, 0.285 mmol, 68.8 % yield) as an off white solid. LCMS (*m/z*): 564.13 [*M*+*H*]⁺, *R_t* = 2.50 min.

¹**H NMR** (400 MHz, CDCl₃): δ ppm 13.10 (br s, 1 H), 7.89 - 8.16 (m, 2 H), 7.72 - 7.80 (m, 1 H), 7.63 - 7.70 (m, 1 H), 7.47 - 7.61 (m, 2 H), 6.42 (d, *J*=7.89 Hz, 1 H), 5.00 (br s, 1 H), 3.92 (qd, *J*=11.91, 4.60 Hz, 2 H), 3.67 - 3.81 (m, 1 H), 3.48 (br s, 2 H), 3.27 - 3.42 (m, 2 H), 2.98 (br d, *J*=12.93 Hz, 2 H), 2.80 (br d, *J*=5.48 Hz, 1 H), 2.25 (br d, *J*=15.13 Hz, 2 H), 1.94 (br d, *J*=12.50 Hz, 2 H), 1.22 - 1.49 (m, 2 H).

Example 65

Synthesis of (9*S*)-3-chloro-N-(6-((*R*)-2,3-dihydroxypropoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide:



5

To a stirred solution of (9*S*)-3-chloro-N-(6-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (270 mg, 0.447 mmol), in methanol (10 mL) was added aq.HCl (1 mL, 32.9 mmol, 36 %) 0 °C and stirred at RT for 4 h. After completion of the reaction, the volatiles were evaporated under reduced pressure to get the crude (TLC eluent system: 100% EtOAc, R_f -0.5, UV active). The crude was diluted with the water (5 ml) and basified with the 10% sodium bicarbonate solution, the precipitated solid was filtered and was washed with the water and dried under vacuum to afford (9*S*)-3-chloro-N-(6-((*R*)-2,3-dihydroxypropoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (142 mg, 0.248 mmol, 55.5 % yield) as an off white solid. LCMS (m/z): 564.13 $[M+H]^+$, R_t = 2.51 min.

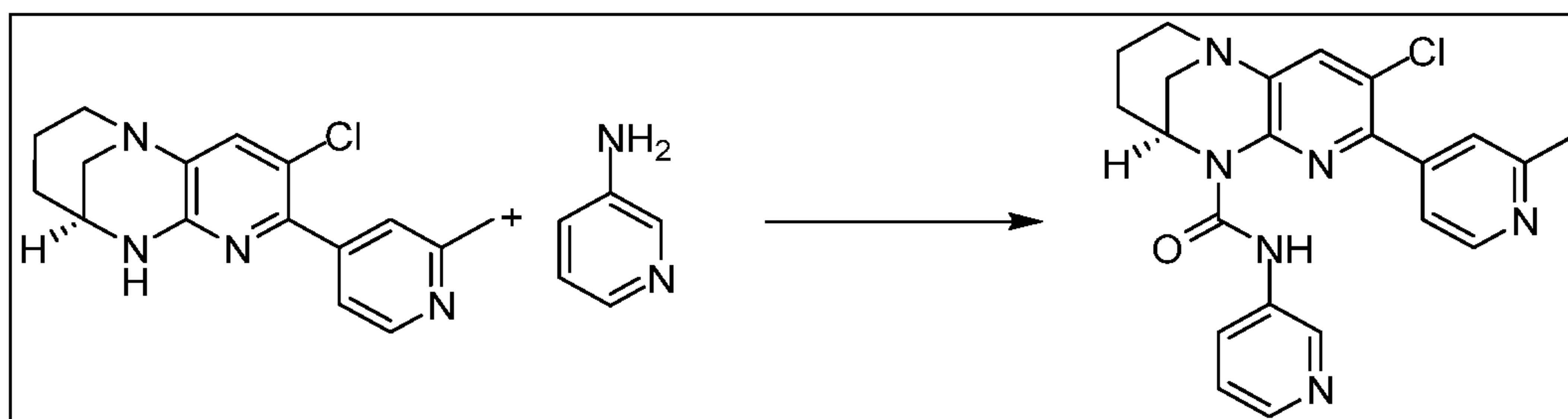
10

15

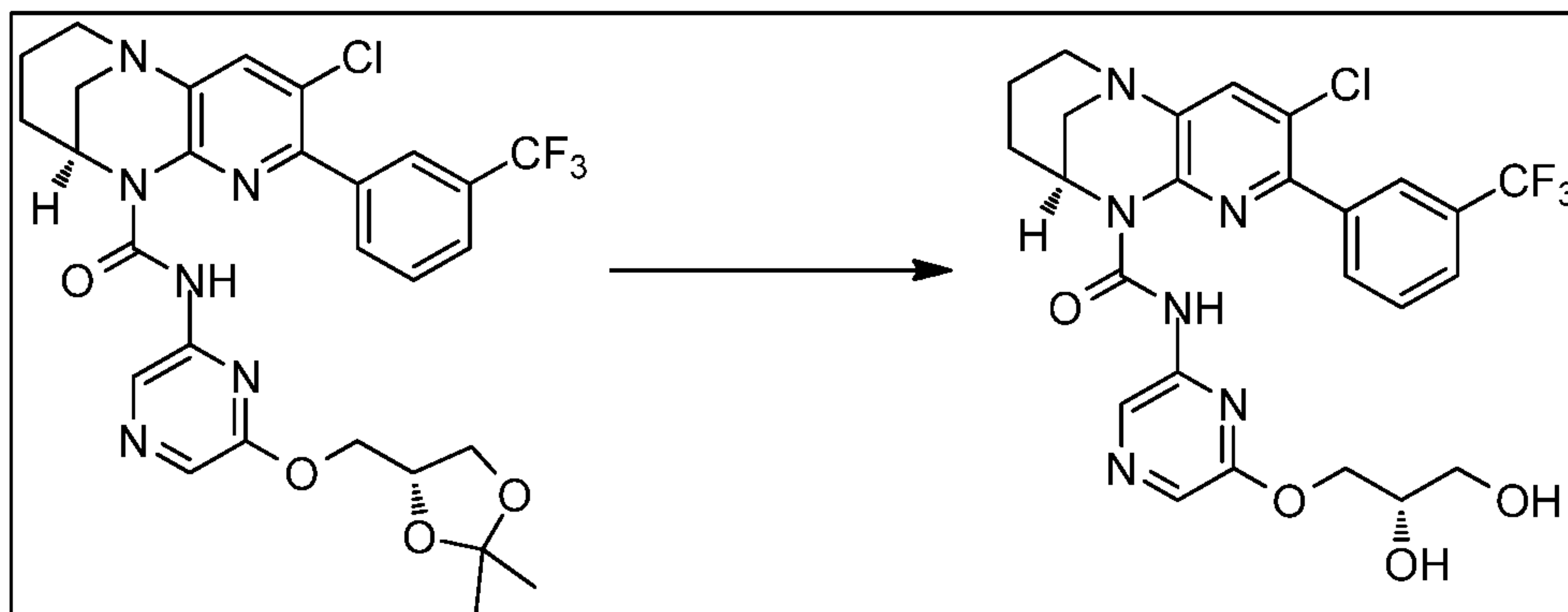
^1H NMR (400 MHz, CDCl_3): δ ppm 13.11 (s, 1 H), 7.89 - 8.14 (m, 2 H), 7.61 - 7.82 (m, 2 H), 7.44 - 7.61 (m, 3 H), 6.42 (d, J =7.89 Hz, 1 H), 5.00 (br s, 1 H), 3.83 - 4.04 (m, 2 H), 3.63 - 3.83 (m, 1 H), 3.42 - 3.57 (m, 2 H), 3.28 - 3.41 (m, 3 H), 2.98 (br d, J =14.03 Hz, 1 H), 2.60 - 2.87 (m, 1 H), 2.17 - 2.39 (m, 2 H), 1.86 - 1.98 (m, 1 H), 1.24 - 1.52 (m, 2 H).

20

25

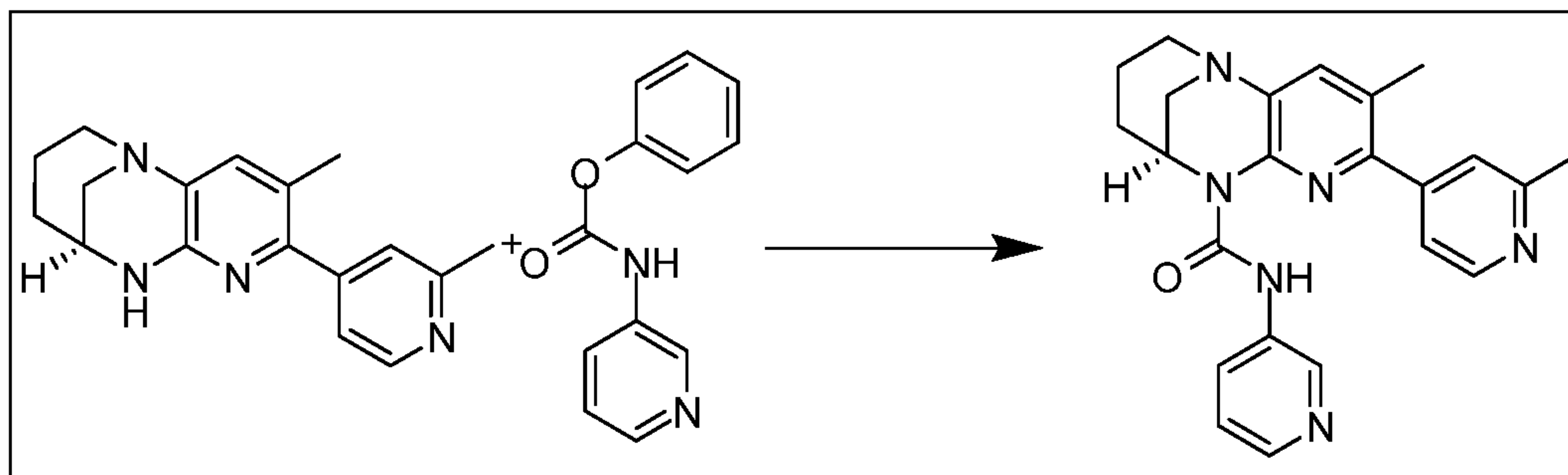
Example 66**Synthesis of (9*S*)-3-chloro-2-(2-methylpyridin-4-yl)-N-(pyridin-3-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

- 5 To a stirred solution of (9*S*)-3-chloro-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (300 mg, 0.997 mmol) in THF (30 mL) under nitrogen at RT was added triethylamine (0.834 mL, 5.98 mmol), triphosgene (296 mg, 0.997 mmol) and stirred for 4 h. then pyridin-3-amine (282 mg, 2.99 mmol) was added and the reaction was heated at 65 °C for 16 h. (TLC eluent: 100% EtOAc: R_f 0.3; UV active).
- 10 The reaction mixture was cooled to RT, concentrated and residue was partitioned between water (5 mL) and EtOAc (20 mL). Organic layer was separated, dried over anhydrous Na_2SO_4 , filtered and filtrate was evaporated to give crude compound. The crude was purified by column chromatography (neutral alumina, eluent: 60% ethyl acetate in hexane) to afford the desired product (9*S*)-3-chloro-2-(2-methylpyridin-4-yl)-N-(pyridin-3-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (135
- 15 mg, 0.319 mmol, 31.9 % yield) as an off- white solid. LCMS (m/z): 550.13 $[\text{M}+\text{H}]^+$, R_t = 1.95 min
- ^1H NMR (400 MHz, CDCl_3): δ ppm 13.11 (s, 1 H), 8.71 (d, $J=5.04$ Hz, 1 H), 8.49 (d, $J=2.19$ Hz, 1 H), 8.29 (dd, $J=4.60, 1.32$ Hz, 1 H), 8.05 - 7.97 (m, 1 H), 7.59 (s, 1 H), 7.54 (s, 1 H), 7.51 (d, $J=5.04$ Hz, 1 H), 7.22 (dd, $J=8.33, 4.82$ Hz, 1 H), 5.00 (br s, 1 H), 3.42 - 3.32 (m, 3 H), 2.98 (br d, $J=14.03$ Hz, 1 H), 2.70 (s, 3 H), 2.25 (br d, $J=14.25$ Hz, 1 H), 2.00 - 1.87 (m, 1 H), 1.51 - 1.40 (m, 2 H)
- 20

Example 67**Synthesis of (9*S*)-3-chloro-N-(6-((*R*)-2,3-dihydroxypropoxy)pyrazin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

To a stirred solution of (9*S*)-3-chloro-N-(6-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (200 mg, 0.331 mmol) in methanol (10 mL) at 0 °C was added aq. HCl (0.201 mL, 6.61 mmol, 36 %) and stirred for 2 h. (TLC eluent: 100% EtOAc: R_f 0.2; UV active). The reaction mixture was basified with saturated sodium bicarbonate solution (till pH-8-9) and solvent was evaporated under reduced pressure. The residue was diluted with water (10 mL) and extracted into dichloromethane (2x15 mL). Combined organic extracts were dried over anhydrous sodium sulphate, filtered and filtrate was evaporated *in vacuo* and the crude was triturated with pentane (10 mL) to afford the desired product (9*S*)-3-chloro-N-(6-((*R*)-2,3-dihydroxypropoxy)pyrazin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (123 mg, 0.214 mmol, 64.6 % yield) as an off-white solid. LCMS (m/z): 565.14 $[M+H]^+$, R_t = 2.33 min

^1H NMR (400 MHz, CDCl_3): δ ppm 13.27 (s, 1 H), 8.88 (s, 1 H), 8.03 (s, 1 H), 7.96 (br d, $J=7.67$ Hz, 1 H), 7.92 (s, 1 H), 7.78 - 7.74 (m, 1 H), 7.72 - 7.66 (m, 1 H), 7.61 (s, 1 H), 5.01 (br s, 1 H), 3.99 - 3.92 (m, 1 H), 3.91 - 3.83 (m, 2 H), 3.66 - 3.59 (m, 2 H), 3.42 - 3.31 (m, 3 H), 2.99 (br d, $J=13.59$ Hz, 1 H), 2.56 (d, $J=4.82$ Hz, 1 H), 2.26 (br d, $J=15.35$ Hz, 1 H), 2.07 (br t, $J=6.03$ Hz, 1 H), 2.00 - 1.86 (m, 1 H), 1.52 - 1.39 (m, 2 H)

Example 68**Synthesis of (9*S*)-3-methyl-2-(2-methylpyridin-4-yl)-N-(pyridin-3-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

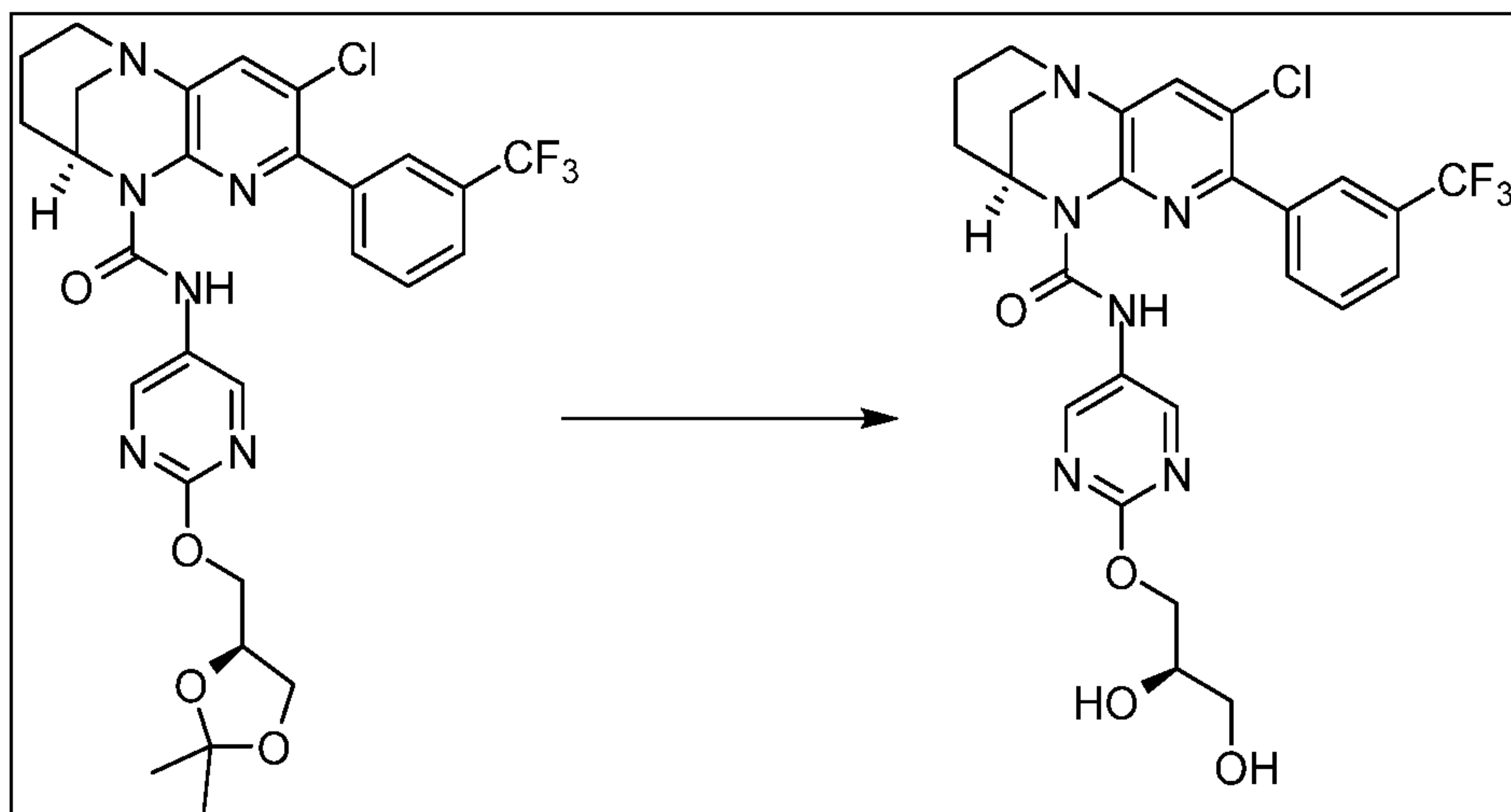
5 To a stirred solution of (9*S*)-3-methyl-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (250 mg, 0.892 mmol) in THF (25 mL) under nitrogen atmosphere at RT was added phenyl pyridin-3-ylcarbamate (573 mg, 2.68 mmol), DMAP (327 mg, 2.68 mmol) and stirred at 65 °C for 48 h. (TLC eluent: 5% MeOH in DCM: R_f 0.4; UV active). The reaction mixture was cooled to RT, concentrated and the

10 residue was partitioned between water (10 mL) and EtOAc (30 mL). Organic layer was separated, dried over anhydrous Na_2SO_4 , filtered and filtrate was evaporated to give crude compound. The crude was purified by column chromatography (neutral alumina, eluent: 70% EtOAc in Hexane) to afford (9*S*)-3-methyl-2-(2-methylpyridin-4-yl)-N-(pyridin-3-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (178

15 mg, 0.437 mmol, 49.0 % yield) as a pale yellow solid. LCMS (m/z): 401.1 $[\text{M}+\text{H}]^+$, R_t = 3.342 min.

¹H NMR (400 MHz, CDCl_3): δ ppm 13.57 (s, 1 H), 8.68 (d, J =5.26 Hz, 1 H), 8.48 (d, J =2.41 Hz, 1 H), 8.26 (dd, J =4.71, 1.43 Hz, 1 H), 8.08 - 7.99 (m, 1 H), 7.42 - 7.36 (m, 2 H), 7.30 (dd, J =5.15, 1.21 Hz, 1 H), 7.20 (dd, J =8.33, 4.60 Hz, 1 H), 4.97 (br s, 1 H), 3.42 - 3.28 (m, 3 H), 3.00 (br d, J =13.59 Hz, 1 H), 2.69 (s, 3 H), 2.36 (s, 3 H), 2.30 - 2.20 (m, 1 H), 2.01 - 1.87 (m, 1 H), 1.54 - 1.35 (m, 2 H)

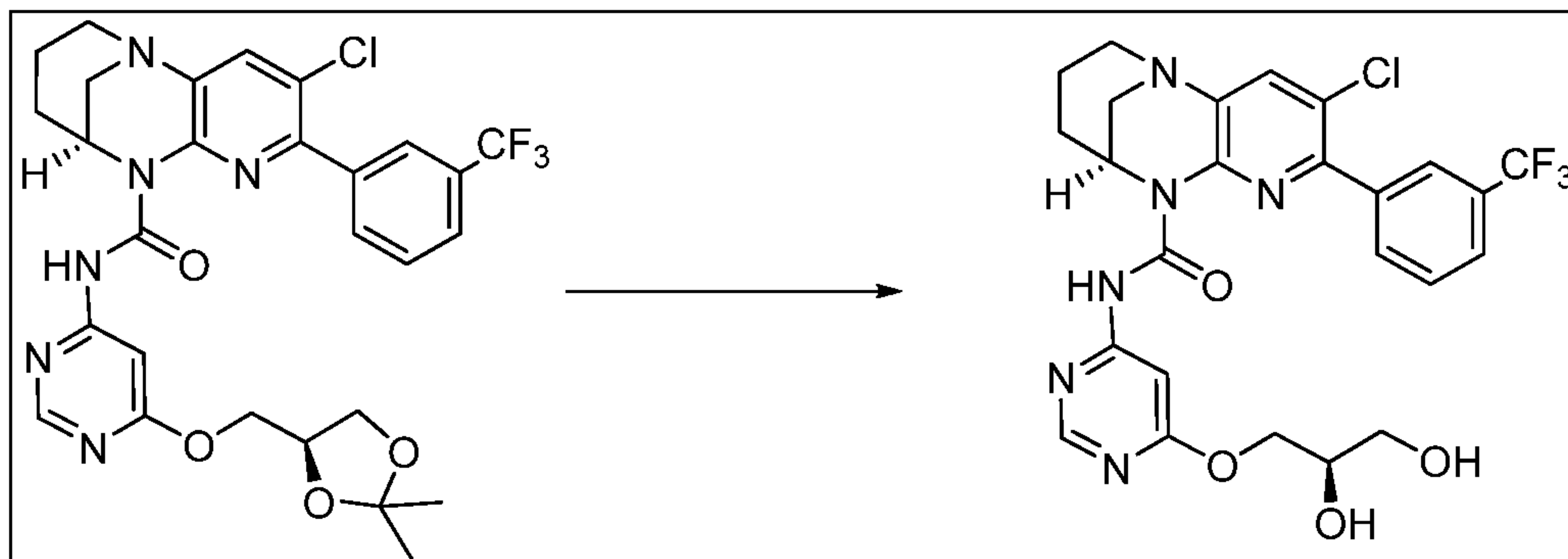
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Example 69**Synthesis of (4*S*)-8-chloro-N-(2-(((*R*)-2,3-dihydroxypropoxy)pyrimidin-5-yl)-7-(3-(trifluoromethyl)phenyl)-3,4-dihydro-1,4-methanopyrido[2,3-*b*][1,4]diazepine-5(2*H*)-carboxamide**

5

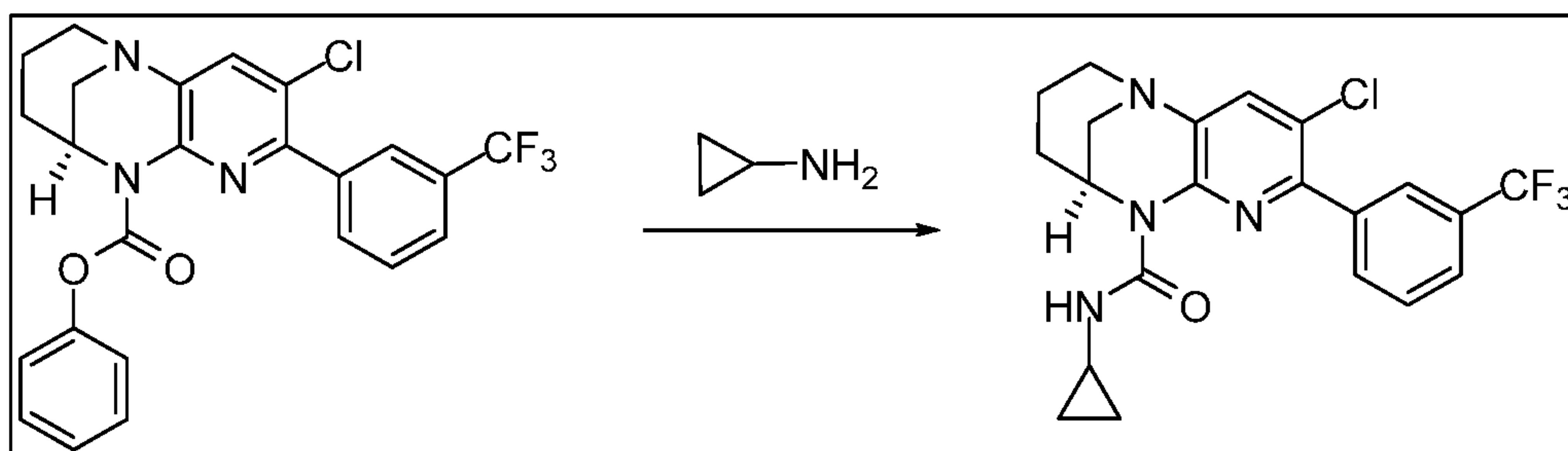
To a stirred solution of (9*S*)-3-chloro-N-(2-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-5-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (150 mg, 0.248 mmol) in methanol (10 mL) at RT was added aq. HCl (1.240 mL, 2.479 mmol, 36 %) and stirred for 2 h. (TLC eluent: 100% EtOAc: R_f 0.2; UV active). The reaction mixture was basified with saturated sodium bicarbonate solution (till pH 8-9) and solvent was evaporated under reduced pressure. The residue was diluted with water (5 mL) and extracted into dichloromethane (2x10 mL). Combined organic extracts were dried over anhydrous sodium sulphate, filtered and filtrate was evaporated *in vacuo* and the crude was triturated with diethylether (10 mL) and pentane (10 mL) to afford the desired product (9*S*)-3-chloro-N-(2-(((*R*)-2,3-dihydroxypropoxy)pyrimidin-5-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (89 mg, 0.153 mmol, 61.6 % yield) as an off-White solid. LCMS (m/z): 565.17 [$M+H$]⁺, R_t = 2.30 min

¹H NMR (400 MHz, CDCl₃): δ ppm 13.10 (s, 1 H), 8.58 (s, 2 H), 8.02 (s, 1 H), 7.94 (d, J =7.89 Hz, 1 H), 7.77 (d, J =7.89 Hz, 1 H), 7.71 - 7.65 (m, 1 H), 7.61 (s, 1 H), 4.98 (br s, 1 H), 4.51 - 4.41 (m, 2 H), 4.15 - 4.06 (m, 1 H), 3.82 - 3.67 (m, 2 H), 3.44 - 3.31 (m, 3 H), 3.14 (d, J =5.26 Hz, 1 H), 2.98 (br d, J =13.59 Hz, 1 H), 2.34 - 2.18 (m, 2 H), 2.00 - 1.88 (m, 1 H), 1.54 - 1.42 (m, 2 H)

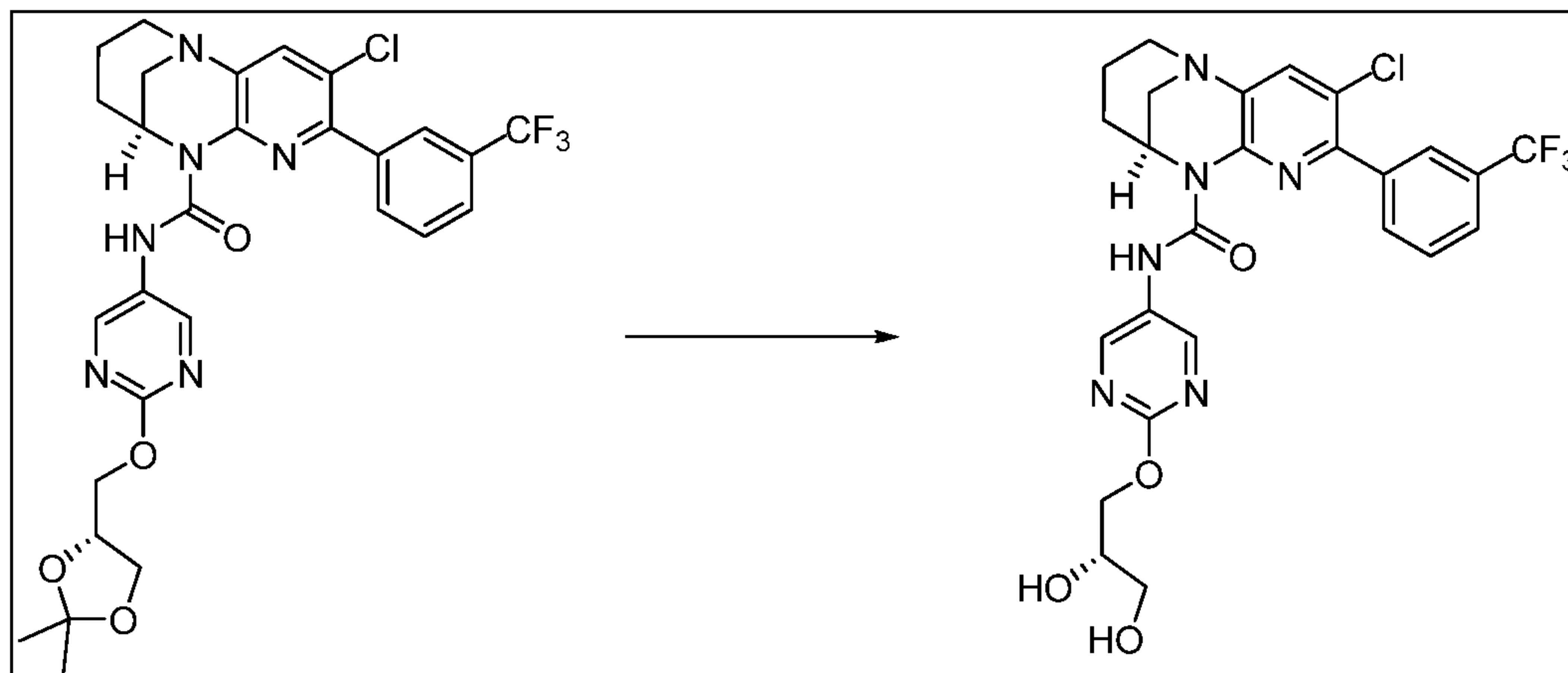
Example 70**Synthesis of (9*S*)-3-chloro-N-(6-((*R*)-2,3-dihydroxypropoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

To a stirred solution of (9*S*)-3-chloro-N-(6-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (0.25 g, 0.413 mmol) in methanol (10 mL) at 0 °C was added aq. HCl (0.517 mL, 6.20 mmol, 36 %) and stirred at RT for 1 h. (TLC eluent: 100% EtOAc; R_f 0.2; UV active). Reaction mixture was basified by adding saturated sodium bicarbonate solution (till pH 8-9) then concentrated. The residue was diluted with water (10 mL) and extracted into EtOAc (2x25 mL). Combined organic extracts were dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to give crude product. The crude was triturated with diethyl ether (10 mL) to afford desired product (9*S*)-3-chloro-N-(6-((*R*)-2,3-dihydroxypropoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (0.18 g, 0.311 mmol, 75 % yield) as off-white solid. LCMS (m/z): 565.14 [M+H]⁺, R_t = 2.51 min.

¹H NMR (400 MHz, CDCl₃): δ ppm 13.64 (s, 1 H), 8.35 (s, 1 H), 8.19 (s, 1 H), 8.11 (br d, J = 7.89 Hz, 1 H), 7.75 (br d, J = 8.11 Hz, 1 H), 7.69 - 7.60 (m, 2 H), 7.55 (s, 1 H), 4.95 (br s, 1 H), 4.51 - 4.46 (m, 2 H), 4.06 - 3.98 (m, 1 H), 3.75 - 3.63 (m, 2 H), 3.48 - 3.31 (m, 4 H), 2.99 (br d, J = 13.59 Hz, 1 H), 2.51 (br t, J = 6.14 Hz, 1 H), 2.23 (br d, J = 12.28 Hz, 1 H), 1.98 - 1.88 (m, 1 H), 1.49 - 1.40 (m, 2 H).

Example 71**Synthesis of (9*S*)-3-chloro-N-cyclopropyl-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

- 5 To a stirred solution of (9*S*)-phenyl 3-chloro-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxylate (400 mg, 0.844 mmol) in THF (30 mL) at RT was added DMAP (516 mg, 4.22 mmol), cyclopropanamine (0.301 mL, 4.22 mmol) and stirred at 65 °C for 16 h. (TLC eluent: 70% Ethyl acetate in pet ether, *R_f*: 0.4). The reaction mixture was cooled to RT, concentrated *in vacuo* and the
- 10 residue was partitioned between water (40 mL) and EtOAc (80 mL). Organic layer was separated and dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to get crude compound. The crude compound was purified by column chromatography (using silica gel, eluent 50% Ethylacetate in hexane) followed by preparative HPLC (Column: XBridge C 18(150x4.6mm, 3.5μ), Mobile Phase: A: 0.01 M Ammonium Bicarbonate B: ACN, Gradient: Time/%B: 0/5, 0.8/5, 5/50, 8/95, 12/95, 12.5/5, 15/5, Temp: Ambient, Flow Rate: 0.8ml/min, Diluent: ACN) to afford (9*S*)-3-chloro-N-cyclopropyl-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (118 mg, 0.269 mmol, 31.9 % yield) as an off white solid. LCMS (*m/z*): 437.05 [M+H]⁺; *R_t* = 2.03 min
- 15
- 20 ¹H NMR (400 MHz, CDCl₃): δ ppm 10.51 (br s, 1 H), 7.97 (s, 1 H), 7.92 (d, *J*=7.67 Hz, 1 H), 7.71 (d, *J*=7.67 Hz, 1 H), 7.64-7.59 (m, 1 H), 7.50 (s, 1 H), 4.95 (t, *J*=2.19 Hz, 1 H), 3.35 - 3.26 (m, 3 H), 2.89 (br d, *J*=13.59 Hz, 1 H), 2.79 (tq, *J*=7.19, 3.68 Hz, 1 H), 2.24 - 2.16 (m, 1 H), 1.92 - 1.81 (m, 1 H), 1.52 - 1.33 (m, 2 H), 0.75 - 0.67 (m, 2 H), 0.46 - 0.40 (m, 2 H).

Example 72**Synthesis of (9*S*)-3-chloro-N-(2-(((*S*)-2,3-dihydroxypropoxy)pyrimidin-5-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

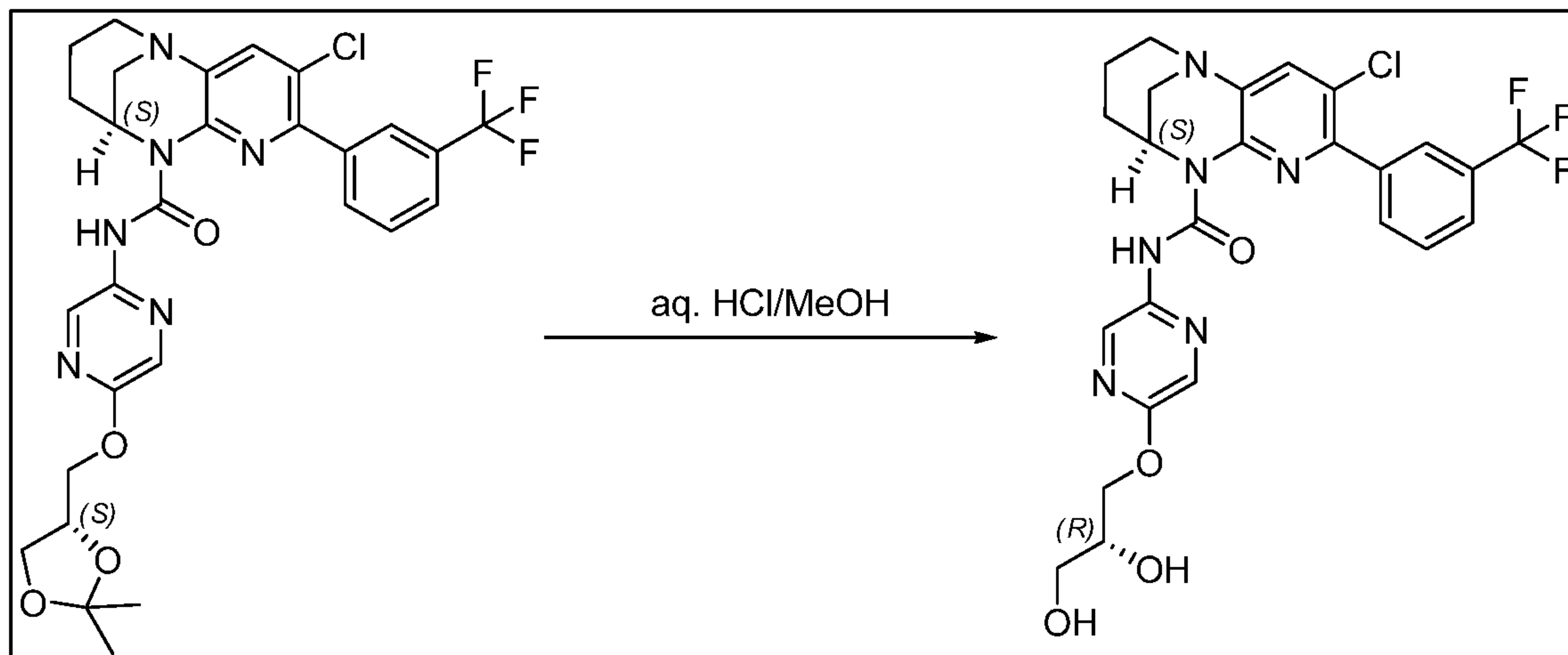
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To a stirred solution of (9*S*)-3-chloro-N-(2-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-5-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (300 mg, 0.496 mmol) in methanol (20 mL) at 0 °C was added aq. HCl (1.2 mL, 14.40 mmol) and stirred for 4 h.

10 (TLC eluent: 100% EtOAc, *R_f*: 0.2). The reaction mixture was concentrated *in vacuo* and the residue was basified with saturated NaHCO₃ solution (20 mL). The resultant solid was filtered and purified by chiral. (Column/dimensions : Chiralpak AD-H (250 X30)mm, 5μ % CO₂ : 50.0% % Co solvent: 50.0% (0.5% DEA in MeOH), Total Flow: 70.0 g/min, Back Pressure: 100.0 bar, UV: 260 nm, Stack time : 14.min, Load/inj : 38.0 mg,

15 *Solubility: Methanol*, Total No of injections : 8 *Instrument details: Make/Model: Thar SFC-80*) to afford (9*S*)-3-chloro-N-(2-(((*S*)-2,3-dihydroxypropoxy)pyrimidin-5-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (139 mg, 0.239 mmol, 48.1 % yield) as an off white solid. LCMS (*m/z*): 565.17 [M+H]⁺, *R_t* = 2.30min.

20 ¹H NMR (400 MHz, CDCl₃): δ ppm 13.10 (s, 1 H), 8.58 (s, 2 H), 8.08 - 7.91 (m, 2 H), 7.78 (s, 1 H), 7.71 - 7.57 (m, 2 H), 4.98 (br s, 1 H), 4.59 - 4.38 (m, 2 H), 4.17 - 4.05 (m, 1 H), 3.87 - 3.63 (m, 2 H), 3.47 - 3.26 (m, 3 H), 2.98 (d, *J*=13.81 Hz, 1 H), 2.23 (d, *J*=14.25 Hz, 2 H), 2.04 - 1.83 (m, 2 H), 1.69 - 1.38 (m, 2 H)

Example 73**Synthesis of (9*S*)-3-chloro-N-(5-((*R*)-2,3-dihydroxypropoxy)pyrazin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

5

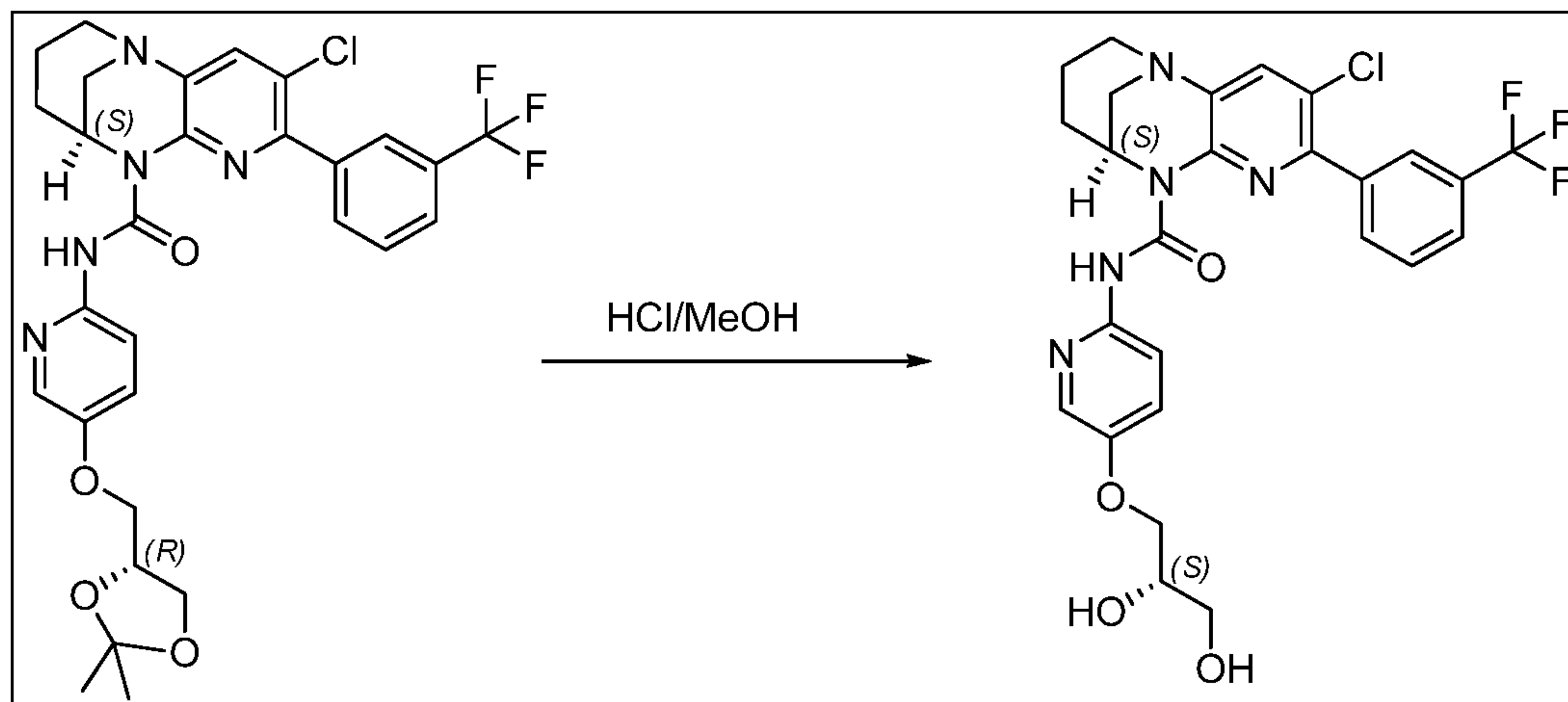
To a stirred solution of (9*S*)-3-chloro-N-(5-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (200 mg, 0.331 mmol) in Methanol (2 mL) was added hydrochloric acid (0.5 mL, 16.46 mmol) drop wise over a period of 5 min at 0 °C. Then the reaction mixture was stirred at room temperature for 2 h. (TLC eluent: 10% MeOH in DCM: R_f 0.3). Evaporated the solvent and neutralized with sodium bicarbonate solution filtered the obtained solid and washed with water to afford the desired product (9*S*)-3-chloro-N-(5-((*R*)-2,3-dihydroxypropoxy)pyrazin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (130 mg, 0.229 mmol, 69.2 % yield) as a white solid. LCMS (m/z): 565.17 $[M+H]^+$, R_t = 2.51 min.

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15

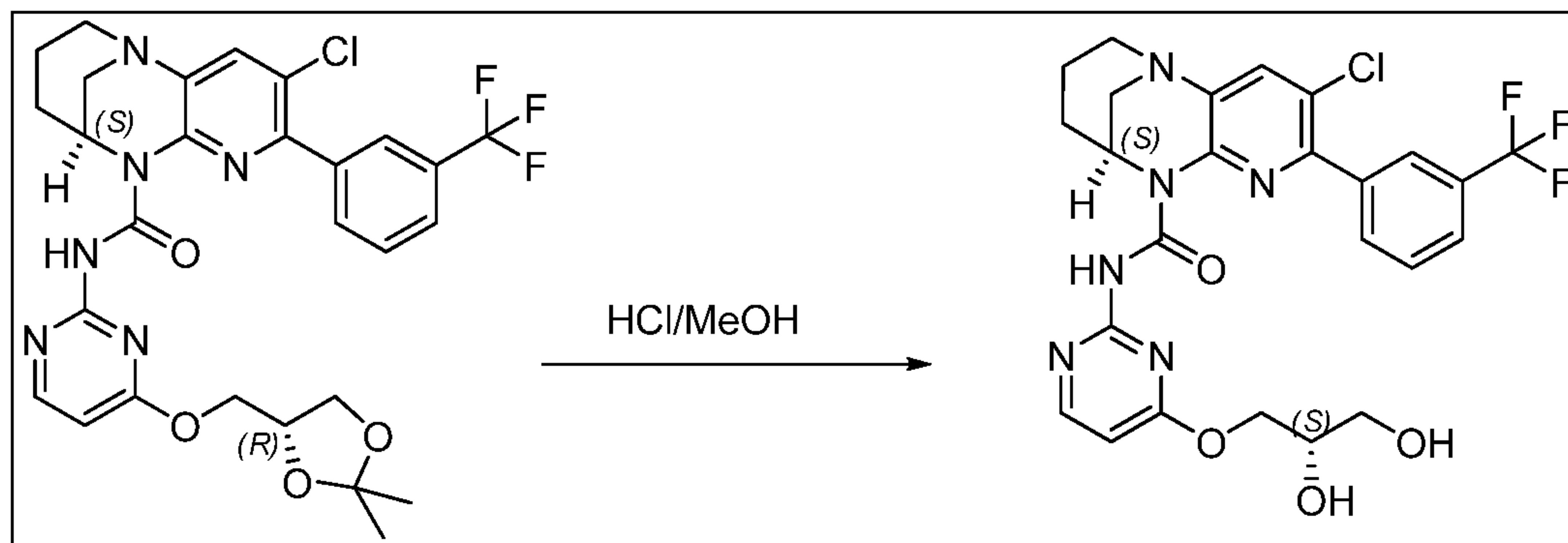
¹H NMR (400 MHz, DMSO-*d*₆): δ ppm 13.35 (s, 1 H), 8.83 (d, J = 1.32 Hz, 1 H), 8.18 - 8.12 (m, 2 H), 7.91 - 7.85 (m, 3 H), 7.83 - 7.76 (m, 1 H), 4.92 (d, J = 5.26 Hz, 1 H), 4.94 - 4.90 (m, 1 H), 4.83 (brs, 1 H), 4.63 (t, J = 5.70 Hz, 1 H), 4.30 (dd, J = 10.85, 4.06 Hz, 1 H), 4.15 (dd, J = 10.74, 6.58 Hz, 1 H), 3.85 - 3.76 (m, 1 H), 3.48 - 3.37 (m, 3 H), 3.32 (d, J = 3.07 Hz, 1 H), 2.91 (d, J = 14.03 Hz, 1 H), 2.11 - 1.76 (m, 2 H), 1.50 - 1.27 (m, 2 H).

20

Example 74**Synthesis of (9*S*)-3-chloro-*N*-(5-(((*S*)-2,3-dihydroxypropoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

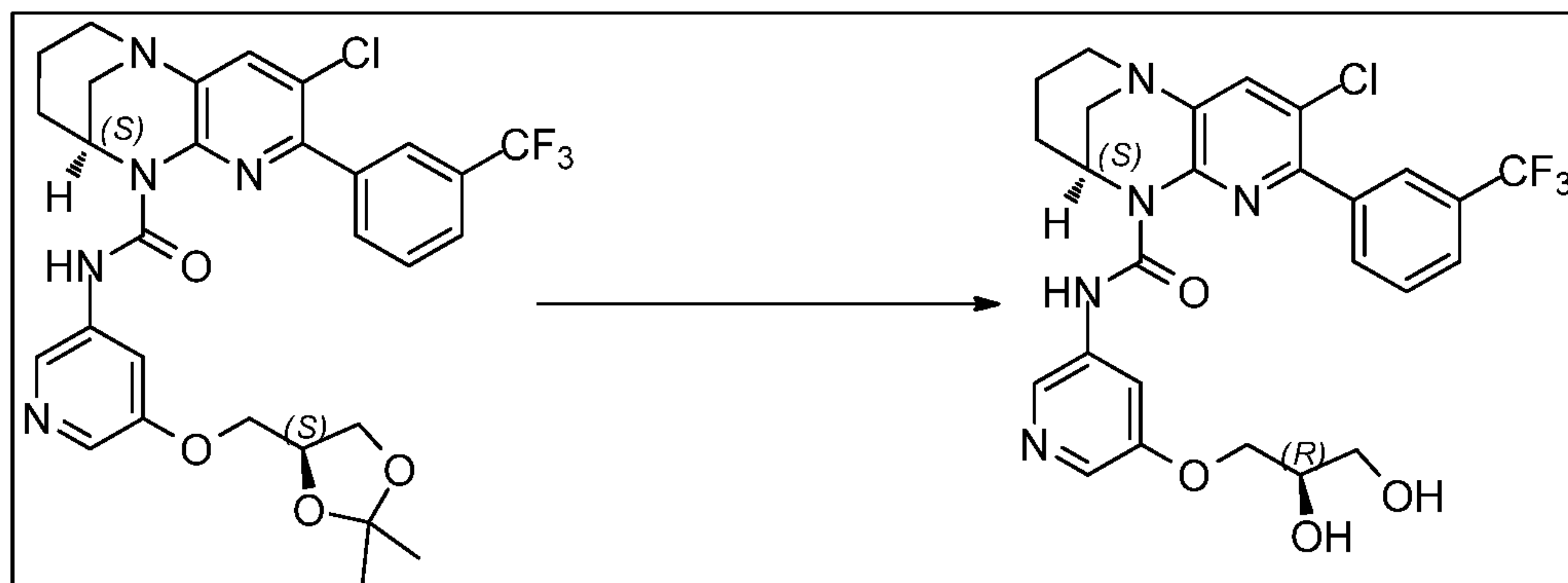
To a stirred solution of (9*S*)-3-chloro-*N*-(5-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido [2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (250 mg, 0.414 mmol) in Methanol (8 mL) and Tetrahydrofuran (5 mL) was added HCl (0.5 mL, 16.46 mmol) at 0 °C then stirred at RT for 2 h. (TLC eluent: neat ethyl acetate, R_f : 0.2). The reaction mixture was concentrated *in vacuo* and the residue was neutralized with saturated NaHCO₃ solution and filtered the obtained solid, washed with *n*-pentane (10 mLx3) to afford the desired product (9*S*)-3-chloro-*N*-(5-(((*S*)-2,3-dihydroxypropoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (201 mg, 0.354 mmol, 86 % yield) as an off white solid. LCMS (m/z): 564.17 [M+H]⁺, Rt = 2.44 min.

¹H NMR (400 MHz, CDCl₃): δ ppm 13.34 (s, 1 H), 8.22 (s, 1 H), 8.14 (d, J =7.89 Hz, 1 H), 8.04 (d, J =8.99 Hz, 1 H), 7.95 (d, J =3.07 Hz, 1 H), 7.73 (d, J =7.89 Hz, 1 H), 7.67 - 7.61(m, 1 H), 7.58 (s, 1 H), 7.25 - 7.22 (m, 1 H), 4.98 (br s, 1 H), 4.14 - 4.03 (m, 3 H), 3.89 - 3.82 (m, 1 H), 3.79 - 3.72 (m, 1 H), 3.40 - 3.31 (m, 3 H), 2.98 (d, J =13.81 Hz, 1 H), 2.54 (d, J =4.38 Hz, 1 H), 2.25 (d, J =12.06 Hz, 1 H), 1.97 - 1.87 (m, 2 H), 1.52 - 1.35 (m, 2 H).

Example 75**Synthesis of (9*S*)-3-chloro-*N*-(4-((*S*)-2,3-dihydroxypropoxy)pyrimidin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

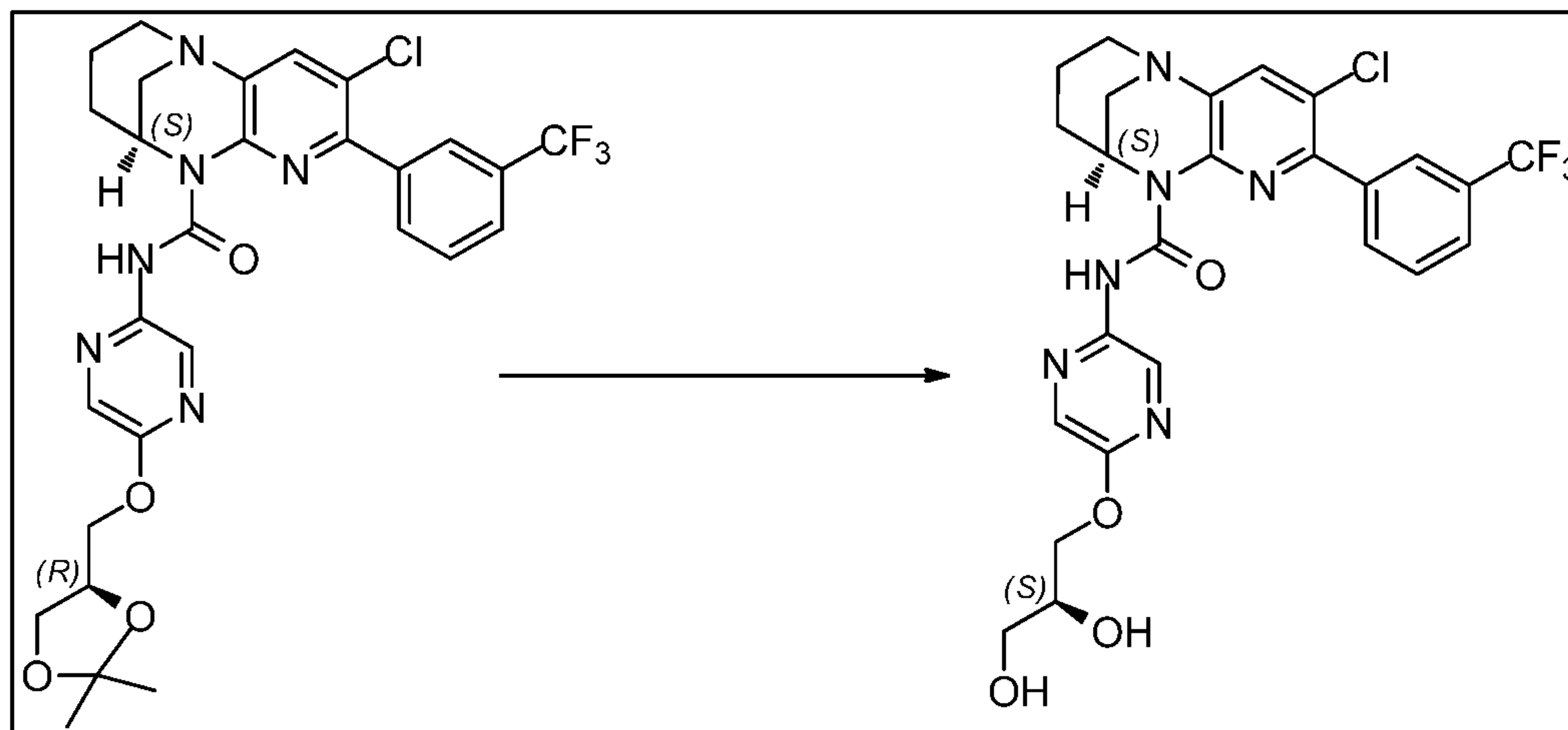
To a stirred solution of (9*S*)-3-chloro-*N*-(4-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (200 mg, 0.331 mmol) in methanol (5 mL) and Tetrahydrofuran (5 mL) was added HCl (0.3 mL, 9.87 mmol) at 0 °C then stirred at RT for 1 h. (TLC system: neat ethyl acetate, R_f 0.1). The reaction mixture was concentrated *in vacuo* and the residue was neutralized with saturated NaHCO_3 solution and filtered the obtained solid, washed with *n*-pentane (10 mLx2) to afford the desired product (9*S*)-3-chloro-*N*-(4-((*S*)-2,3-dihydroxypropoxy)pyrimidin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (114 mg, 0.200 mmol, 60.4 % yield) as an off white solid. LCMS (m/z): 565.17 $[\text{M}+\text{H}]^+$, R_t = 2.05 min.

^1H NMR (400 MHz, CDCl_3): δ ppm 13.90 (s, 1 H), 8.21 - 8.16 (m, 2 H), 8.10 (d, $J=7.67$ Hz, 1 H), 7.73 (d, $J=7.89$ Hz, 1 H), 7.66 - 7.59 (m, 2 H), 6.42 (d, $J=5.70$ Hz, 1 H), 5.03 (br s, 1 H), 4.64 (dd, $J=11.95, 5.37$ Hz, 1 H), 4.46 (dd, $J=11.95, 4.49$ Hz, 1 H), 4.11 (d, $J=6.36$ Hz, 1 H), 3.94 - 3.88 (m, 1 H), 3.64 - 3.57 (m, 2 H), 3.46 - 3.30 (m, 4 H), 2.97 (d, $J=14.03$ Hz, 1 H), 2.27 (d, $J=12.50$ Hz, 1 H), 1.97 - 1.86 (m, 1 H), 1.52 - 1.39 (m, 2 H).

Example 76**Synthesis of (9*S*)-3-chloro-*N*-(5-((*R*)-2,3-dihydroxypropoxy)pyridin-3-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

To a stirred solution of (9*S*)-3-chloro-*N*-(5-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (240 mg, 0.397 mmol) in Methanol (10 mL) was added aq.HCl (0.345 mL, 3.97 mmol) drop wise over a period of 2 min at 0 °C and stirred at room temperature for 1 h. (TLC system: 100 % Ethyl acetate. *R_f* value: 0.3). Then the reaction mixture was partitioned between saturated NaHCO₃ solution (20 mL) and DCM (30 mL), the separated organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to obtain crude compound. The crude product was purified by flash column chromatography (100-200 silicagel eluted with 0-2% of MeOH in Ethyl acetate) to afford the desired product (9*S*)-3-chloro-*N*-(5-((*R*)-2,3-dihydroxypropoxy)pyridin-3-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (170 mg, 0.299 mmol, 75 % yield) as an off white solid. LCMS (*m/z*): 564.20 [M+H]⁺, *R_t* = 2.17 min.

¹H NMR (400 MHz, CDCl₃): δ ppm 13.21 (s, 1 H), 8.04 (s, 1 H), 7.99 (d, *J*=2.41 Hz, 1 H), 7.94 (d, *J*=7.45 Hz, 1 H), 7.88 (t, *J*=2.30 Hz, 1 H), 7.83 (d, *J*=1.97 Hz, 1 H), 7.76 - 7.80 (m, 1 H), 7.67 - 7.74 (m, 1 H), 7.61 (s, 1 H), 4.97 (brs, 1 H), 4.01 - 4.13 (m, 3 H), 3.79 - 3.86 (m, 2 H), 3.71 - 3.77 (m, 1 H), 3.30 - 3.42 (m, 3 H), 2.99 (br d, *J*=13.81 Hz, 1 H), 2.63 (brs, 1 H), 2.24 (brd, *J*=14.69 Hz, 1 H), 1.88 - 1.99 (m, 1 H), 1.39 - 1.48 (m, 1 H), 1.26 (s, 1 H).

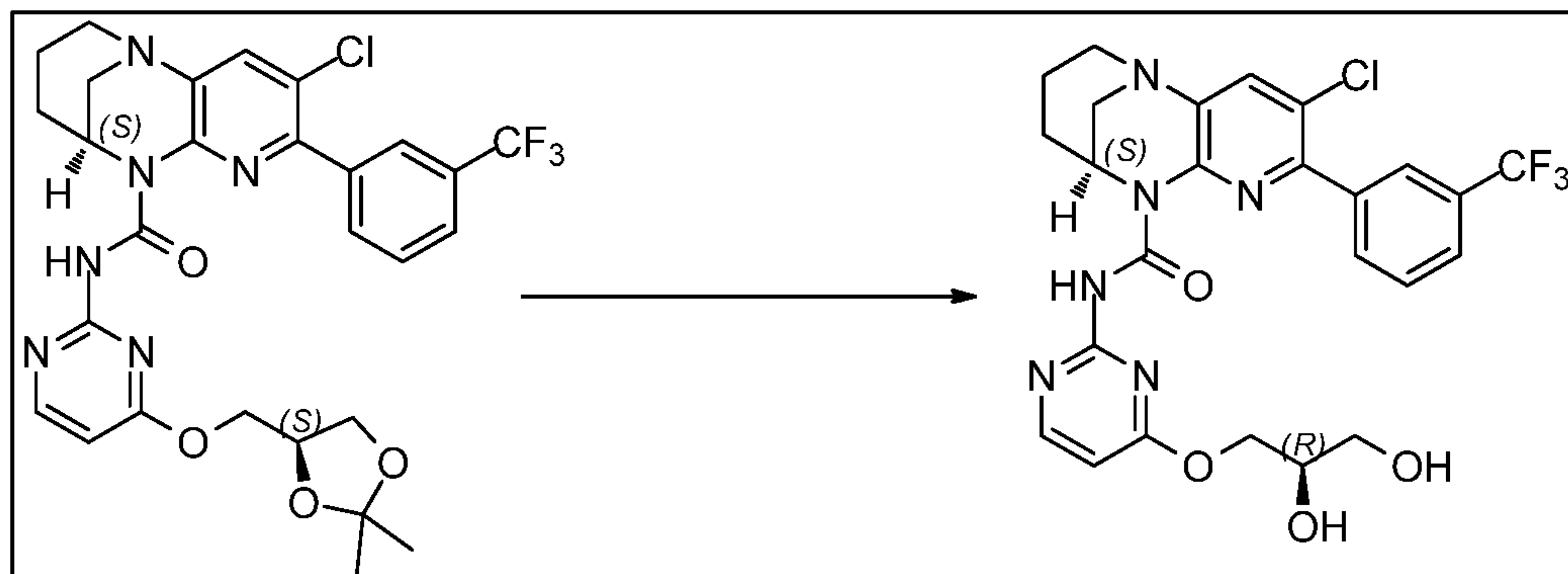
Example 77**Synthesis of (9*S*)-3-chloro-*N*-(5-((*S*)-2,3-dihydroxypropoxy)pyrazin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

5

To a stirred solution of (9*S*)-3-chloro-*N*-(5-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrazin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (200 mg, 0.331 mmol) in Methanol (10 mL) was added aq.HCl (0.287 mL, 3.31 mmol), drop wise over a period of 2 min at 0 °C and stirred at room temperature for 1 h. (TLC system: 100 % Ethyl acetate. *R_f* value: 0.4). Then the reaction mixture was partitioned between saturated NaHCO₃ solution (20 mL) and DCM (30 mL) dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford the desired product (9*S*)-3-chloro-*N*-(5-((*S*)-2,3-dihydroxypropoxy)pyrazin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (171 mg, 0.299 mmol, 91 % yield) as an off white solid. LCMS (*m/z*): 565.17 [M+H]⁺, *R_t*=2.51 min.

¹H NMR (400 MHz, DMSO-*d*₆): δ ppm 13.35 (s, 1 H), 8.83 (s, 1 H), 8.15 (s, 2 H), 7.26 - 7.96 (m, 4 H), 4.92 (d, *J*=5.26 Hz, 1 H), 4.83 (br s, 1 H), 4.63 (br t, *J*=5.81 Hz, 1 H), 4.30 (br dd, *J*=10.85, 3.84 Hz, 1 H), 4.15 (br dd, *J*=10.63, 6.69 Hz, 1 H), 3.81 (br dd, *J*=10.19, 5.15 Hz, 1 H), 3.30 - 3.63 (m, 5 H), 2.91 (br d, *J*=13.37 Hz, 1 H), 1.88 - 2.11 (m, 2 H), 1.34 (br d, *J*=4.38 Hz, 2 H).

20

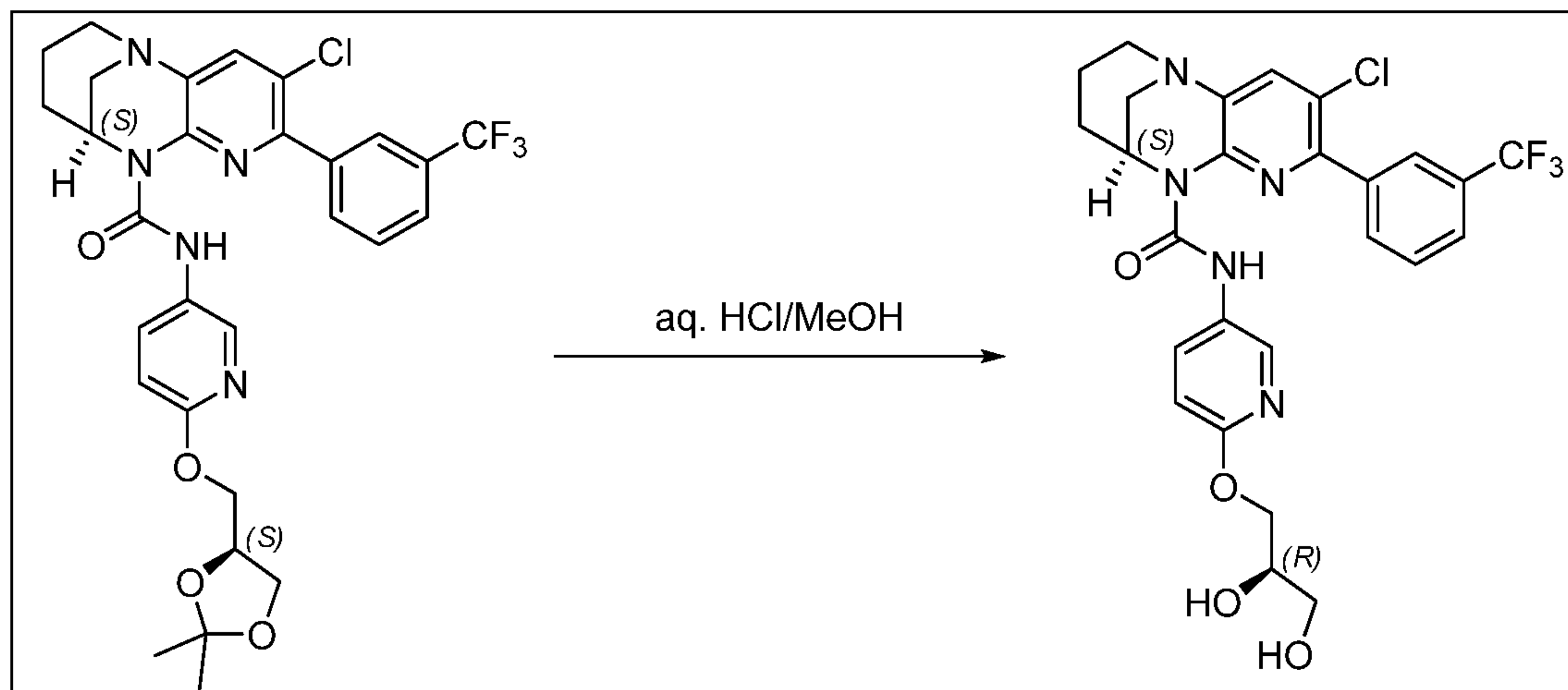
Example 78**Synthesis of (9*S*)-3-chloro-*N*-(4-((*R*)-2,3-dihydroxypropoxy)pyrimidin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

5

To a solution of (9*S*)-3-chloro-*N*-(4-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (290 mg, 0.479 mmol) in Methanol (10 mL) was added aq.HCl (0.416 mL, 4.79 mmol) drop wise over a period of 5 min at 0 °C and stirred at room temperature for 1 h. (TLC system: 100 % Ethyl acetate. *R_f* value: 0.3). Then the reaction mixture was partitioned between saturated aq NaHCO₃ solution (20 mL) and DCM (30 mL). The separated organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford the desired product (9*S*)-3-chloro-*N*-(4-((*R*)-2,3-dihydroxypropoxy)pyrimidin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (100 mg, 0.173 mmol, 36.1 % yield) as an off white solid. LCMS (*m/z*): 565.10 [M+H]⁺, *R_t* = 2.07 min.

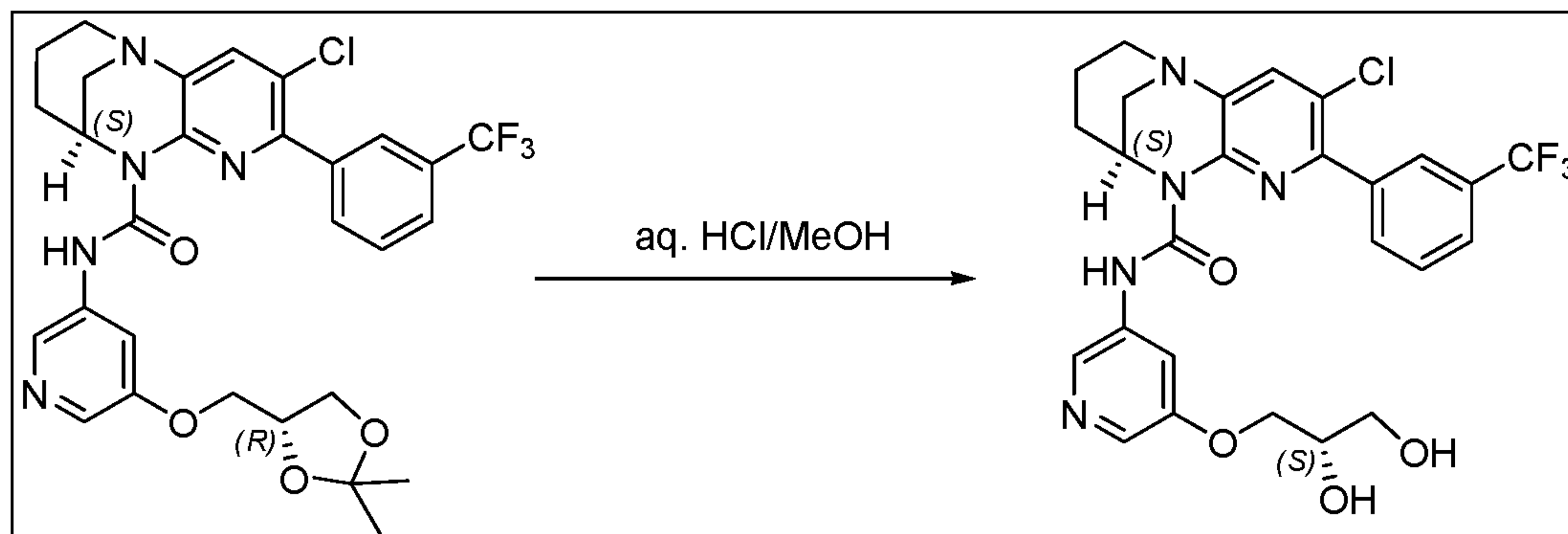
¹H NMR (400 MHz, DMSO-*d*₆): δ ppm 13.42 (s, 1 H), 8.31 - 8.18 (m, 1 H), 8.17 - 7.97 (m, 2 H), 7.95 - 7.80 (m, 1 H), 6.58 - 6.41 (m, 1 H), 4.92 (br d, *J*=5.04 Hz, 1 H), 4.80 (br s, 1 H), 4.62 (br t, *J*=5.70 Hz, 1 H), 4.24 - 4.13 (m, 2 H), 4.08 (br dd, *J*=10.85, 5.81 Hz, 1 H), 3.79 - 3.67 (m, 2 H), 3.52 - 3.31 (m, 3 H), 3.34 - 3.30 (m, 2 H), 2.90 (br d, *J*=12.72 Hz, 1 H), 2.04 - 1.78 (m, 2 H), 1.42 - 1.19 (m, 2 H).

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Example 79**Synthesis of (9*S*)-3-chloro-*N*-(6-((*R*)-2,3-dihydroxypropoxy)pyridin-3-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

To a stirred solution of (9*S*)-3-chloro-*N*-(6-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (300 mg, 0.497 mmol) in Methanol (10 mL) was added HCl (0.151 mL, 4.97 mmol) at 0 °C and stirred at 25 °C for 2 h. (TLC system: 100% ethylacetate, *R_f* value: 0.2). The reaction mixture was quenched with saturated NaHCO₃ solution (10 mL) and extracted with DCM (2x30 mL). The combined organic layer was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to obtain crude compound. The crude compound was triturated with *n*-pentane (3 x 10mL). to afford the desired product (9*S*)-3-chloro-*N*-(6-((*R*)-2,3-dihydroxypropoxy)pyridin-3-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (140 mg, 0.248 mmol, 49.8 % yield) as a white solid. LCMS (*m/z*): 564.17 [M+H]⁺, *R_t* = 2.42 min.

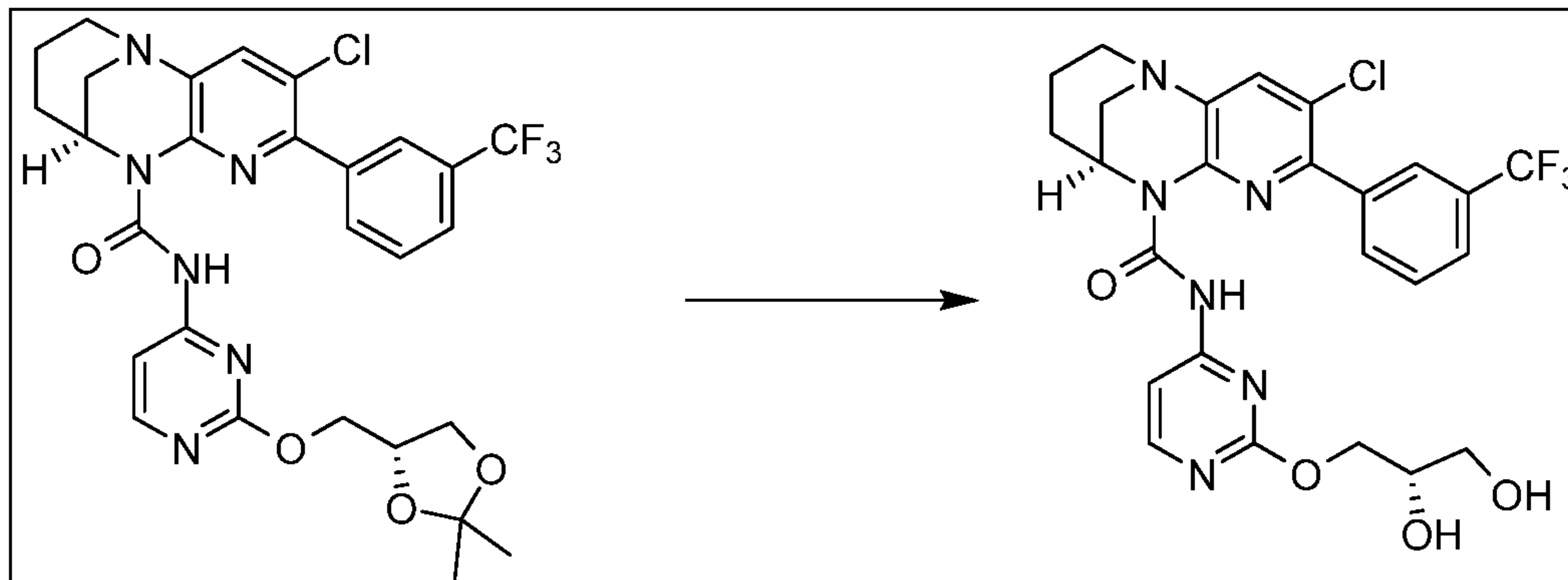
¹H NMR (400 MHz, CDCl₃): δ ppm 12.92 (s, 1 H), 8.09 (d, *J*=2.85 Hz, 1 H), 8.03 (s, 1 H), 7.96 (d, *J*=7.67 Hz, 1 H), 7.77 (dd, *J*=8.88, 2.74 Hz, 2 H), 7.71 - 7.64 (m, 1 H), 7.59 (s, 1 H), 6.73 (d, *J*=8.99 Hz, 1 H), 4.98 (brs, 1 H), 4.43 - 4.39 (m, 2 H), 4.01 - 3.95 (m, 2 H), 3.70 - 3.61 (m, 2 H), 3.42 - 3.30 (m, 3 H), 2.98 (d, *J*=13.81 Hz, 1 H), 2.69 (t, *J*=6.47 Hz, 1 H), 2.24 (d, *J*=12.06 Hz, 1 H), 1.98 - 1.87 (m, 1 H), 1.53 - 1.40 (m, 2 H).

Example 80**Synthesis of (9*S*)-3-chloro-N-(5-((*S*)-2,3-dihydroxypropoxy)pyridin-3-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

To a stirred solution of (9*S*)-3-chloro-N-(5-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido [2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (200 mg, 0.331 mmol) in Methanol (5 mL) was added *aq* HCl (1.0 mL, 32.9 mmol), over a period of 5 min. at 0 °C.

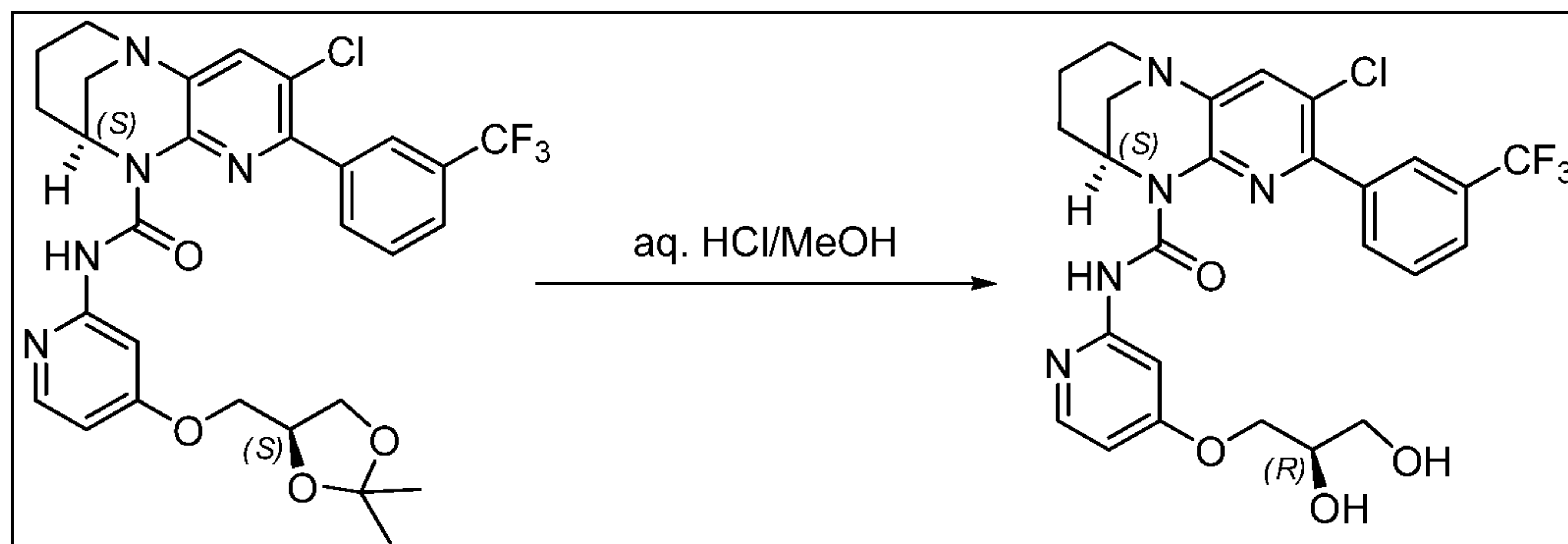
Then the reaction mixture was stirred at room temperature for 1 h. (TLC eluent: 10% MeOH in DCM R_f: 0.3; UV active) and the reaction mixture was poured in to ice cold water (10 mL) the the *pH* of the reaction mixture was adjusted to neutral with saturated NaHCO₃ solution and extracted with EtoAc (2x25 mL). The combined organic layer was washed with water (10 mL), brine solution (10mL) and dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to obtain crude compound. The crude compound was purified by flash column chromatography (silicagel: 100-200 mesh, Eluent: 5% MeOH in DCM) to afford the desired product (9*S*)-3-chloro-N-(5-((*S*)-2,3-dihydroxypropoxy)pyridin-3-yl)-2-(3-(trifluoromethyl) phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (110 mg, 0.190 mmol, 57.4 % yield) as an off-white solid. LCMS (*m/z*): 564.38 [M+H]⁺, *R*_t = 2.17 min.

¹H NMR (400 MHz, DMSO-*d*₆): δ ppm 13.07 (s, 1 H), 8.18 - 8.10 (m, 2 H), 7.95 - 7.81 (m, 5 H), 7.64 (br s, 1 H), 4.96 (d, *J*=5.04 Hz, 1 H), 4.82 (br s, 1 H), 4.67 (t, *J*=5.59 Hz, 1 H), 3.99 (dd, *J*=9.32, 3.84 Hz, 1 H), 3.87 - 3.75 (m, 2 H), 3.45 (t, *J*=5.59 Hz, 3 H), 3.40 (s, 1 H), 2.89 (d, *J*=13.59 Hz, 1 H), 2.04 - 1.90 (m, 2 H), 1.41- 1.29 (m, 3 H).

Example 81**Synthesis of (9*S*)-3-chloro-N-(2-(((*S*)-2,3-dihydroxypropoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

To a solution of (9*S*)-3-chloro-N-(2-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (170 mg, 0.281 mmol) in methanol (10 mL) under nitrogen at 0 °C was added aq. HCl (1 mL, 32.9 mmol, 36 %) and stirred at RT for 2 h. (TLC eluent: 100% Ethyl acetate: R_f 0.2; UV active). The reaction mixture was basified with saturated sodium bicarbonate solution (till pH-8-9) at 0°C and solvent was evaporated under reduced pressure. The residue was diluted with water (30 mL) and extracted into DCM (2x100 mL). Combined organic extracts were dried over anhydrous sodium sulphate, filtered and filtrate was evaporated to give crude as a white solid. The crude was triturated with ether and filtered to afford (9*S*)-3-chloro-N-(2-(((*S*)-2,3-dihydroxypropoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (70 mg, 0.121 mmol, 43.0 % yield) as off white solid. LCMS (m/z): 565.17 [$M+H$]⁺, R_t = 2.32 min.

¹H NMR (400 MHz, CDCl₃): δ ppm 13.57 (s, 1 H), 8.32 (d, J =5.70 Hz, 1 H), 8.15 - 7.97 (m, 2 H), 7.87 - 7.67 (m, 3 H), 7.62 (s, 1 H), 4.95 (br s, 1 H), 4.31 - 4.07 (m, 2 H), 4.00 - 3.85 (m, 1 H), 3.77 - 3.52 (m, 2 H), 3.43 - 3.21 (m, 3 H), 3.16 (br d, J =5.26 Hz, 1 H), 3.00 (br d, J =13.81 Hz, 1 H), 2.41 (br t, J =6.25 Hz, 1 H), 2.24 (br d, J =14.69 Hz, 1 H), 2.03 - 1.85 (m, 1 H), 1.54 - 1.37 (m, 2 H).

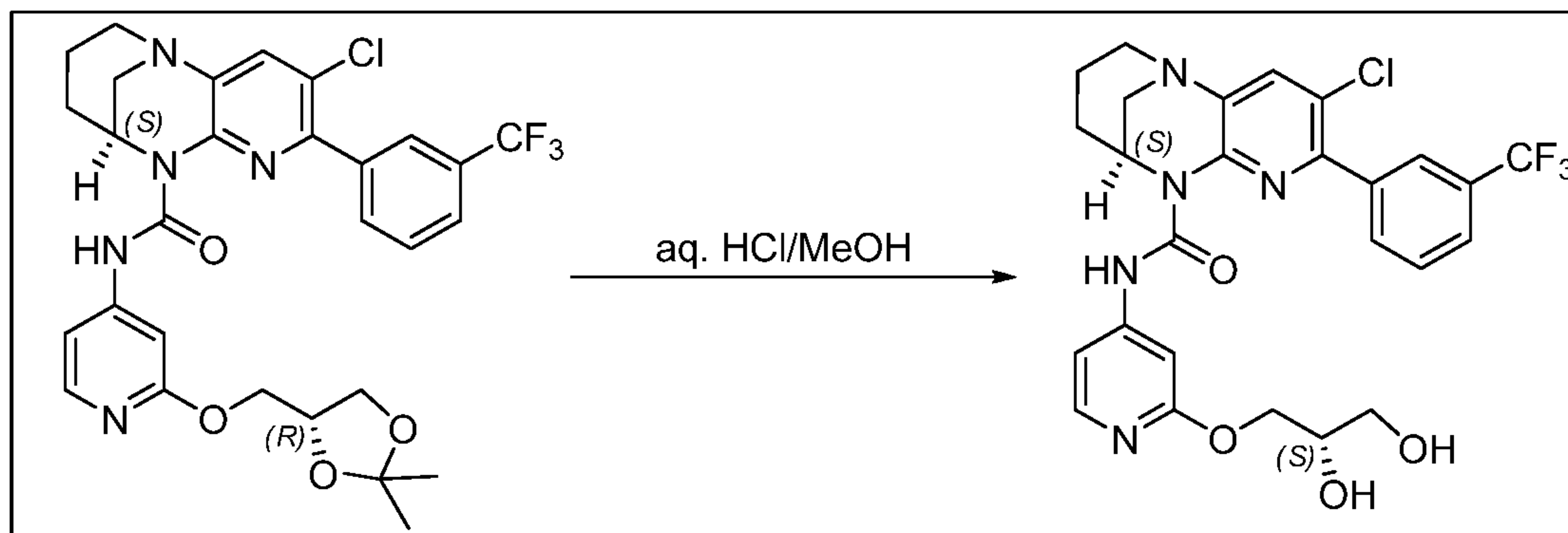
Example 82**Synthesis of (9*S*)-3-chloro-N-(4-((*R*)-2,3-dihydroxypropoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

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To a stirred solution of (9*S*)-3-chloro-N-(4-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido [2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (180 mg, 0.298 mmol) in Methanol (10 mL) was added hydrochloric acid (5 mL, 165 mmol) at 0 °C over a period of 5 min. Then the reaction mixture was stirred at 30 °C for 2 h. (TLC eluent: 5% MeOH in DCM: R_f 0.5; UV active). The solvent was evaporated and the reaction mixture was neutralized with sodium bicarbonate solution, filtered the obtained solid and washed with water (10 mL) and with *n*-pentane (2 x 20 mL) to afford the desired product (9*S*)-3-chloro-N-(4-((*R*)-2,3-dihydroxypropoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (120 mg, 0.212 mmol, 35.5 % yield) as an off-white solid. LCMS (m/z): 564 $[M+H]^+$, R_t = 6.0 min.

¹H NMR (400 MHz, CDCl₃): δ ppm 13.43 (s, 1 H), 8.23 (s, 1 H), 8.16 (d, J =8.11 Hz, 1 H), 8.04 (d, J =5.70 Hz, 1 H), 7.71 - 7.79 (m, 2 H), 7.60 - 7.67 (m, 1 H), 7.58 (s, 1 H), 6.53 (dd, J =5.70, 2.19 Hz, 1 H), 4.96 (s, 1 H), 4.08 - 4.18 (m, 2 H), 3.79 - 3.87 (m, 1 H), 3.70 - 3.77 (m, 1 H), 3.31 - 3.40 (m, 3 H), 3.00 (d, J =13.59 Hz, 1 H), 2.60 (s, 3 H), 2.24 (d, J =12.93 Hz, 1 H), 2.05 (d, J =7.67 Hz, 1 H), 1.86 - 1.98 (m, 1 H), 1.38 - 1.52 (m, 1 H).

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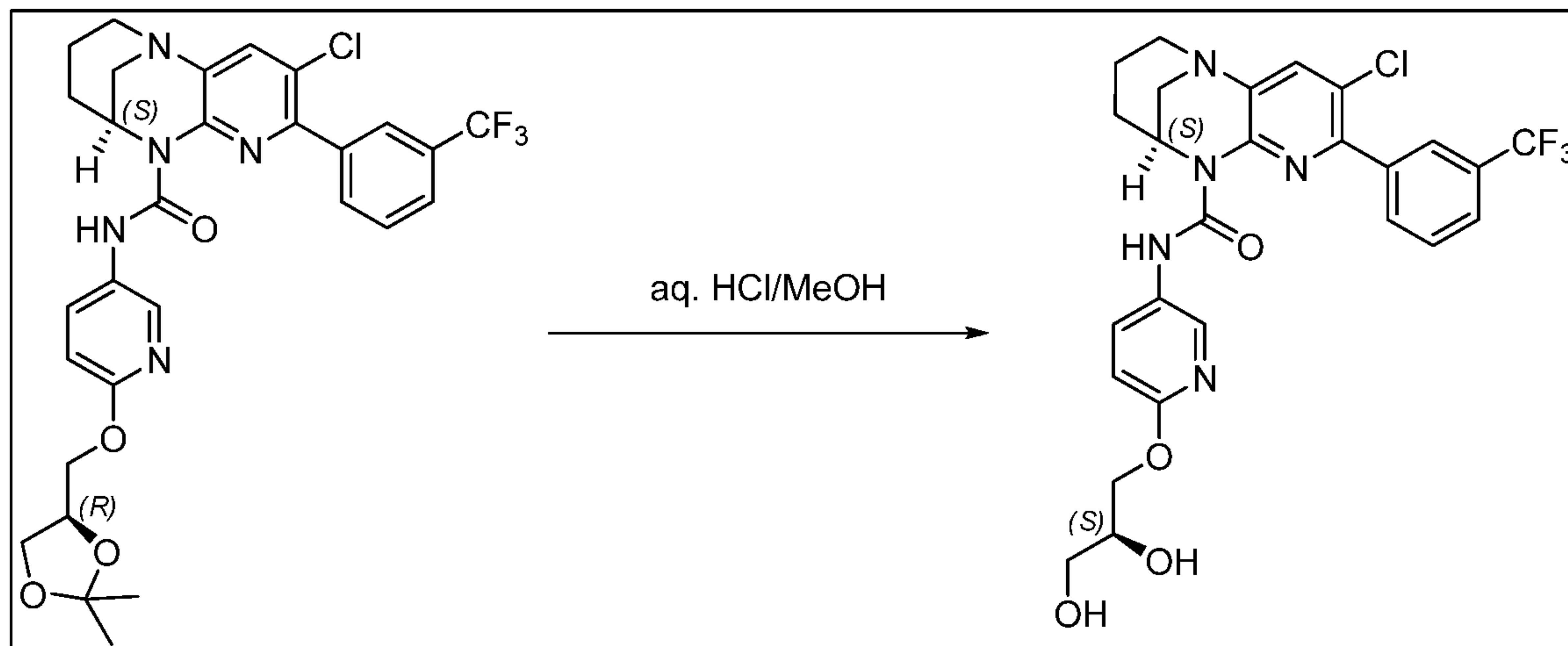
Example 83**Synthesis of (9*S*)-3-chloro-N-(2-(((*S*)-2,3-dihydroxypropoxy)pyridin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

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To a stirred solution of (9*S*)-3-chloro-N-(2-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido [2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (420 mg, 0.695 mmol) in Methanol (10 mL) was added hydrochloric acid (5 mL, 165 mmol) at 0 °C over a period of 5min. Then the reaction mixture was stirred at 30 °C for 30 min. (TLC System: 5% MeOH in DCM: R_f 0.5; UV active). Then the solvent was evaporated and the reaction mixture was neutralized with sodium bicarbonate solution, filtered and washed with water and *n*-pentane (2x 20 mL) to afford the desired product (9*S*)-3-chloro-N-(2-(((*S*)-2,3-dihydroxypropoxy)pyridin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (299 mg, 0.517 mmol, 37.1 % yield) as an off-white solid. LCMS (m/z): 564.20 $[M+H]^+$, R_t = 2.35 min.

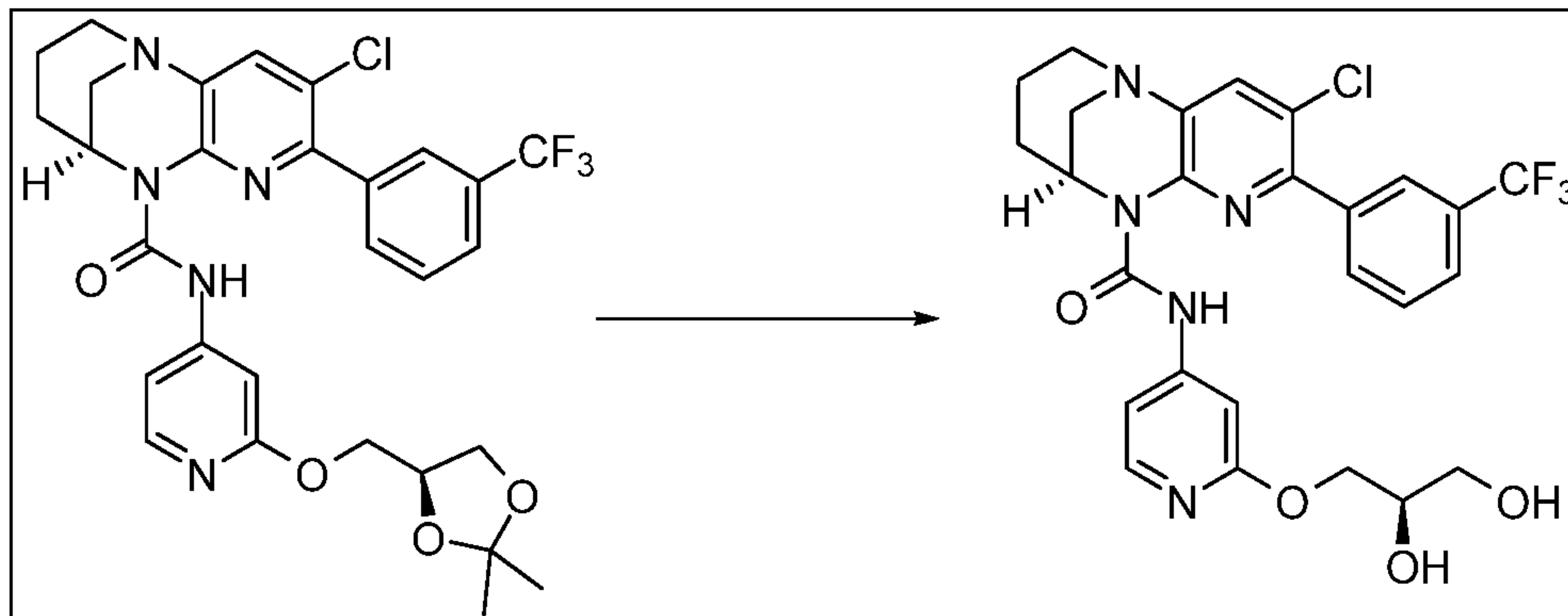
¹H NMR (400 MHz, CDCl₃): δ ppm 13.29 (s, 1 H), 8.02 (s, 1 H), 7.97 (d, J =7.67 Hz, 1 H), 7.85 (d, J =5.70 Hz, 1 H), 7.81 (d, J =7.45 Hz, 1 H), 7.73-7.65 (m, 1 H), 7.61 (s, 1 H), 7.06 (d, J =1.53 Hz, 1 H), 6.69 (dd, J =5.70, 1.75 Hz, 1 H), 4.97 (s, 1 H), 4.46-4.42 (m, 2 H), 4.28 (d, J =4.82 Hz, 1 H), 3.96 (d, J =4.38 Hz, 1 H), 3.69-3.60 (m, 2 H), 3.41-3.32 (m, 3 H), 2.98 (d, J =13.81 Hz, 1 H), 2.85 (t, J =6.36 Hz, 1 H), 2.23 (d, J =14.03 Hz, 1 H), 2.01-1.87 (m, 1 H), 1.49-1.38 (m, 2 H).

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Example 84**Synthesis of (9*S*)-3-chloro-N-(6-(((*S*)-2,3-dihydroxypropoxy)pyridin-3-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

To a stirred solution of (9*S*)-3-chloro-N-(6-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-3-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (150 mg, 0.248 mmol) in Methanol (10mL) was added Hydrochloric acid (4 mL, 132 mmol) over a period of 5 min. at 0 °C. Then the reaction mixture was stirred at 30 °C for 30 min. (TLC eluent: 5% MeOH in DCM: R_f 0.5; UV active). The solvent was evaporated and reaction mixture was neutralized with sodium bicarbonate solution, extracted with DCM (2 x 50.mL). The combined organic layer washed with brine solution and dried over anhydrous sodium sulphate, filtered and evaporated to obtain crude compound. The crude product was purified by combi-flash chromatography (120g reverse phase column: Eluent: 100% acetonitrile) and the obtained product was washed with *n*-pentane (2 x 20 mL) to afford the desired product (9*S*)-3-chloro-N-(6-(((*S*)-2,3-dihydroxypropoxy)pyridin-3-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (108 mg, 0.190 mmol, 77 % yield) as an off white solid. LC-MS (m/z): 564.17 [$M+H$]⁺, R_t = 2.42 min.

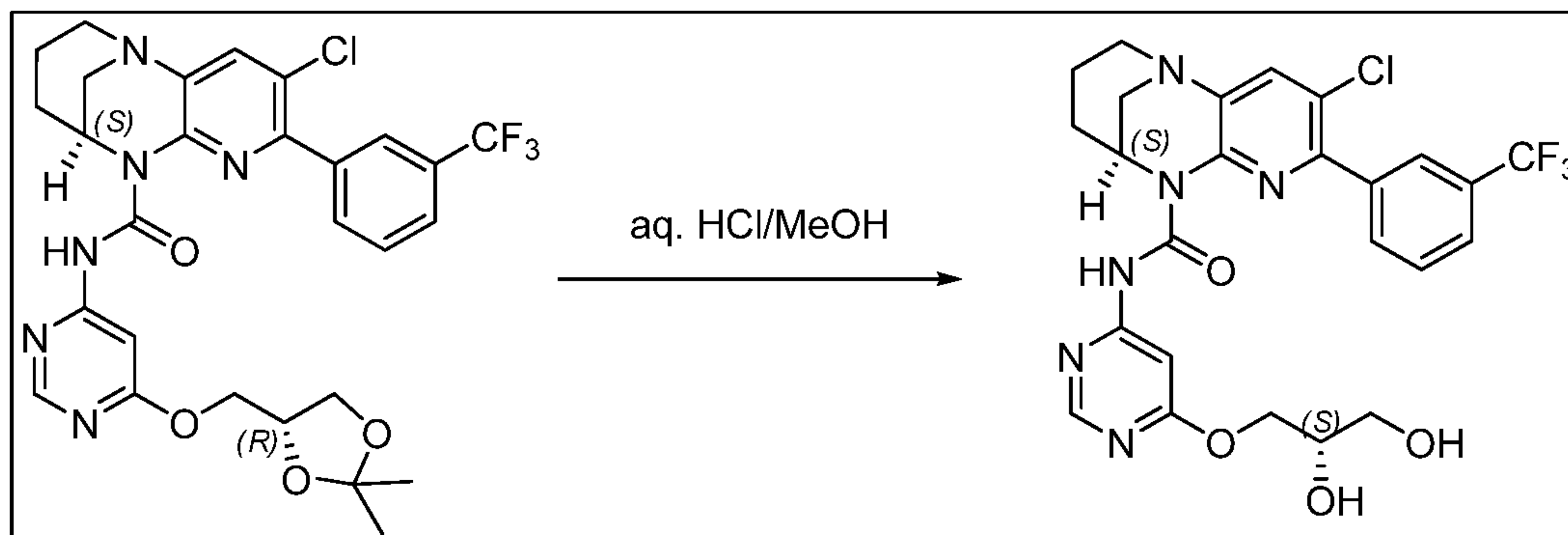
¹H NMR (400 MHz, CDCl₃): δ ppm 12.92 (s, 1 H), 8.09 (d, J =2.85 Hz, 1 H), 8.03 (s, 1 H), 7.96 (d, J =7.67 Hz, 1 H), 7.77 (dd, J =8.99, 2.63 Hz, 2 H), 7.71- 7.63 (m, 1 H), 7.59 (s, 1 H), 6.73 (d, J =8.77 Hz, 1 H), 4.99 (s, 1 H), 4.42 (d, J =4.17 Hz, 2 H), 3.97 (s, 2 H), 3.73- 3.61 (m, 2 H), 3.40- 3.31 (m, 3 H), 2.98 (d, J =13.59 Hz, 1 H), 2.67 (t, J =6.14 Hz, 1 H), 2.23 (d, J =14.03 Hz, 1 H), 1.99- 1.87 (m, 1 H), 1.49- 1.38 (m, 2 H).

Example 85**Synthesis of (9*S*)-3-chloro-N-(2-(((*R*)-2,3-dihydroxypropoxy)pyridin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

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To a stirred suspension of (9*S*)-3-chloro-N-(2-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (250 mg, 0.414 mmol) in methanol (5.0 mL) was added aq. HCl (0.349 mL, 4.14 mmol, 36 %) at 0 °C and stirred at
 10 RT for 5 h. After completion of the reaction the volatiles were evaporated under reduced pressure to get the crude (TLC eluent system: 5% Methanol in DCM, R_f -0.2, UV active). The crude was diluted with water (5 ml) and basified (up to pH 8) with the aqueous sodium bicarbonate solution. The precipitated solid was filtered, washed with the water and dried under vacuum to afford (9*S*)-3-chloro-N-(2-(((*R*)-2,3-dihydroxypropoxy)pyridin-
 15 4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (149.0 mg, 0.262 mmol, 63.4 % yield) as an off white solid. LCMS (m/z): 564.20 $[M+H]^+$, R_t = 2.37 min.

¹H NMR (400 MHz, CDCl₃): δ ppm 13.30 (s, 1 H), 8.02 (s, 1 H), 7.97 (br d, J =7.67 Hz, 1 H) 7.78 - 7.88 (m, 2 H), 7.66 - 7.73 (m, 1 H), 7.61 (s, 1 H), 7.06 (d, J =1.32 Hz, 1 H), 6.69
 20 (dd, J =5.70, 1.53 Hz, 1 H), 4.96 (br s, 1 H), 4.43 (d, J =4.60 Hz, 2 H), 4.30 (br s, 1 H), 3.97 (br d, J =3.73 Hz, 1 H), 3.65 (br d, J =4.38 Hz, 2 H), 3.29 - 3.42 (m, 3 H), 2.87 - 3.01 (m, 2 H), 2.23 (br d, J =14.47 Hz, 1 H), 1.87 - 2.00 (m, 1 H), 1.39 - 1.55 (m, 2 H).

Example 86**Synthesis of (9*S*)-3-chloro-N-(6-((*S*)-2,3-dihydroxypropoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

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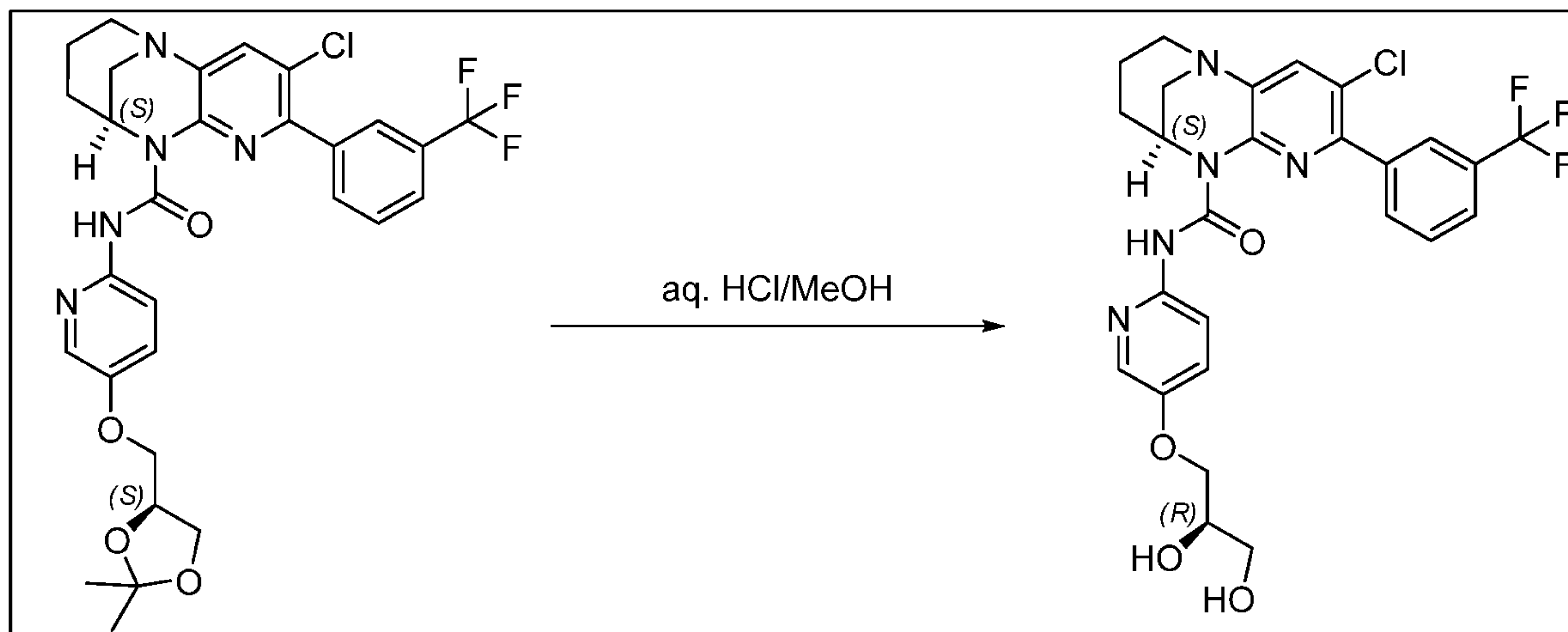
To a stirred solution of (9*S*)-3-chloro-N-(6-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (200 mg, 0.331 mmol) in Methanol (5 mL) at 0 °C was added hydrochloric acid (1 mL, 32.9 mmol) drop wise over a period of 5 min. Then the reaction mixture was stirred at 28 °C for 30 min. (TLC eluent: 10% MeOH in DCM : R_f 0.2; UV active). The reaction mixture was neutralized with sodium bicarbonate solution and filtered the obtain solid, triturated with diethylether to afford the desired product (9*S*)-3-chloro-N-(6-((*S*)-2,3-dihydroxypropoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (110 mg, 0.189 mmol, 57.1 % yield) as an off-white solid. LCMS (m/z): 565.10 $[M+H]^+$, R_t : 2.52 min.

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^1H NMR (400 MHz, CDCl_3): δ ppm 13.64 (s, 1 H), 8.35 (d, $J=0.66$ Hz, 1 H), 8.19 (s, 1 H), 8.11 (d, $J=7.89$ Hz, 1 H), 7.75 (d, $J=8.11$ Hz, 1 H), 7.67 - 7.62 (m, 1 H), 7.61 (s, 1 H), 7.55 (d, $J=0.88$ Hz, 1 H), 4.95 (br s, 1 H), 4.55 - 4.42 (m, 2 H), 3.98 - 4.08 (m, 1 H), 3.75 - 3.62 (m, 2 H), 3.50 - 3.31 (m, 4 H), 2.99 (d, $J=13.59$ Hz, 1 H), 2.52 (t, $J=6.25$ Hz, 1 H), 2.22 (br d, $J=14.69$ Hz, 1 H), 2.02 - 1.82 (m, 1 H), 1.53 - 1.37 (m, 2 H).

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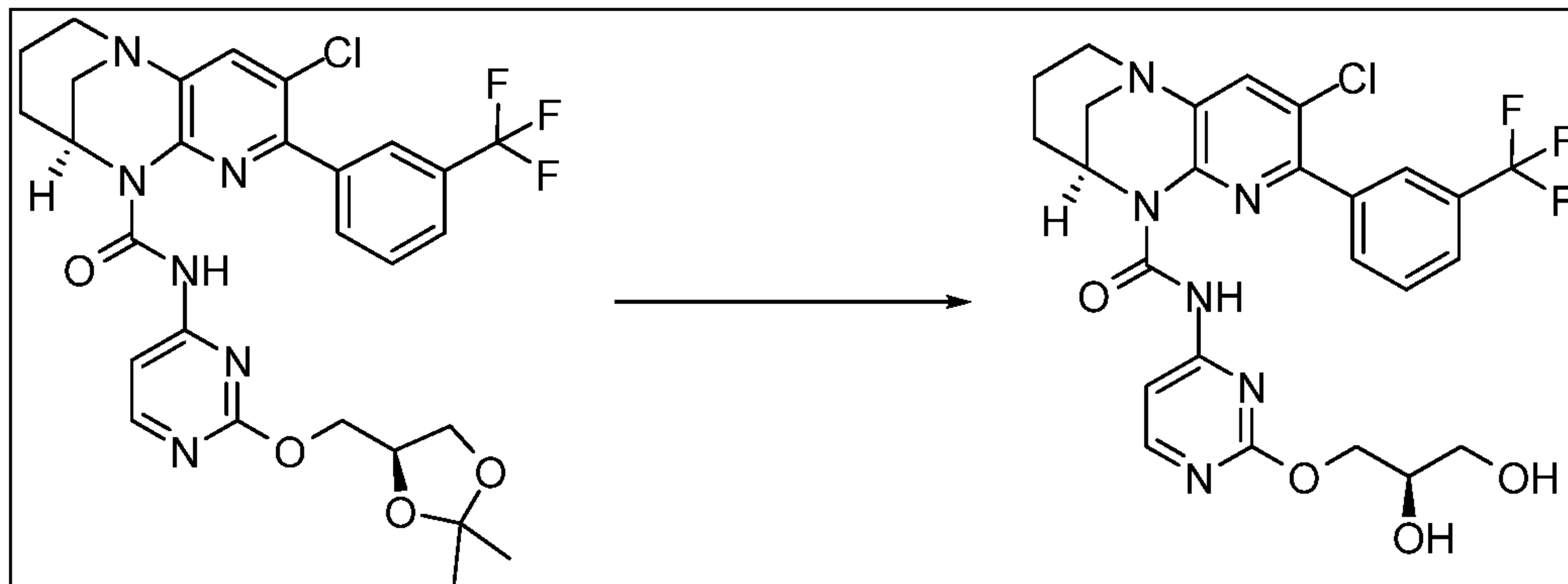
Example 87**Synthesis of (9*S*)-3-chloro-*N*-(5-((*R*)-2,3-dihydroxypropoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

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To a stirred solution of (9*S*)-3-chloro-*N*-(5-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (250 mg, 0.414 mmol) in Methanol (5 mL) was added aq. HCl (0.126 mL, 4.14 mmol) at 0 °C. The reaction mixture was stirred for 1 h. (TLC System: R_f : 0.4, EtOAc). at room temperature and concentrated under reduced pressure to obtain residue. The residue was neutralized with saturated sodium bicarbonate solution and filtered the obtained solid and dried to afford the desired product (9*S*)-3-chloro-*N*-(5-((*R*)-2,3-dihydroxypropoxy)pyridin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (160 mg, 0.274 mmol, 66.1 % yield) as an off white solid. LCMS (m/z): 564.1 $[M+H]^+$, R_t = 2.44 min.

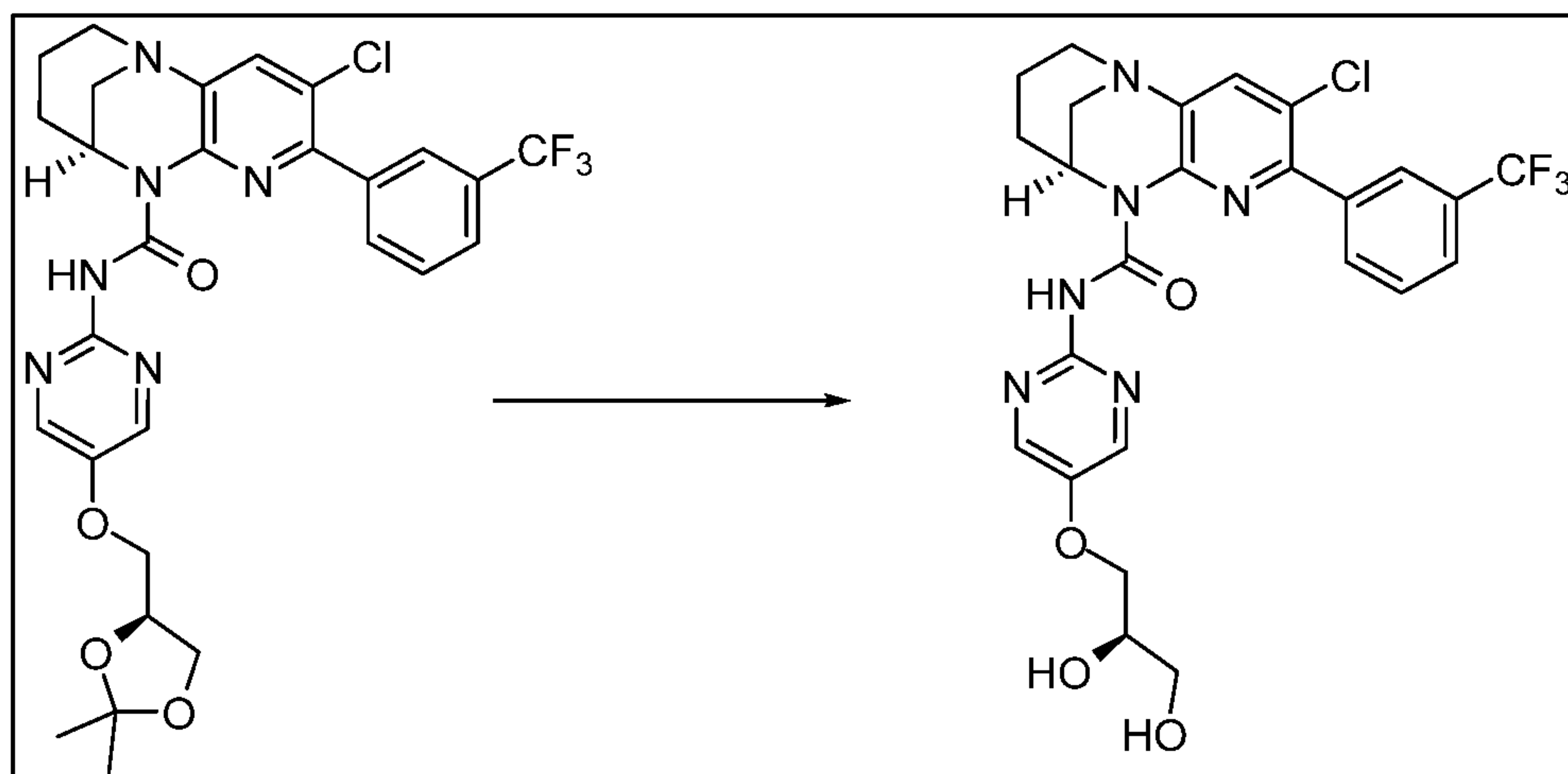
$^1\text{H NMR}$ (400 MHz, DMSO- d_6): δ ppm 13.22 (s, 1 H), 8.23- 8.12 (m, 2 H), 7.99 (d, J =9.21 Hz, 1 H), 7.90- 7.84 (m, 3 H), 7.83 -7.78 (m, 1 H), 7.43 (dd, J =9.21, 3.07 Hz, 1 H), 4.95 (br s, 1 H), 4.83 (br s, 1 H), 4.65 (br s, 1 H), 4.02 (dd, J =9.87, 3.95 Hz, 1 H), 3.92- 3.85 (m, 1 H), 3.82 -3.74 (m, 1 H), 3.48- 3.31 (m, 5 H), 2.89 (d, J =13.81 Hz, 1 H), 2.06- 1.89 (m, 2 H), 1.42 -1.30 (m, 2 H).

20

Example 88**Synthesis of (9*S*)-3-chloro-N-(2-(((*R*)-2,3-dihydroxypropoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

To a stirred solution of (9*S*)-3-chloro-N-(2-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (300 mg, 0.496 mmol) in methanol (10 mL) under nitrogen at 0 °C was added aq. HCl (1 mL, 4.00 mmol, 36 %) and stirred at RT for 1 h. (TLC eluent: 5% Methanol in DCM, R_f : 0.3, UV active). To the reaction mixture was added saturated NaHCO₃ solution (till pH-8-9) and extracted into EtOAc (3x10 mL). The combined organic extracts were dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to obtain crude product. The crude compound was purified by chromatography (GRACE instrument using C-18 column, Mobile phase A: 0.1% Formic Acid in water; B: ACN, the product was eluted at 90% ACN/0.1% Formic Acid in water.) Subsequently required fractions were concentrated and basified with saturated NaHCO₃ and extracted into DCM. Combined extracts were dried over anhydrous Na₂SO₄, filtered and evaporated to afford (9*S*)-3-chloro-N-(2-(((*R*)-2,3-dihydroxypropoxy)pyrimidin-4-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (170 mg, 0.292 mmol, 58.9 % yield) as an off-white solid. LCMS (m/z): 565.2 [M+H]⁺, R_t = 2.30 min.

¹H NMR (400 MHz, CDCl₃): δ ppm 13.57 (s, 1 H), 8.32 (d, J =5.70 Hz, 1 H), 8.10 - 8.05 (m, 2 H), 7.79 - 7.69 (m, 3 H), 7.62 (s, 1 H), 4.95 (br s, 1 H), 4.24 - 4.12 (m, 2 H), 3.97 (dq, J =10.06, 5.05 Hz, 1 H), 3.72 - 3.58 (m, 2 H), 3.41 - 3.32 (m, 3 H), 3.11 (d, J =5.26 Hz, 1 H), 3.00 (br d, J =14.03 Hz, 1 H), 2.37 (t, J =6.36 Hz, 1 H), 2.24 (br d, J =14.03 Hz, 1 H), 2.00 - 1.88 (m, 1 H), 1.53 - 1.39 (m, 2 H)

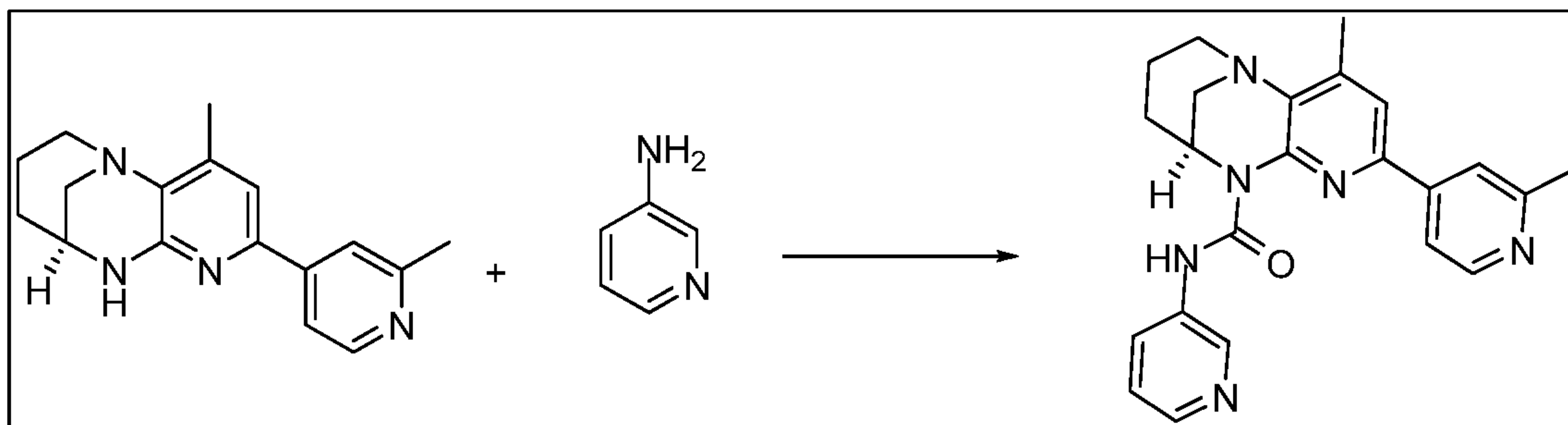
Example 89**Synthesis of (9*S*)-3-chloro-N-(5-((*R*)-2,3-dihydroxypropoxy)pyrimidin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

To a stirred solution of (9*S*)-3-chloro-N-(5-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (120 mg, 0.198 mmol) in methanol (15 mL) was added aq.HCl (6.03 μ L, 0.198 mmol, 36 %) at 0 $^{\circ}$ C and stirred at RT for 1 h. (TLC eluent: 10%MeOH in EtOAc : R_f 0.1; UV active). The reaction mixture was basified with saturated sodium bicarbonate solution (till pH-8-9) at 0 $^{\circ}$ C and concentrated. The residue was diluted with water (8 mL) and extracted into DCM (2x25 mL). Combined organic extracts were dried over anhydrous sodium sulphate, filtered and filtrate was evaporated under reduced pressure to afford (9*S*)-3-chloro-N-(5-((*R*)-2,3-dihydroxypropoxy)pyrimidin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (40 mg, 0.068 mmol, 34.4 % yield) as brown solid. LCMS (m/z): 565.17 [$M+H$] $^{+}$, R_t = 2.25 min.

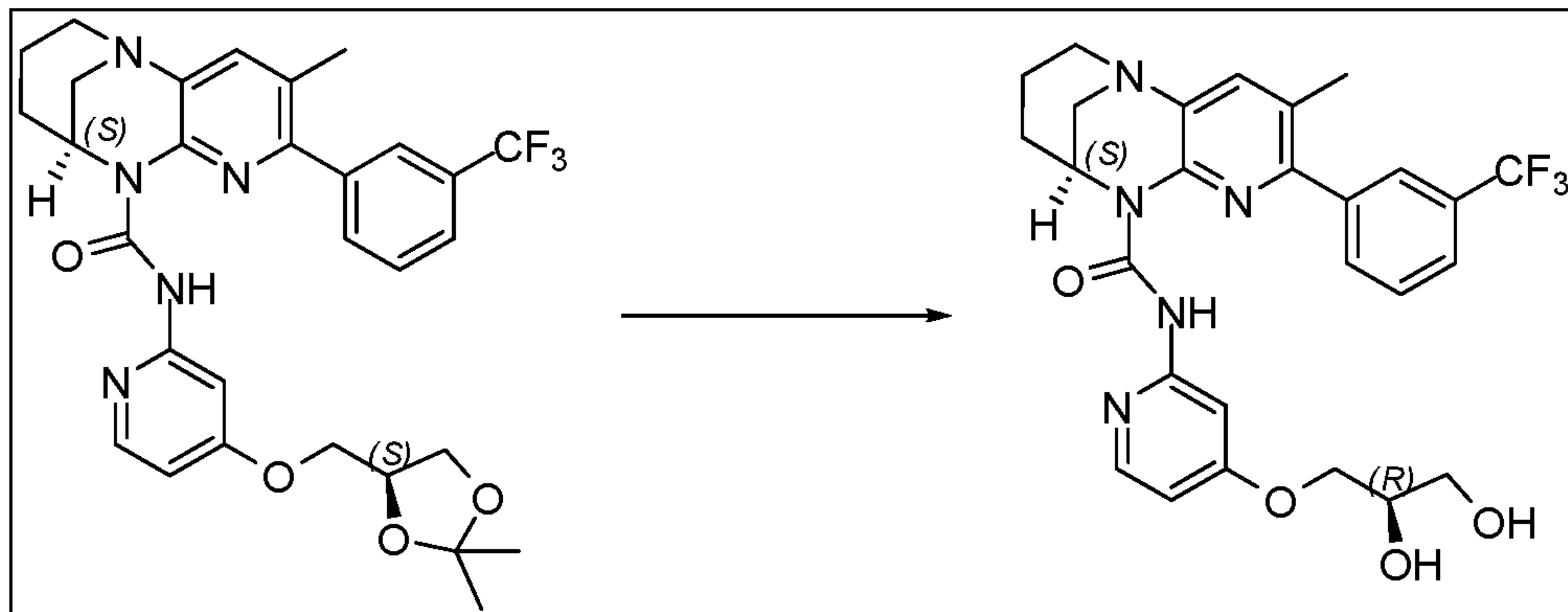
$^1\text{H NMR}$ (400 MHz, DMSO- d_6): δ ppm 13.43 (s, 1 H), 8.33 (s, 2 H), 8.00 - 8.24 (m, 2 H), 7.83 - 7.91 (m, 2 H), 7.83 - 7.91 (m, 1 H), 5.01 (br d, J =3.95 Hz, 1 H), 4.80 (br s, 1 H), 4.47 - 4.74 (m, 1 H), 4.12 (dd, J =10.08, 3.73 Hz, 2 H), 3.89 - 4.06 (m, 1 H), 3.36 - 3.48 (m, 3 H), 3.30 - 3.34 (m, 2 H), 2.89 (br d, J =13.59 Hz, 1H), 1.98 (br s, 2 H), 1.35 (br s, 2 H).

Example 90

Synthesis of (9*S*)-4-methyl-2-(2-methylpyridin-4-yl)-N-(pyridin-3-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide.



- 5 To a stirred solution of (9*S*)-4-methyl-2-(2-methylpyridin-4-yl)-7,8,9,10-tetrahydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine (500 mg, 1.783 mmol) in THF (30 mL) at RT was added DIPEA (0.934 mL, 5.35 mmol), triphosgene (318 mg, 1.070 mmol) and stirred for 1 h. then added pyridin-3-amine (252 mg, 2.68 mmol) and the reaction mixture was heated to 75 °C for 16 h. (TLC eluting system: 10% MeOH in DCM; R_f 0.3; UV active). The
- 10 reaction mixture was cooled to RT and quenched with water (15 mL) and extracted into EtOAc (2x15 mL). Organic layer was separated, dried over anhydrous sodium sulphate, filtered and filtrate was evaporated to get crude compound. The crude was purified by chromatography (GRACE using C-18 reserval column, Mobile phase A: 0.1% Formic Acid in water; B: ACN, eluent 56% B in A). Combined fractions were evaporated and
- 15 basified with saturated NaHCO₃ solution. The aqueous layer was extracted with DCM, DCM layer was dried over anhydrous Na₂SO₄, filtered and filtrate was evaporated to afford (9*S*)-4-methyl-2-(2-methylpyridin-4-yl)-N-(pyridin-3-yl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (160 mg, 0.395 mmol, 22.17 % yield) as yellow solid. LCMS (m/z): 400.9 [M+H]⁺, R_t = 4.45 min.
- 20 ¹H NMR (400 MHz, CDCl₃): δ ppm 13.83 (s, 1 H), 8.57 - 8.76 (m, 2 H), 8.31 (dd, J =4.71, 1.43 Hz, 1 H), 8.02 - 8.23 (m, 1 H), 7.61 (s, 1 H), 7.51 (dd, J =5.04, 1.53 Hz, 1 H), 7.20 - 7.33 (m, 2 H), 4.99 (br s, 1 H), 3.38 (dd, J =13.59, 1.75 Hz, 1 H), 3.12 - 3.28 (m, 2 H), 2.98 (br d, J =13.59 Hz, 1 H), 2.68 (s, 3 H), 2.44 (s, 3 H), 2.13 - 2.41 (m, 1 H), 1.85 - 2.00 (m, 1 H), 1.44 (dt, J =6.08, 3.21 Hz, 2 H).

Example 91**Synthesis of (9*S*)-N-(4-((*R*)-2,3-dihydroxypropoxy)pyridin-2-yl)-3-methyl-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide**

5

To a stirred solution of (9*S*)-N-(4-(((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyridin-2-yl)-3-methyl-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (300 mg, 0.514 mmol) in methanol (10 mL) was added HCl (1 mL, 32.9 mmol) drop wise over a period of 5 min. at 0 °C. Then the reaction mixture was stirred at 30 °C for 1 h. (TLC eluent: 5% MeOH in DCM: R_f 0.3; UV active). Evaporated the solvent under reduced pressure and neutralized with sodium bicarbonate solution and filtered the obtain solid, it was triturated with 1:1 ratio of diethyleher (50 ml) and pentane (50 ml) to afford pure compound (9*S*)-N-(4-((*R*)-2,3-

10 dihydroxypropoxy)pyridin-2-yl)-3-methyl-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6*H*-5,9-methanopyrido[2,3-*b*][1,4]diazocine-10(7*H*)-carboxamide (250 mg, 0.453 mmol, 88 %

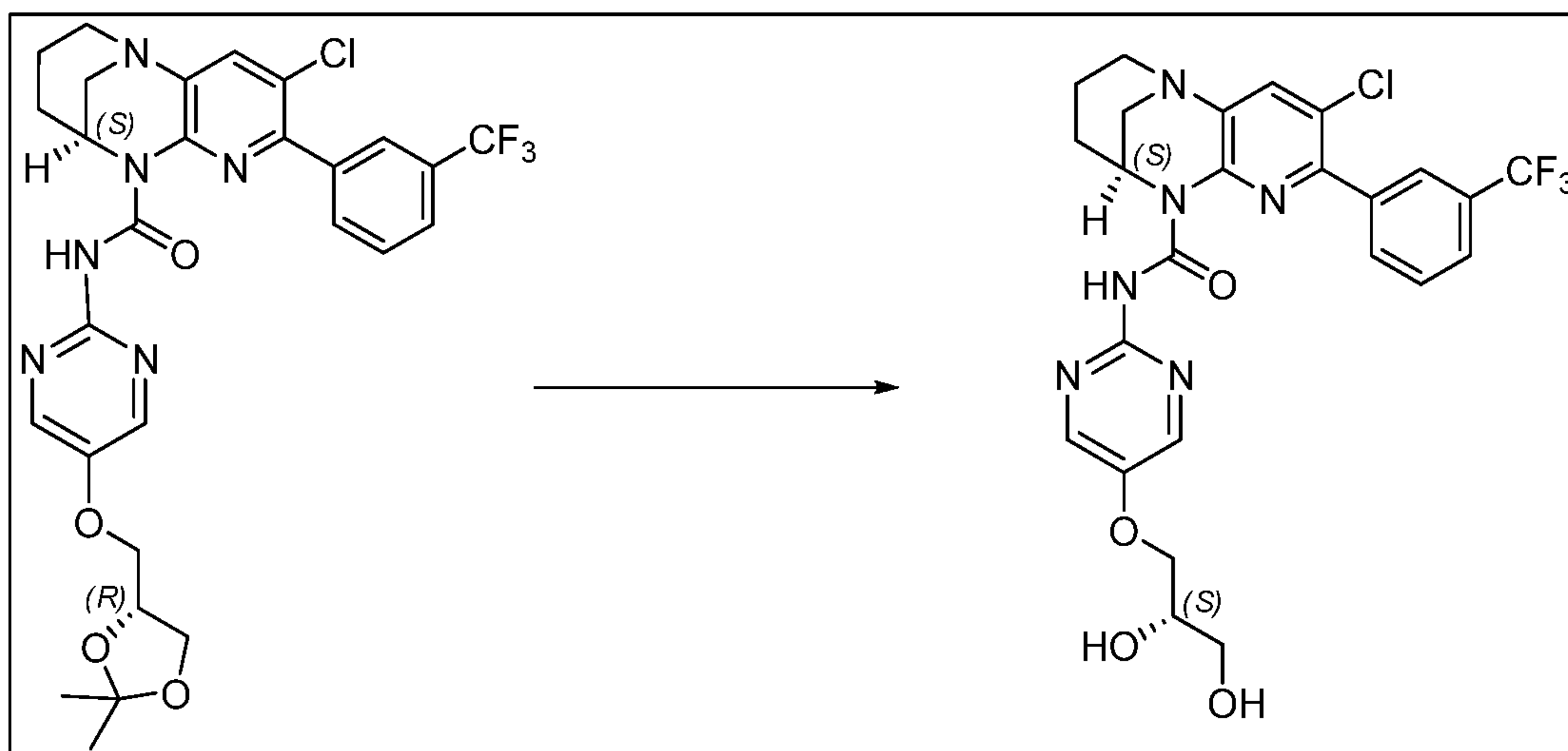
15 yield) as an off white solid. LCMS (m/z): 544.26 $[M+H]^+$, R_t = 2.23 min.

$^1\text{H NMR}$ (400 MHz, CDCl_3): δ : 13.76 (s, 1 H), 8.08 - 8.00 (m, 2 H), 7.97 - 7.88 (m, 2 H), 7.73 - 7.57 (m, 2 H), 7.38 (d, J = 0.7 Hz, 1 H), 7.23 (dd, J = 9.0, 3.1 Hz, 1 H), 4.95 (d, J = 3.1 Hz, 1 H), 4.16 - 4.00 (m, 3 H), 3.85 (dd, J = 11.4, 3.8 Hz, 2 H), 3.76 (dd, J = 11.4, 5.4 Hz, 3 H), 3.01 (d, J = 13.6 Hz, 1 H), 2.55 - 2.4 (m, 1 H), 2.39 (s, 3 H), 2.25 (d, J = 14.4 Hz, 1 H), 1.98 - 1.84 (m, 2 H), 1.42 - 1.33 (m, 1 H).

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Example 92

Synthesis of (9S)-3-chloro-N-(5-((S)-2,3-dihydroxypropoxy)pyrimidin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide



- 5 To a stirred solution of (9S)-3-chloro-N-(5-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)pyrimidin-2-yl)-2-(3-(trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (0.420g, 0.694 mmol) in methanol (5 mL) was added aq. HCl (0.5 mL, 6.00 mmol, 12M) at 0 °C and stirred at RT for 1 h. (TLC eluent 100% Ethylacetate: R_f = 0.1; UV active). Reaction mixture was
- 10 basified by adding saturated sodium bicarbonate solution (till pH-8-9) then concentrated. The residue was diluted with water (10 mL) and extracted into ethyl acetate (20 mL). Combined organic extracts were dried over anhydrous sodium sulphate, filtered and filtrate was evaporated *in vacuo* and the crude was triturated with diethylether (10 mL) to afford the desired product (9S)-3-chloro-N-(5-((S)-2,3-dihydroxypropoxy)pyrimidin-2-yl)-2-(3-
- 15 (trifluoromethyl)phenyl)-8,9-dihydro-6H-5,9-methanopyrido[2,3-b][1,4]diazocine-10(7H)-carboxamide (0.083g, 0.145 mmol, 20.90 % yield) as an off white solid. LCMS (m/z): 565.14 $[M+H]^+$, R_t = 2.25 min.
- $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 13.74 (s, 1 H), 8.29 (s, 2 H), 8.18 (s, 1 H), 8.10 (d, J =7.89 Hz, 1 H), 7.77-7.75 (m, 1 H), 7.65 -7.56 (m, 2 H), 5.03 (br s, 1 H), 4.21 -4.00 (m, 3 H), 3.86 (br dd, J =11.18, 2.63 Hz, 1 H), 3.76 (br dd, J =11.18, 4.82 Hz, 1 H), 3.44 -3.24 (m, 3 H), 2.98 (br d, J =13.59 Hz, 1 H), 2.62 (br s, 1 H), 2.38 - 2.21 (m, 1 H), 2.01 (br s, 2 H), 1.98 -1.75 (m, 2 H).
- 20

Example 93. Full-length SIRT1 production

Full-length human SIRT1 (hSIRT1) proteins were expressed with a C-terminal His₆ tag and purified as described in Hubbard. et al. (2013) Science 339, 1216. Each cell paste was resuspended in buffer A (50 mM Tris-HCl pH 7.5, 250 mM NaCl, 25 mM imidazole, and 0.1 mM TCEP) with 1,000 U Benzonase nuclease (Sigma Aldrich) supplemented with cOmplete, EDTA-free Protease Inhibitor Cocktail Tablets (Roche) on ice. Cells were disrupted by pulse sonication with 50% on and 50% off for 12 minutes total at 40 W. Insoluble debris was removed by centrifugation. Clarified supernatant was directly loaded onto a 1 mL HisTrap FF Crude column (GE Lifesciences). After washing with buffer A, SIRT1 was eluted with buffer B (50 mM Tris-HCl pH 7.5, 250 mM NaCl, 500 mM imidazole and 0.1 mM TCEP). Protein was further purified by size exclusion chromatography in buffer C (50 mM Tris-HCl pH 7.5, 300 mM NaCl, 0.1 mM TCEP) using a Hi-load Superdex 200 16/60 column (GE Lifesciences). Enzyme concentrations were determined by Bradford assay using BSA as a standard. Final protein purity was assessed by gel densitometry. Proteins were confirmed by LC/MS. All proteins were greater than 90 % pure.

Example 94. SIRT1 deacetylation reactions

SIRT1 deacetylation reactions were performed in reaction buffer (50 mM HEPES-NaOH, pH 7.5, 150 mM NaCl, 1 mM DTT, and 1 % DMSO) at 25°C monitoring either nicotinamide production using the continuous PNC1/GDH coupled assay (Smith, B. C. et al. (2009) Anal Biochem 394, 101) or O-acetyl ADP ribose (OAcADPr) production by mass spectrometry (Hubbard. et al. (2013) Science 339, 1216). Final concentrations of the PNC1/GDH coupling system components used were 20 units/mL bovine GDH (Sigma-Aldrich), 1 uM yeast PNC1, 3.4 mM α -ketoglutarate, and 220 μ M NADH or NADPH. An extinction coefficient of 6.22 mM⁻¹cm⁻¹ and a pathlength of 0.81 cm was used to convert the absorbance at 340 nm to product concentration for the 150 uL reactions used. Assays monitoring OAcADPr production were performed in reaction buffer with 0.05% BSA and time points were taken by quenching the deacetylation reaction with a stop solution which gave a final concentration of 1 % formic acid and 5 mM nicotinamide. Quenched reactions were diluted 5-fold with 1:1 acetonitrile:methanol and spun at 5,000 x g for 10 minutes to precipitate protein before being analyzed with an Agilent RapidFire 200 High-Throughput Mass Spectrometry System (Agilent, Wakefield, MA) coupled to an ABSciex API 4000 mass spectrometer fitted with an electrospray ionization source. The p53-based

Ac-p53(W5) (Ac-RHKK^{Ac}W-NH₂) and TAMRA (Ac-EE-K(biotin)-GQSTSSHSK(Ac)NleSTEG-K(5TMR)-EE-NH₂) peptides were obtained from American Century Peptide and Biopeptide, Inc, respectively.). Substrate K_M determinations were performed by varying one substrate concentration at a fixed, saturating concentration of the second substrate. SIRT1 activation and inhibition assays were run in reaction buffer with 0.05 % BSA at 25 °C and analyzed using the OAcADPr assay. Enzyme and compound were pre-incubated for 20 minutes before addition of substrates. For the activation screen of full-length hSIRT1, compounds were tested in duplicate with a dose response. In order to be sensitive to K_M-modulating activators, substrate concentrations of approximately one-tenth their K_M values were used. The dose-dependence of five compounds was tested and the fold-activation data were described by Eq. 1

$$\frac{v_x}{v_0} = b + \frac{RV_{\max} - b}{1 + \frac{EC_{50}}{[X]}} \quad (\text{Eq.1})$$

where v_x/v_0 is the ratio of the reaction rate in the presence (v_x) versus absence (v_0) of activator (X), $RV_{\max}$ is the relative velocity at infinite activator concentration, EC_{50} is the concentration of activator required to produce one-half $RV_{\max}$ and b is the minimum value of v_x/v_0 .

Example 95. Biochemical Activity

Mass spectrometry based assays were used to identify modulators of SIRT1 activity. The TAMRA based assay utilized a peptide having 20 amino acid residues as follows: Ac-EE-K(biotin)-GQSTSSHSK(Ac)NleSTEG-K(5TMR)-EE-NH₂ (SEQ ID NO: 1), wherein K(Ac) is an acetylated lysine residue and Nle is a norleucine. The peptide was labeled with the fluorophore 5TMR (excitation 540 nm/emission 580 nm) at the C-terminus. The sequence of the peptide substrate was based on p53 with several modifications. In addition, the methionine residue naturally present in the sequence was replaced with the norleucine because the methionine may be susceptible to oxidation during synthesis and purification. The Trp based assay utilized a peptide having an amino acid residues as follows: Ac-R-H-K-K(Ac)-W-NH₂ (SEQ ID NO: 2).

The TAMRA based mass spectrometry assay was conducted as follows: 0.5 μM peptide substrate and 120 μM βNAD⁺ was incubated with 10 nM SIRT1 for 25 minutes at 25°C in a reaction buffer (50 mM Tris-acetate pH 8, 137 mM NaCl, 2.7 mM KCl, 1 mM

MgCl₂, 5 mM DTT, 0.05% BSA). The SIRT1 protein was obtained by cloning the SirT1 gene into a T7-promoter containing vector, which was then transformed and expressed in BL21(DE3) bacterial cells. Test compound was added at varying concentrations to this reaction mixture and the resulting reactions were monitored. After the 25 minute

5 incubation with SIRT1, 10 μ L of 10% formic acid was added to stop the reaction. The resulting reactions were sealed and frozen for later mass spec analysis. Determination of the amount of deacetylated substrate peptide formed (or, alternatively, the amount of O-acetyl-ADP-ribose (OAADPR) generated) by the sirtuin-mediated NAD-dependent deacetylation reaction allowed for the precise measurement of relative SIRT1 activity in
10 the presence of varying concentrations of the test compound versus control reactions lacking the test compound.

The Trp mass spectrometry assay was conducted as follows. 0.5 μ M peptide substrate and 120 μ M β NAD⁺ were incubated with 10 nM SIRT1 for 25 minutes at 25°C in a reaction buffer (50 mM HEPES pH 7.5, 1500 mM NaCl, 1 mM DTT, 0.05% BSA).

15 The SIRT1 protein was obtained by cloning the SirT1 gene into a T7-promoter containing vector, which was then expressed in BL21(DE3) bacterial cells and purified as described in further detail below. Test compound was added at varying concentrations to this reaction mixture and the resulting reactions were monitored. After the 25 minute incubation with SIRT1, 10 μ L of 10% formic acid was added to stop the reaction. The
20 resulting reactions were sealed and frozen for later mass spec analysis. The relative SIRT1 activity was then determined by measuring the amount of O-acetyl-ADP-ribose (OAADPR) formed (or, alternatively, the amount of deacetylated Trp peptide generated) by the NAD-dependent sirtuin deacetylation reaction in the presence of varying concentrations of the test compound versus control reactions lacking the test compound.
25 The degree to which the test agent activated deacetylation by SIRT1 was expressed as EC_{1.5} (i.e., the concentration of compound required to increase SIRT1 activity by 50% over the control lacking test compound), and Percent Maximum Activation (i.e., the maximum activity relative to control (100%) obtained for the test compound).

A control for inhibition of sirtuin activity was conducted by adding 1 μ L of 500
30 mM nicotinamide as a negative control at the start of the reaction (e.g., permits determination of maximum sirtuin inhibition). A control for activation of sirtuin activity was conducted using 10 nM of sirtuin protein, with 1 μ L of DMSO in place of compound,

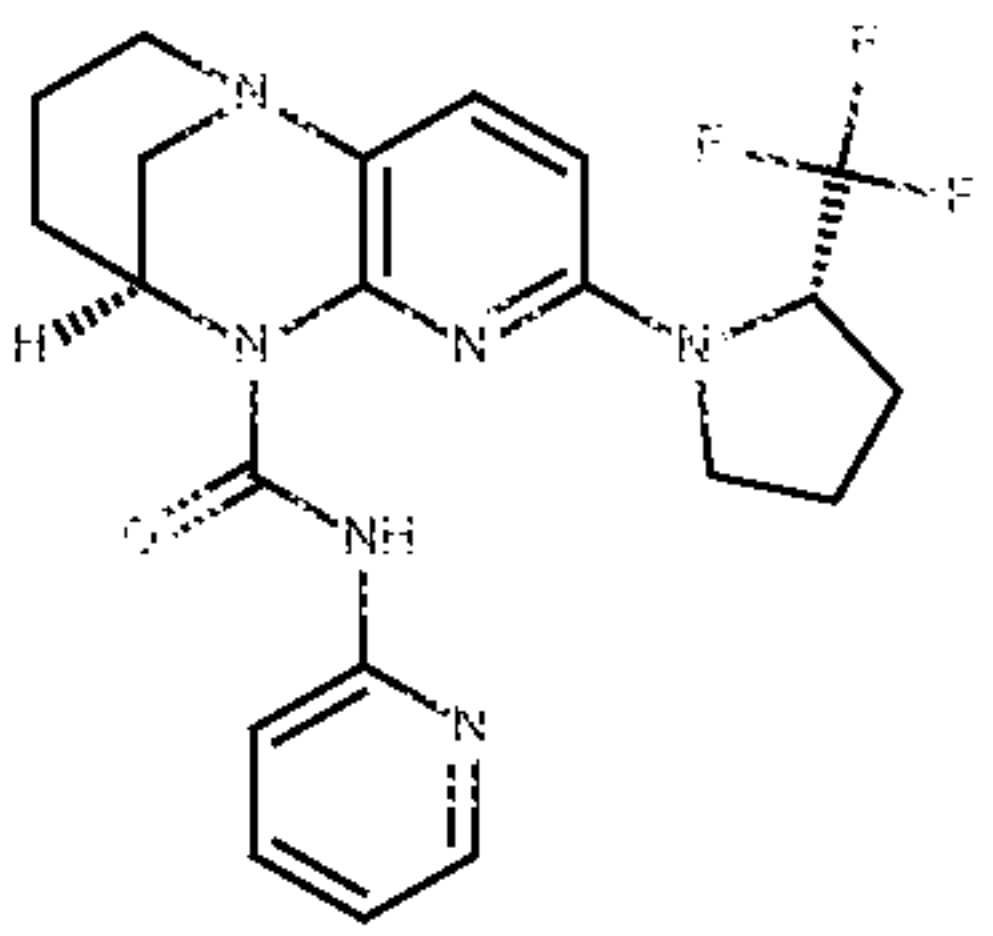
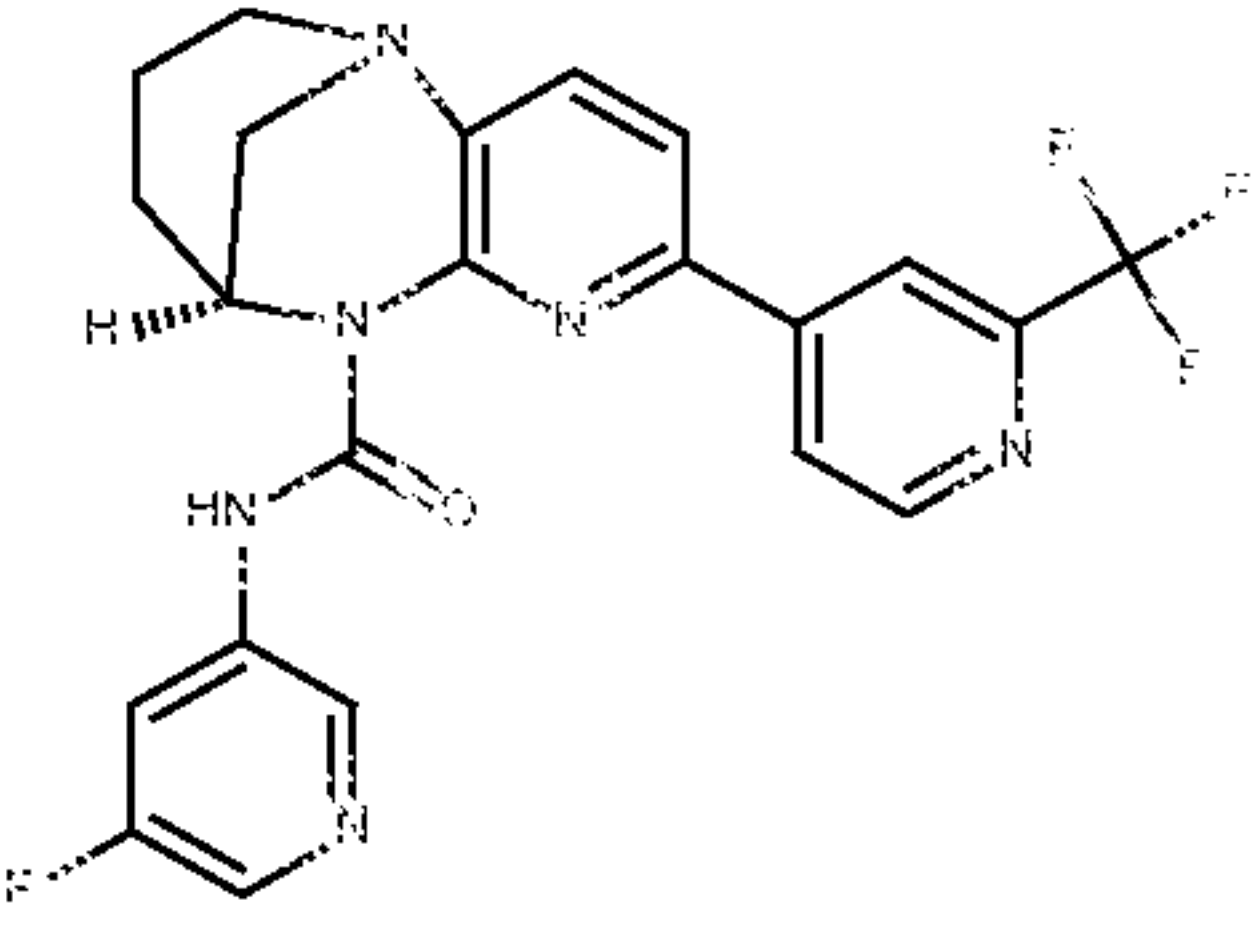
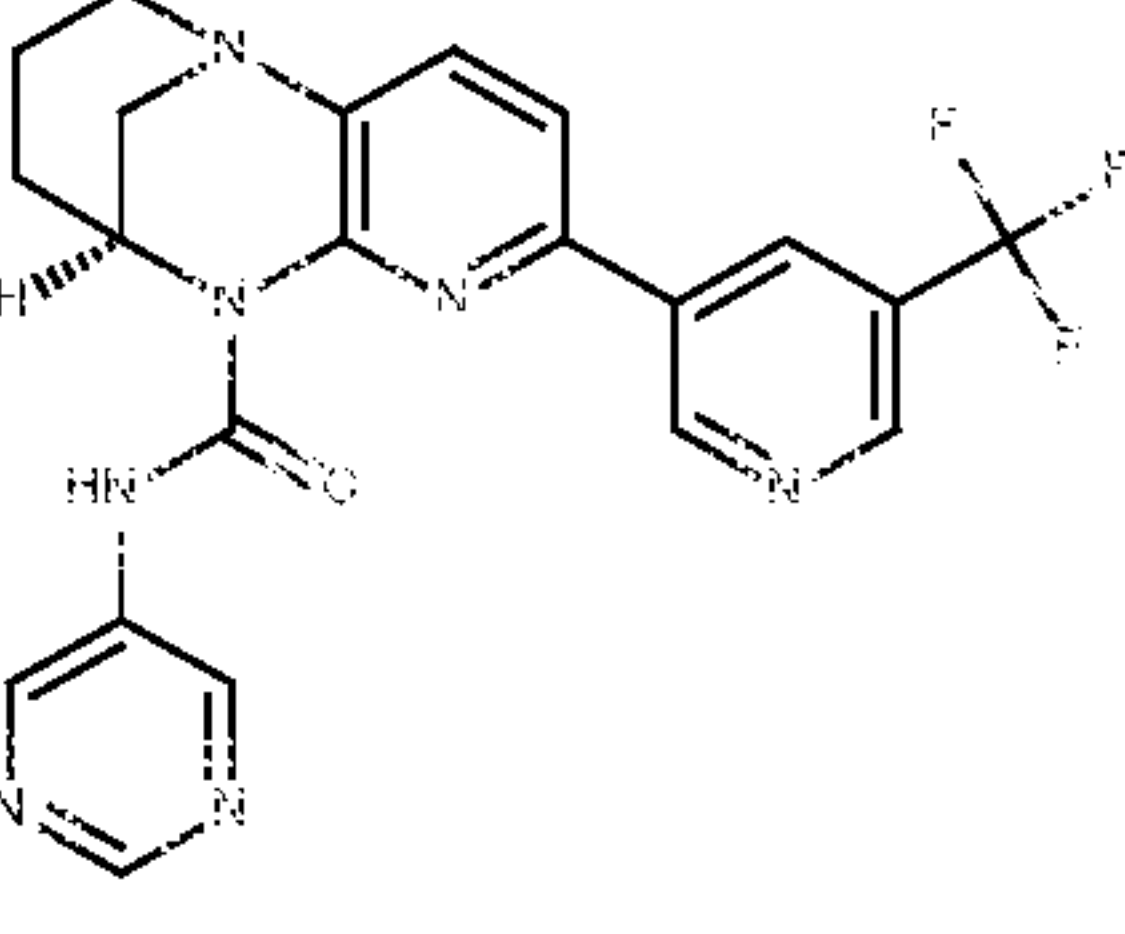
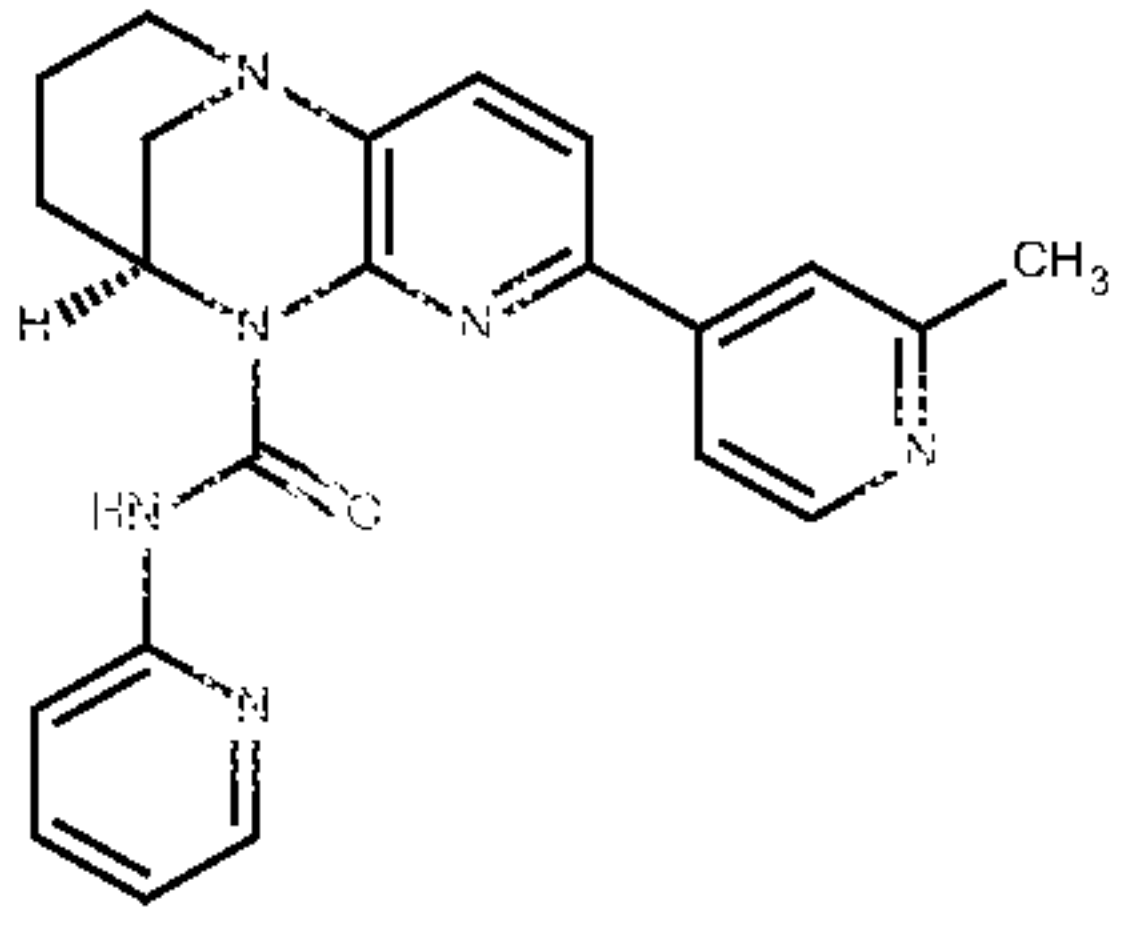
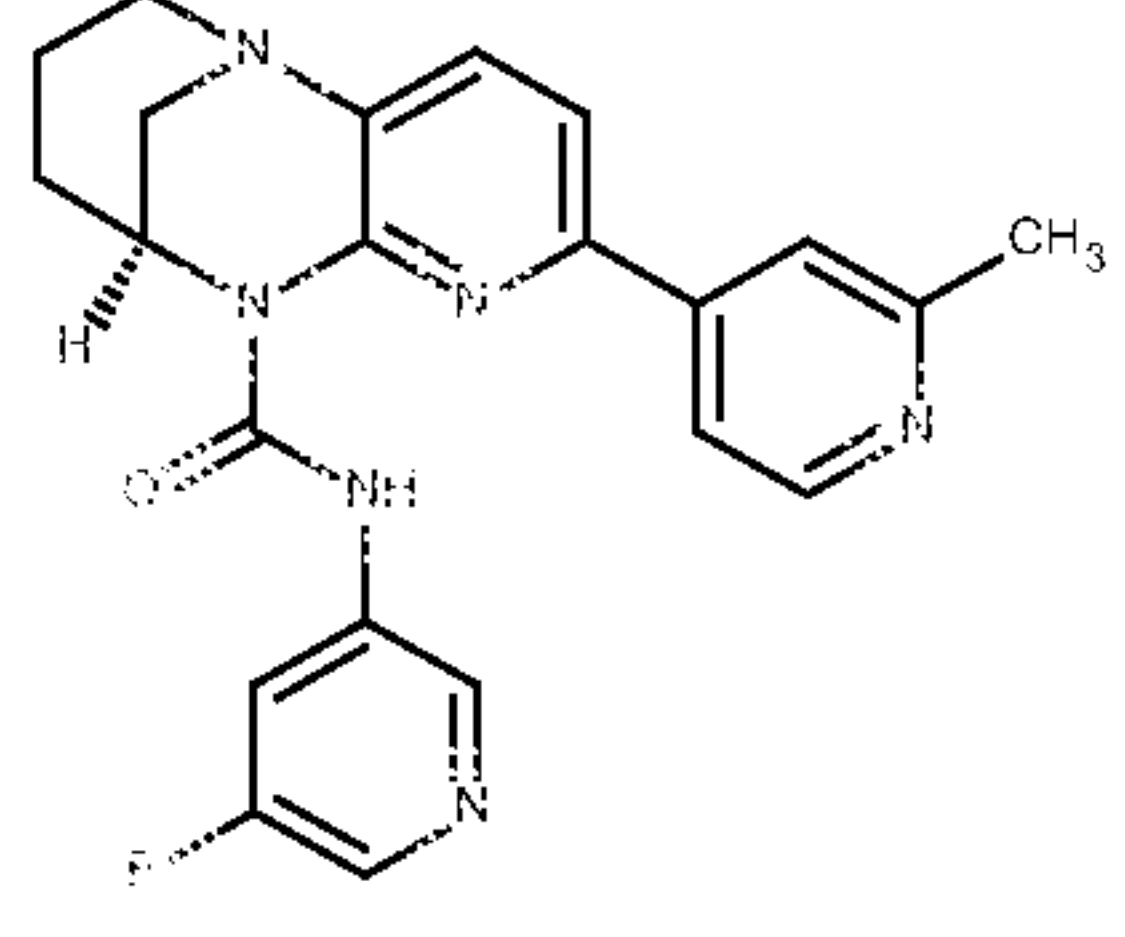
to determine the amount of deacetylation of the substrate at a given time point within the linear range of the assay. This time point was the same as that used for test compounds and, within the linear range, the endpoint represents a change in velocity.

For the above assay, SIRT1 protein was expressed and purified as follows. The
 5 SirT1 gene was cloned into a T7-promoter containing vector and transformed into BL21(DE3). The protein was expressed by induction with 1 mM IPTG as an N-terminal His-tag fusion protein at 18°C overnight and harvested at 30,000 x g. Cells were lysed with lysozyme in lysis buffer (50 mM Tris-HCl, 2 mM Tris[2-carboxyethyl] phosphine (TCEP), 10 µM ZnCl₂, 200 mM NaCl) and further treated with sonication for 10 min for complete
 10 lysis. The protein was purified over a Ni-NTA column (Amersham) and fractions containing pure protein were pooled, concentrated and run over a sizing column (Sephadex S200 26/60 global). The peak containing soluble protein was collected and run on an Ion-exchange column (MonoQ). Gradient elution (200 mM - 500 mM NaCl) yielded pure protein. This protein was concentrated and dialyzed against dialysis buffer (20 mM Tris-
 15 HCl, 2 mM TCEP) overnight. The protein was aliquoted and frozen at -80°C until further use.

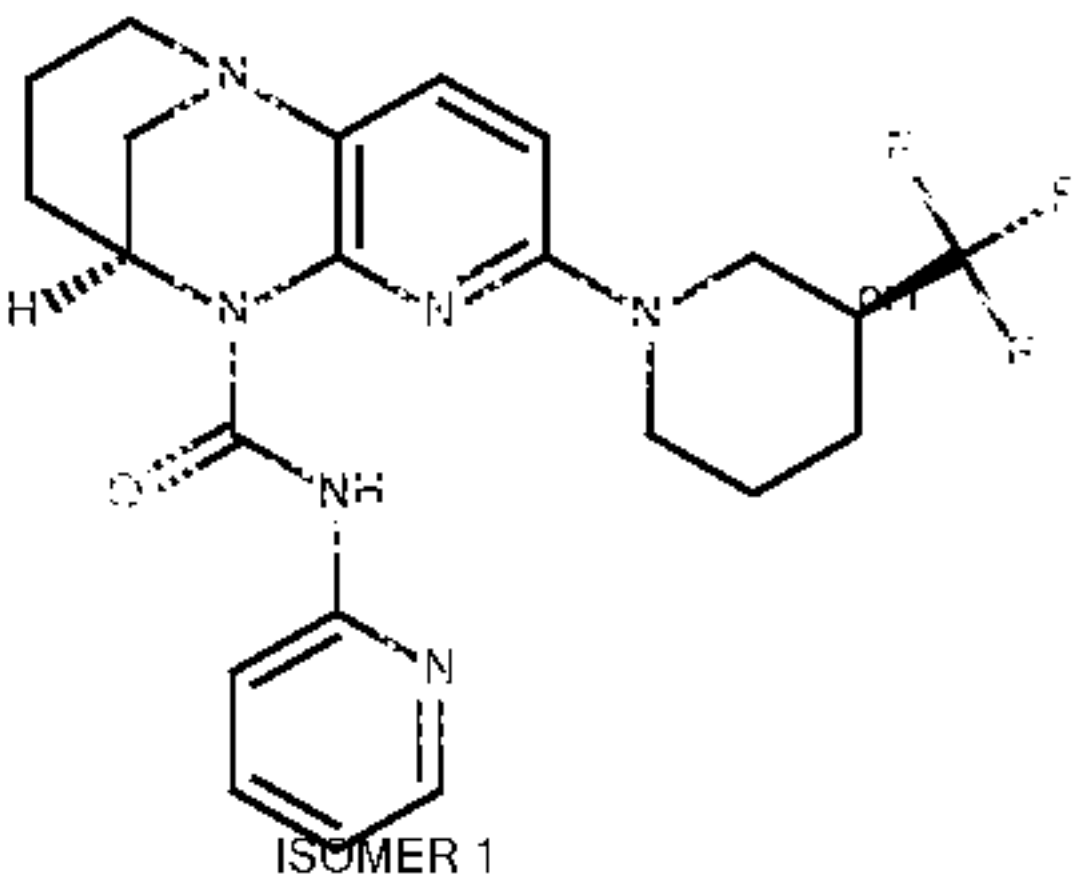
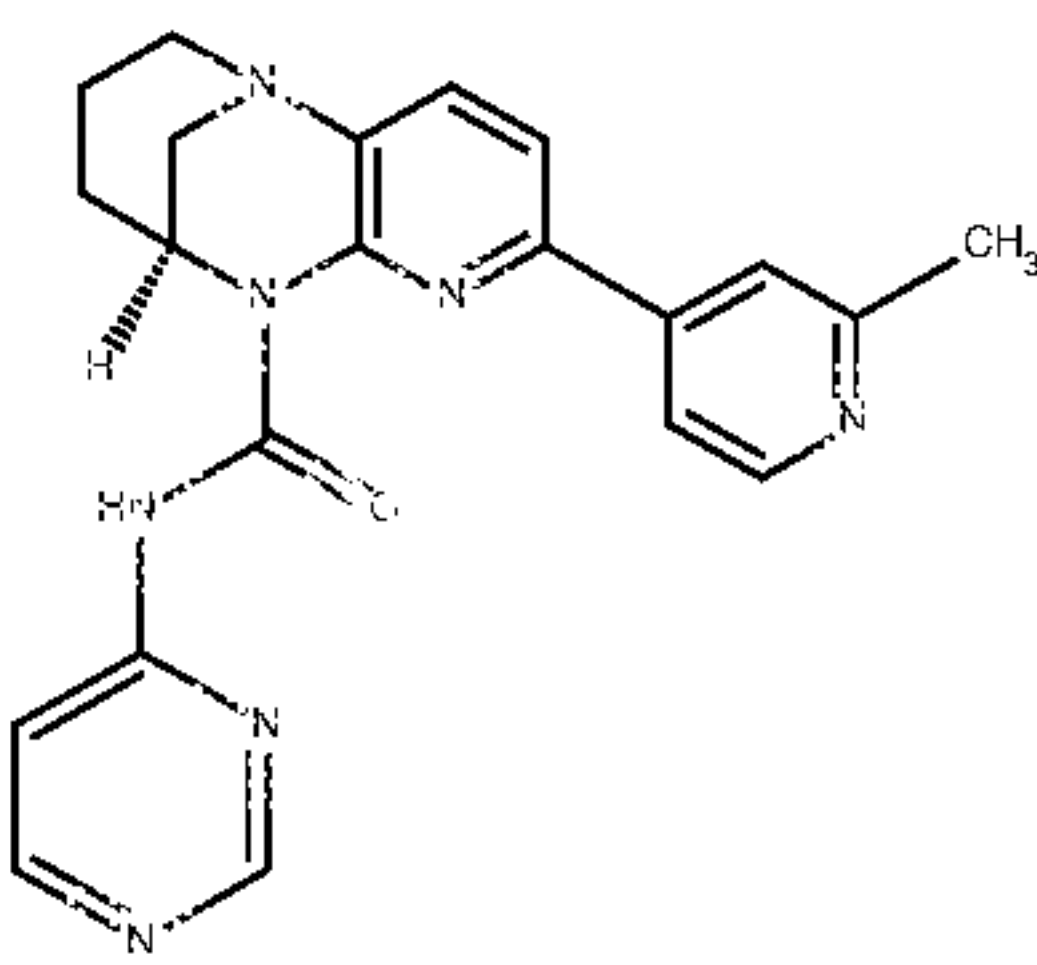
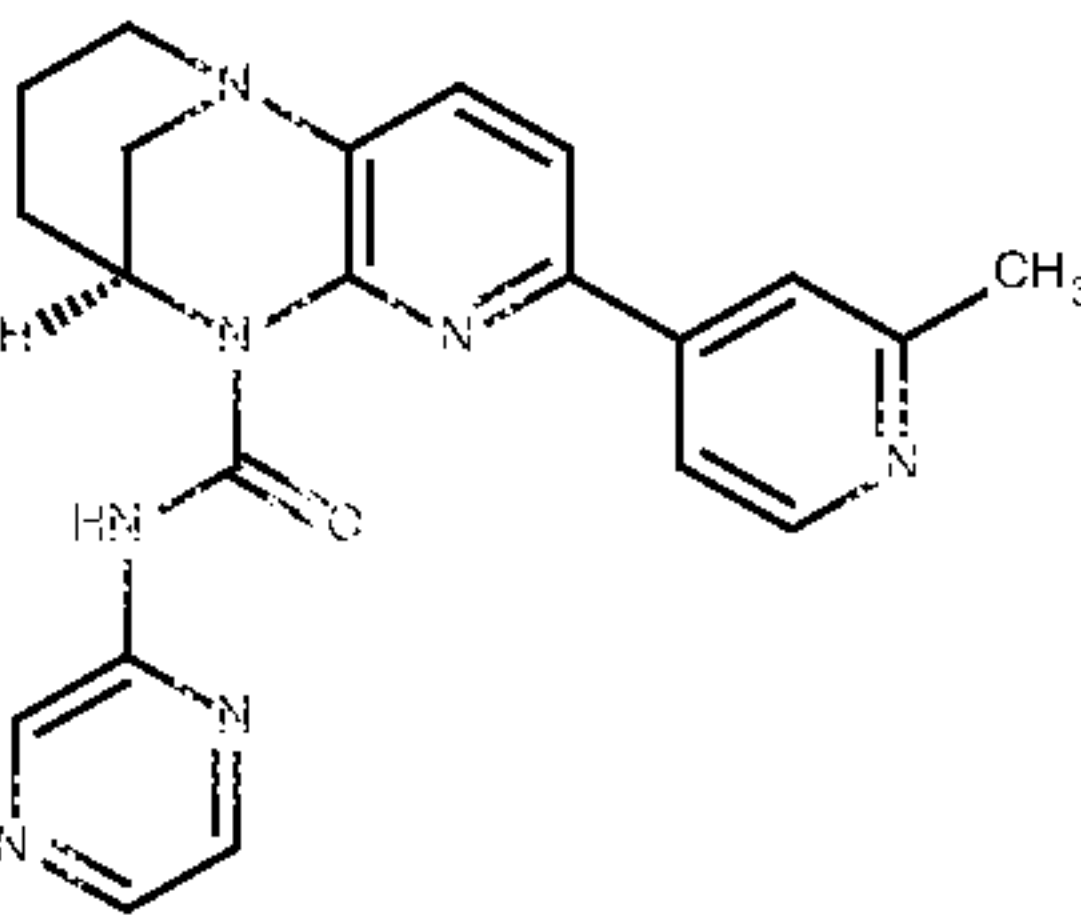
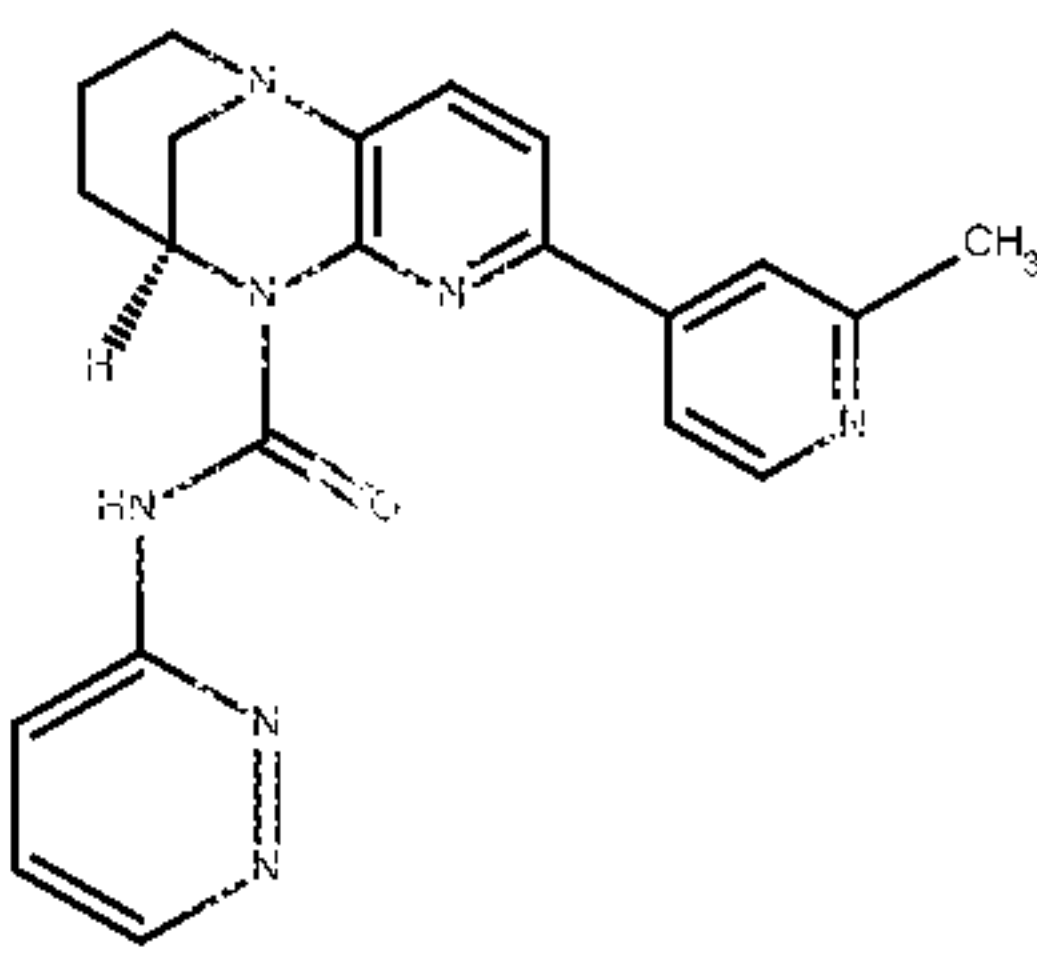
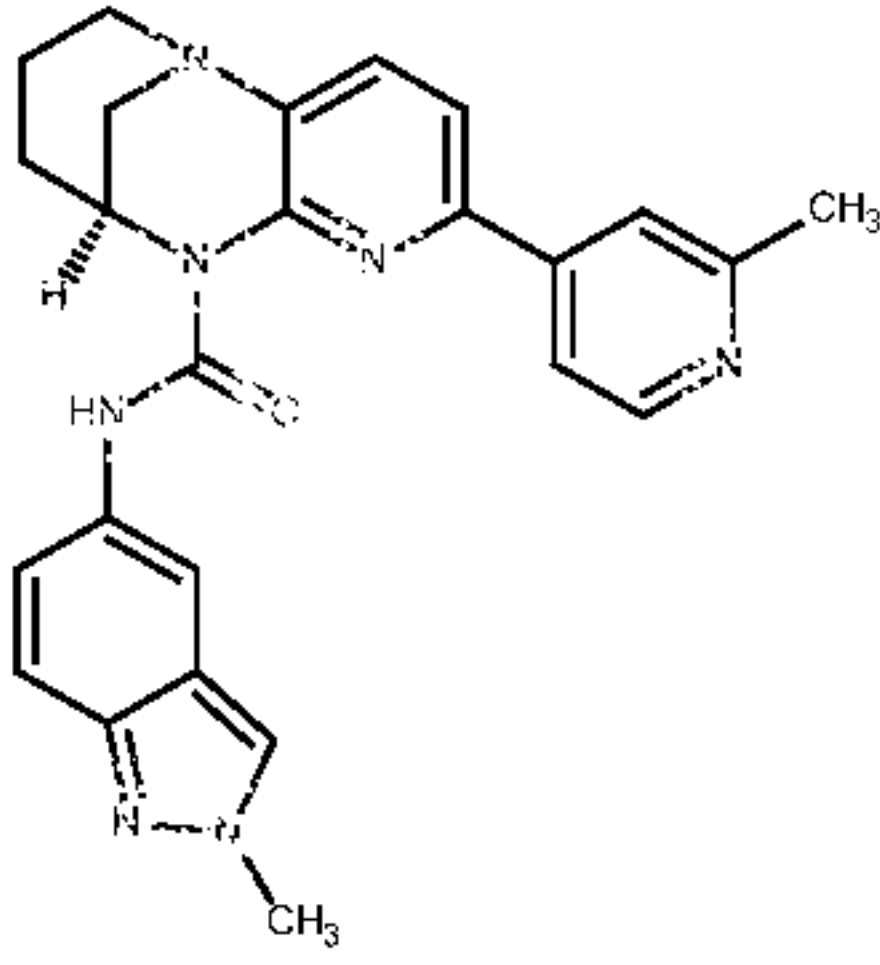
Sirtuin-modulating compounds of Formula (I) that activated SIRT1 were identified using the assay described above and are shown below in Table 1. The EC_{1.5} values represent the concentration of test compounds that result in 150% activation of SIRT1.
 20 The EC_{1.5} values for the activating compounds of Formula (I) are represented by A (EC_{1.5} <1 µM), B (EC_{1.5} 1–25 µM), C (EC_{1.5} >25 µM). The percent maximum fold activation is represented by A (Fold activation ≥150%) or B (Fold Activation <150%). “NT” means not tested; “ND” means not determinable. The compound numbering in the table starts with compound number 10, and parenthetic numbering (#) corresponding to the STAC
 25 numbering system in Figure 4 and Examples 90-106 (i.e., compound no. 68 is also STAC 1, so it is shown as 68(1), and further STACs: 546(3), 444(4), 314(5), 816(7), 76(8), and 81(9)).

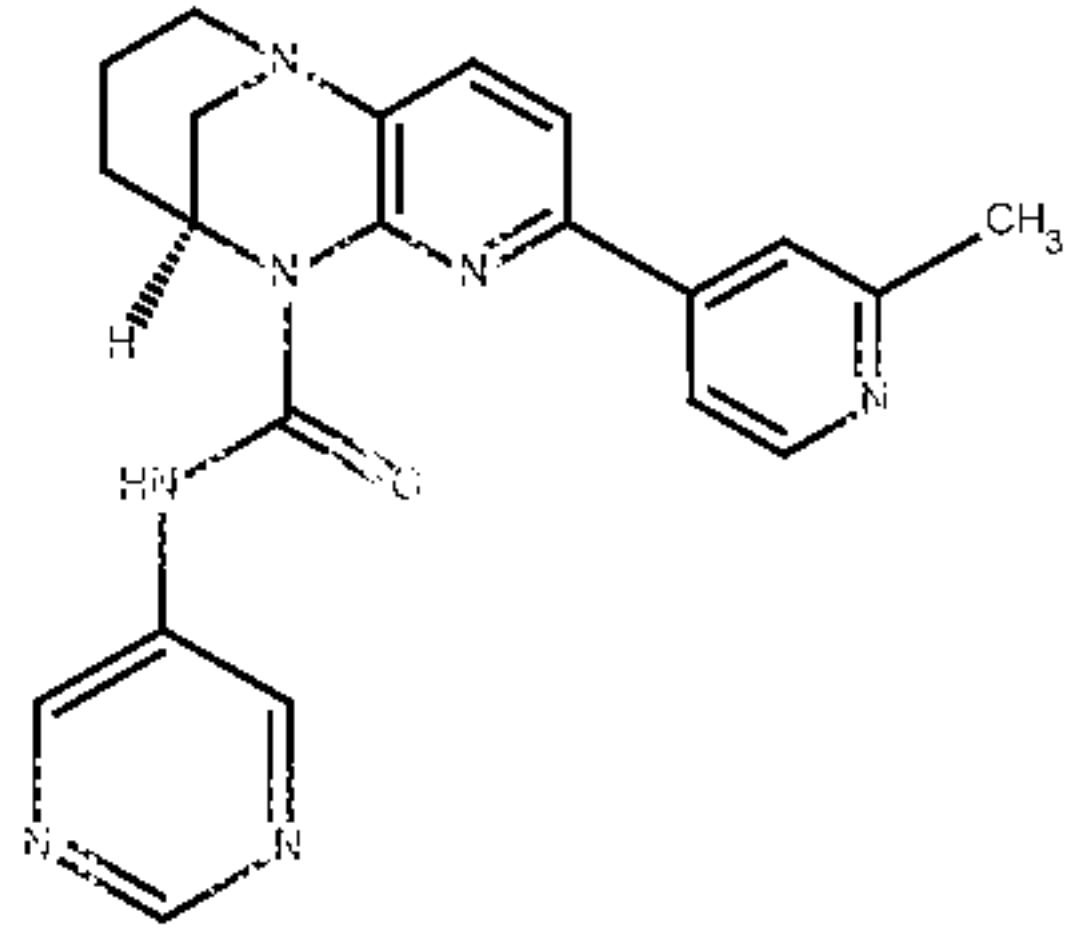
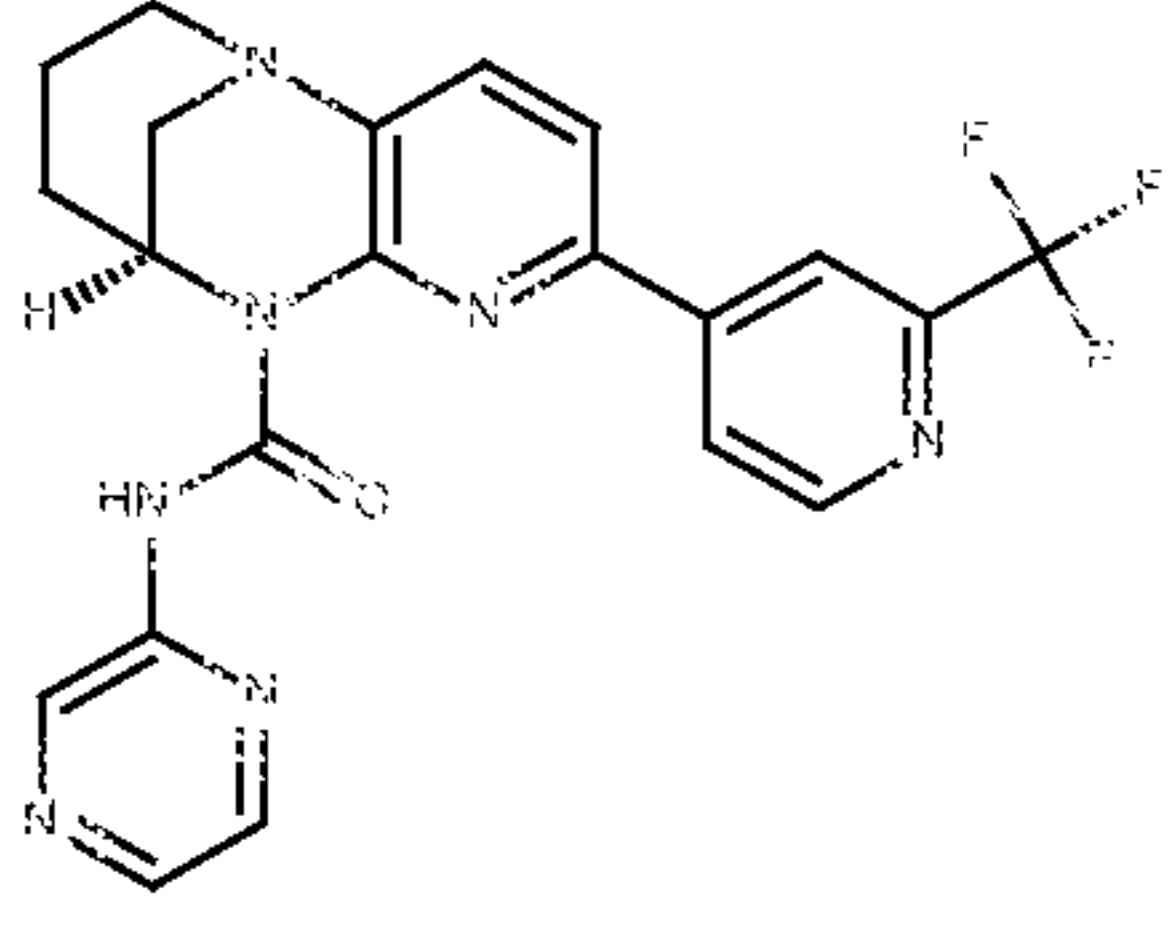
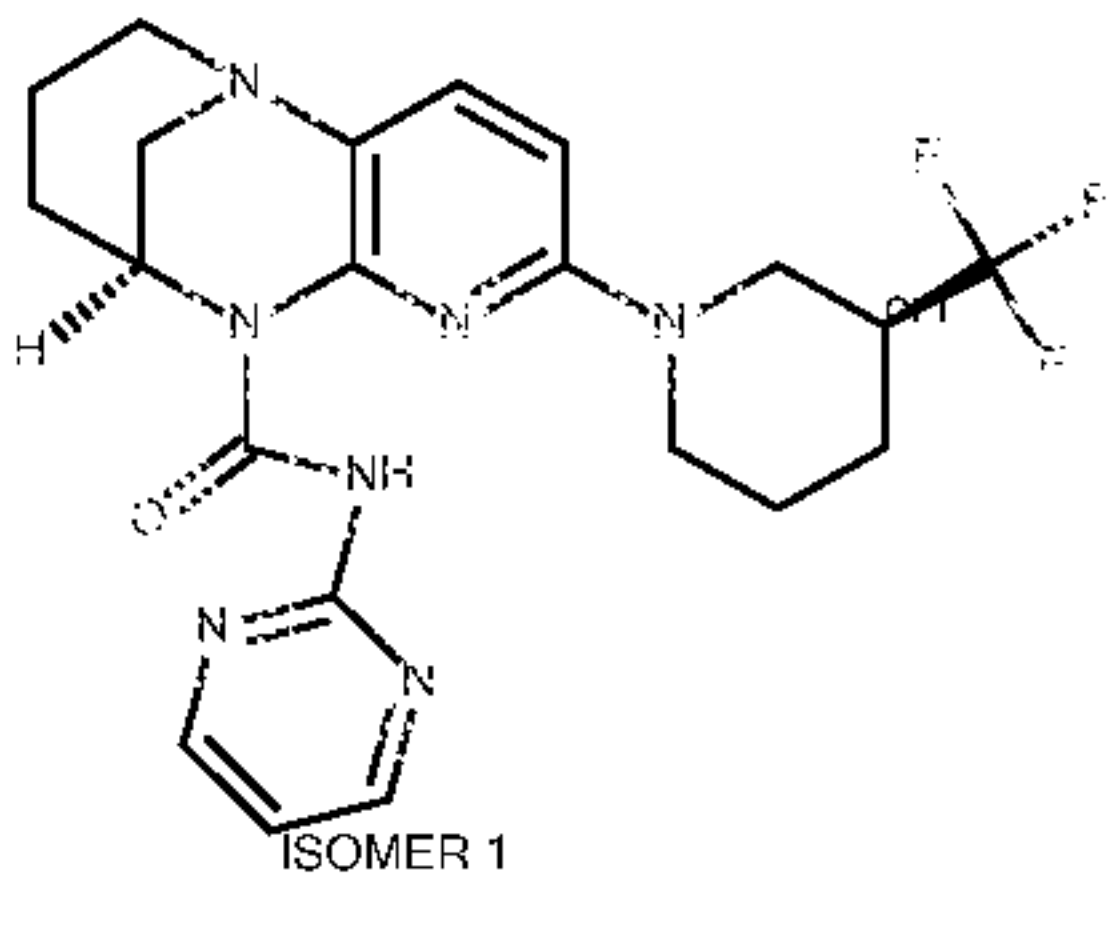
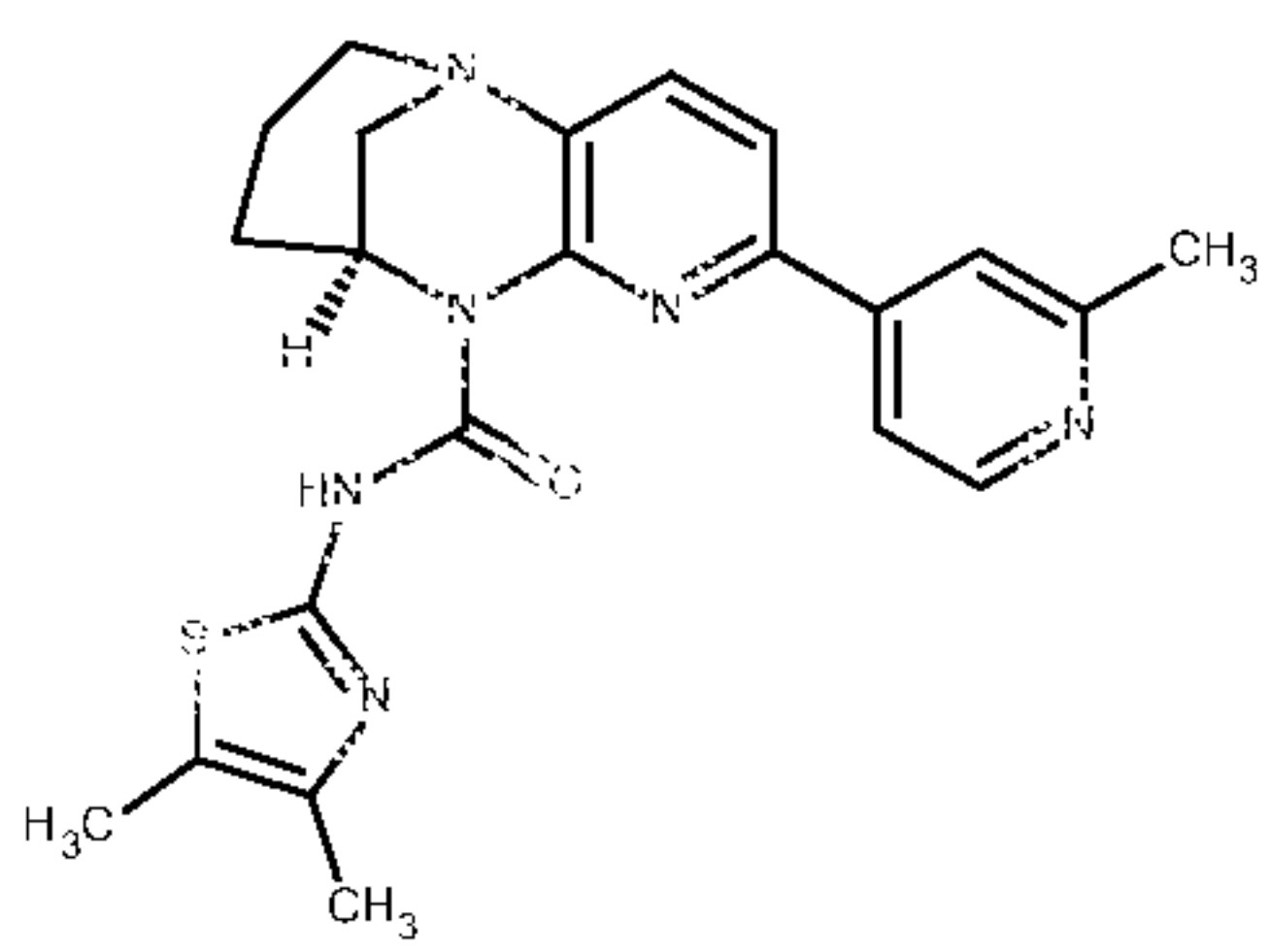
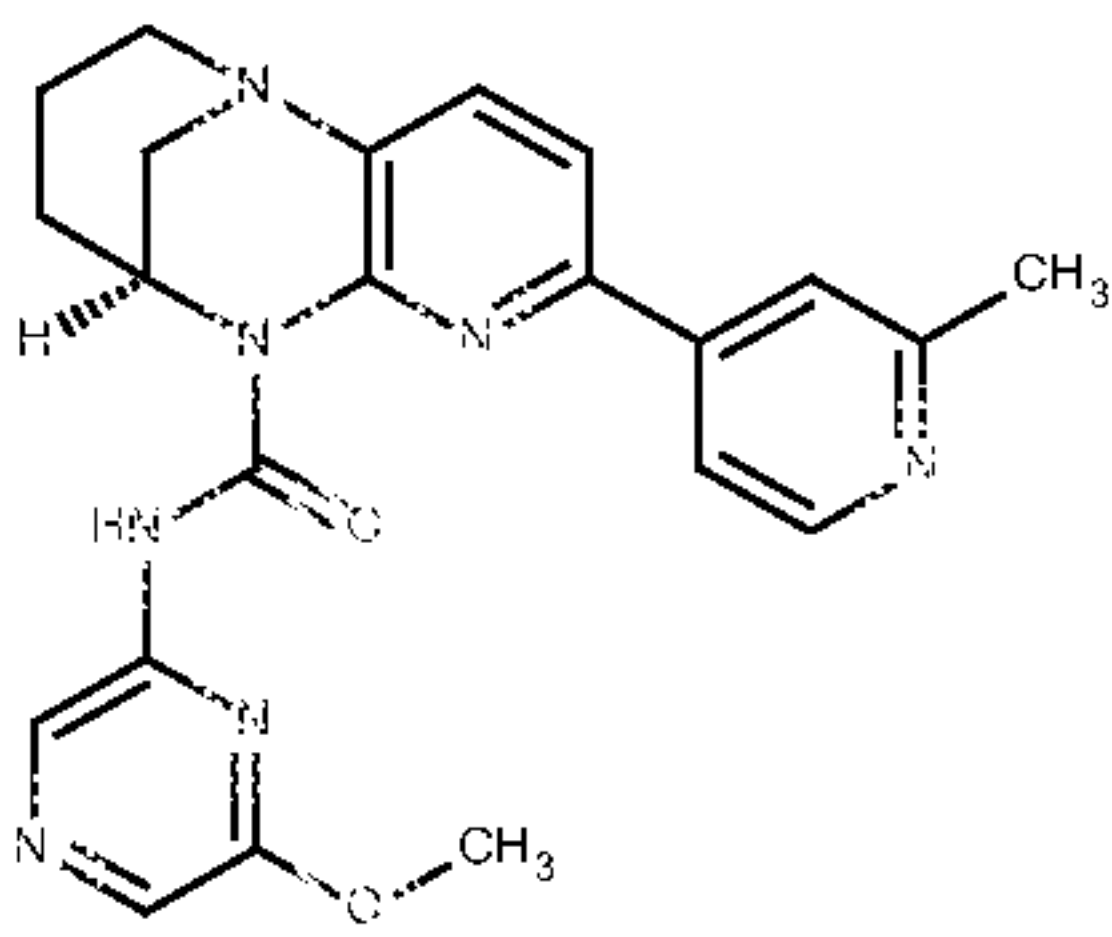
It is noted that compounds of the present invention have been named by two different chemical nomenclature conventions as generated by two different chemical drawing and/or
 30 chemical naming computer programs, i.e., generated by Chem Axon (JChem-Excel) and Cambridge Soft (ChemDraw[®]), respective companies.

TABLE 1

Example number	Structure	Chemical Name: Generated by ChEMAxon	TRP Activi ty	TRP MAX RESP
1		(9S)-N-(pyridin-2-yl)-5-[(2S)-2-(trifluoromethyl)pyrrolidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	B	A
2		(9S)-N-(5-fluoropyridin-3-yl)-5-[2-(trifluoromethyl)pyridin-4-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
3		(9S)-N-(pyrimidin-5-yl)-5-[5-(trifluoromethyl)pyridin-3-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
4		(9S)-5-(2-methylpyridin-4-yl)-N-(pyridin-2-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
5		(9S)-N-(5-fluoropyridin-3-yl)-5-(2-methylpyridin-4-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2,4,6-triene-8-carboxamide	A	A

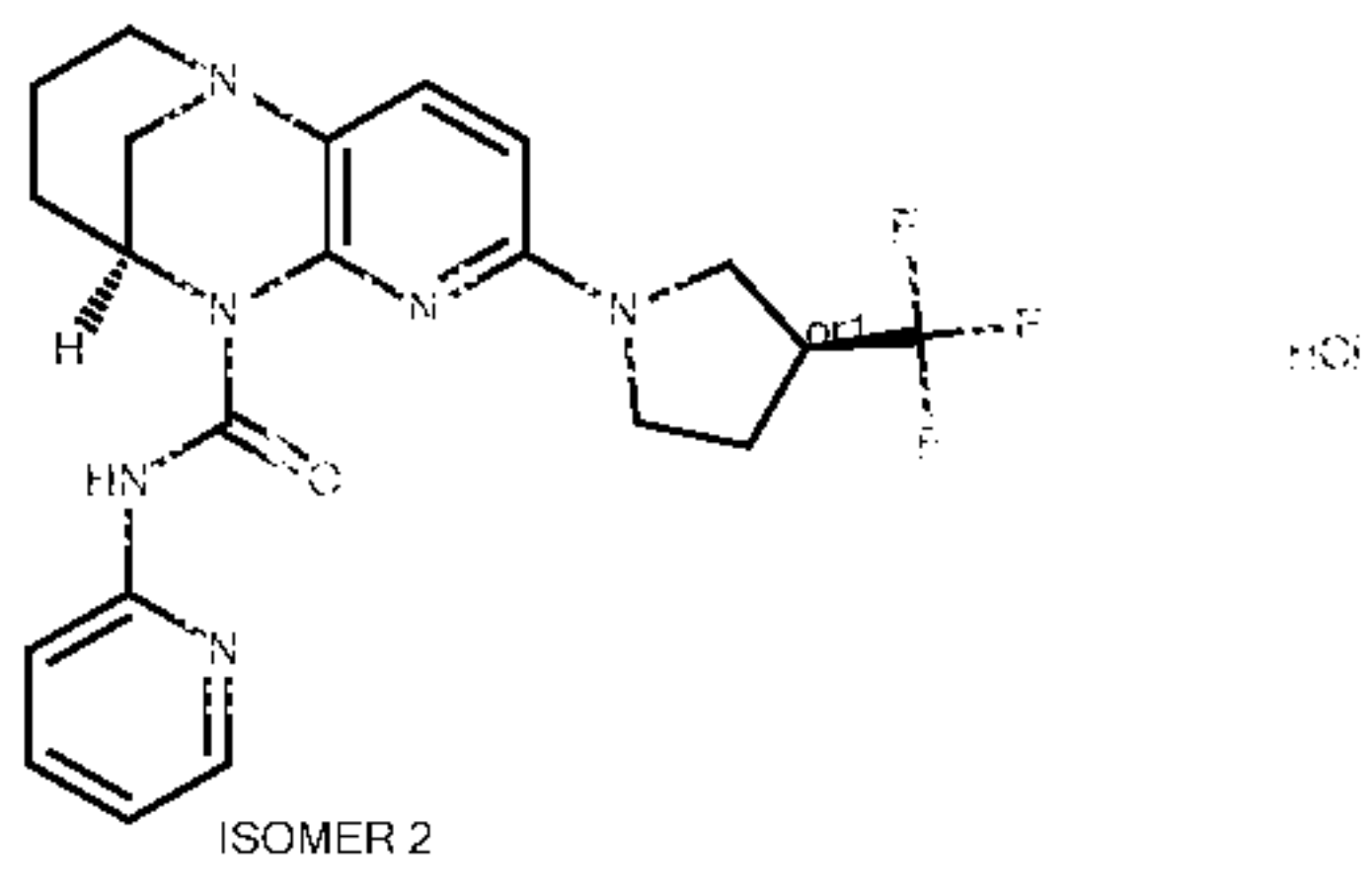
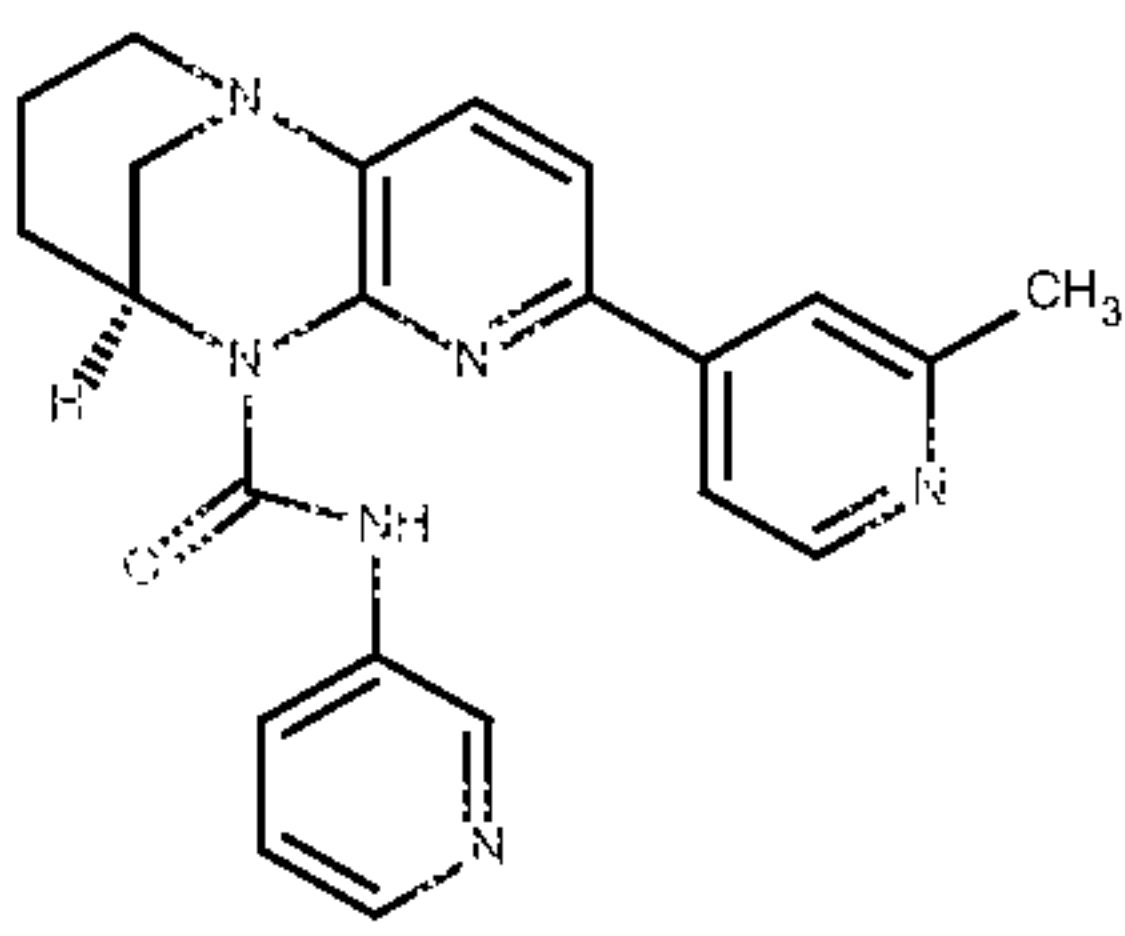
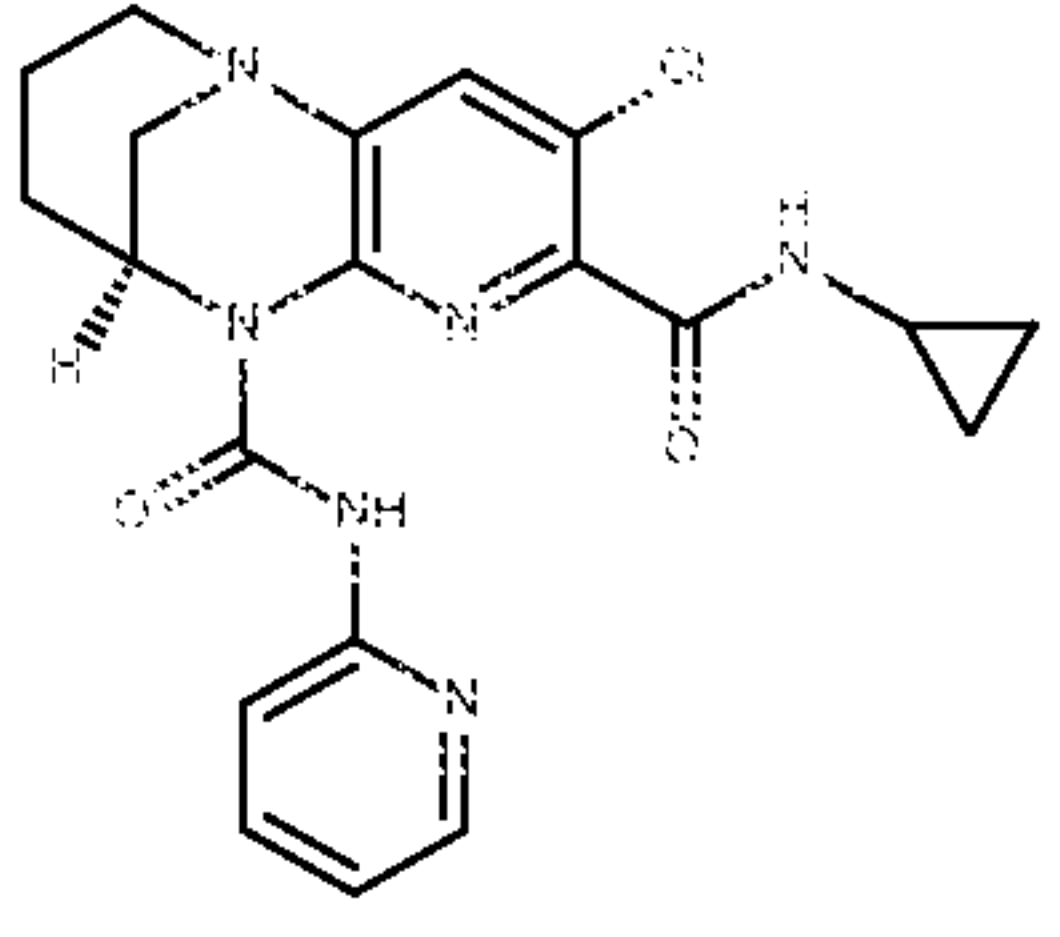
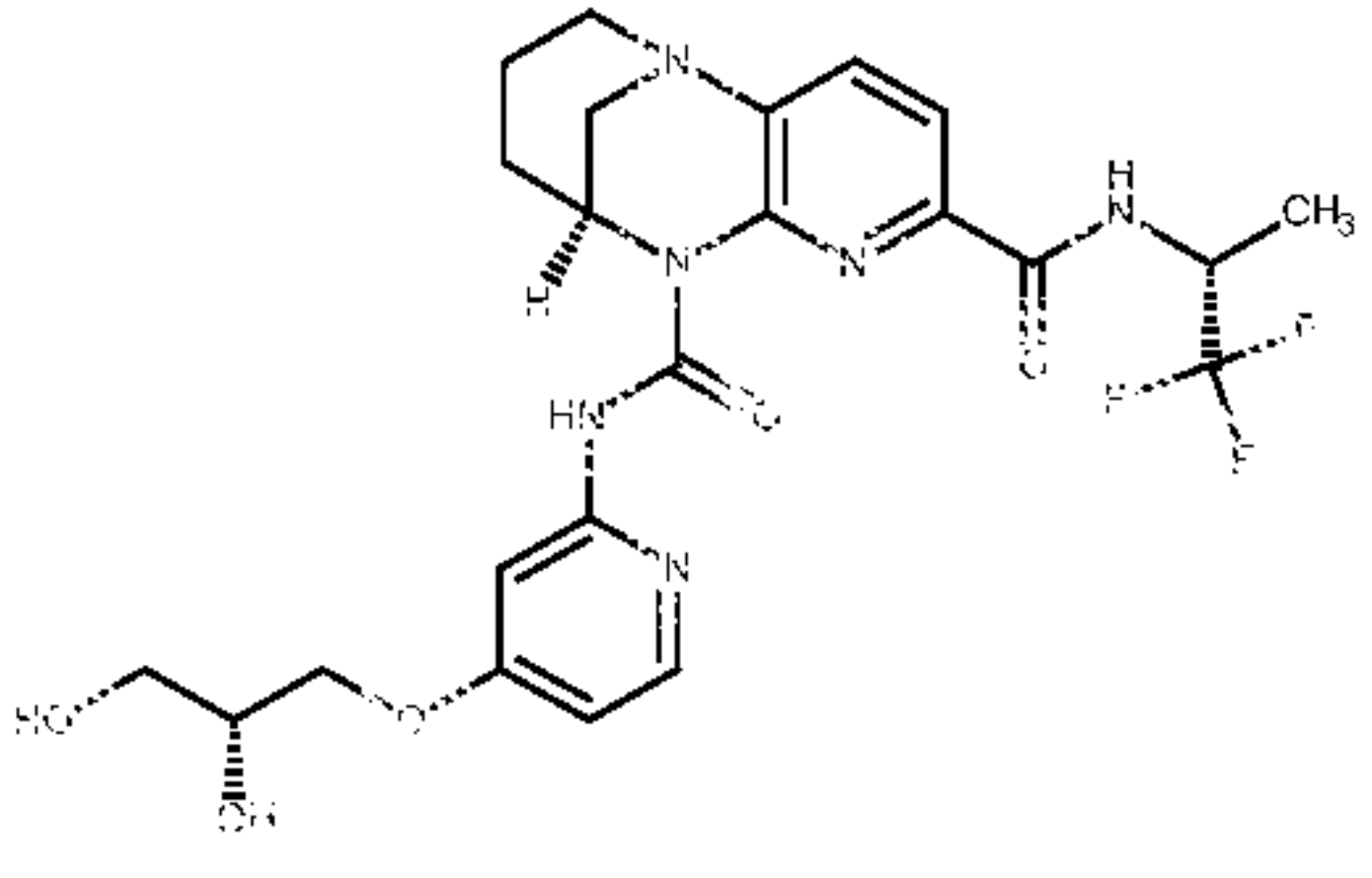
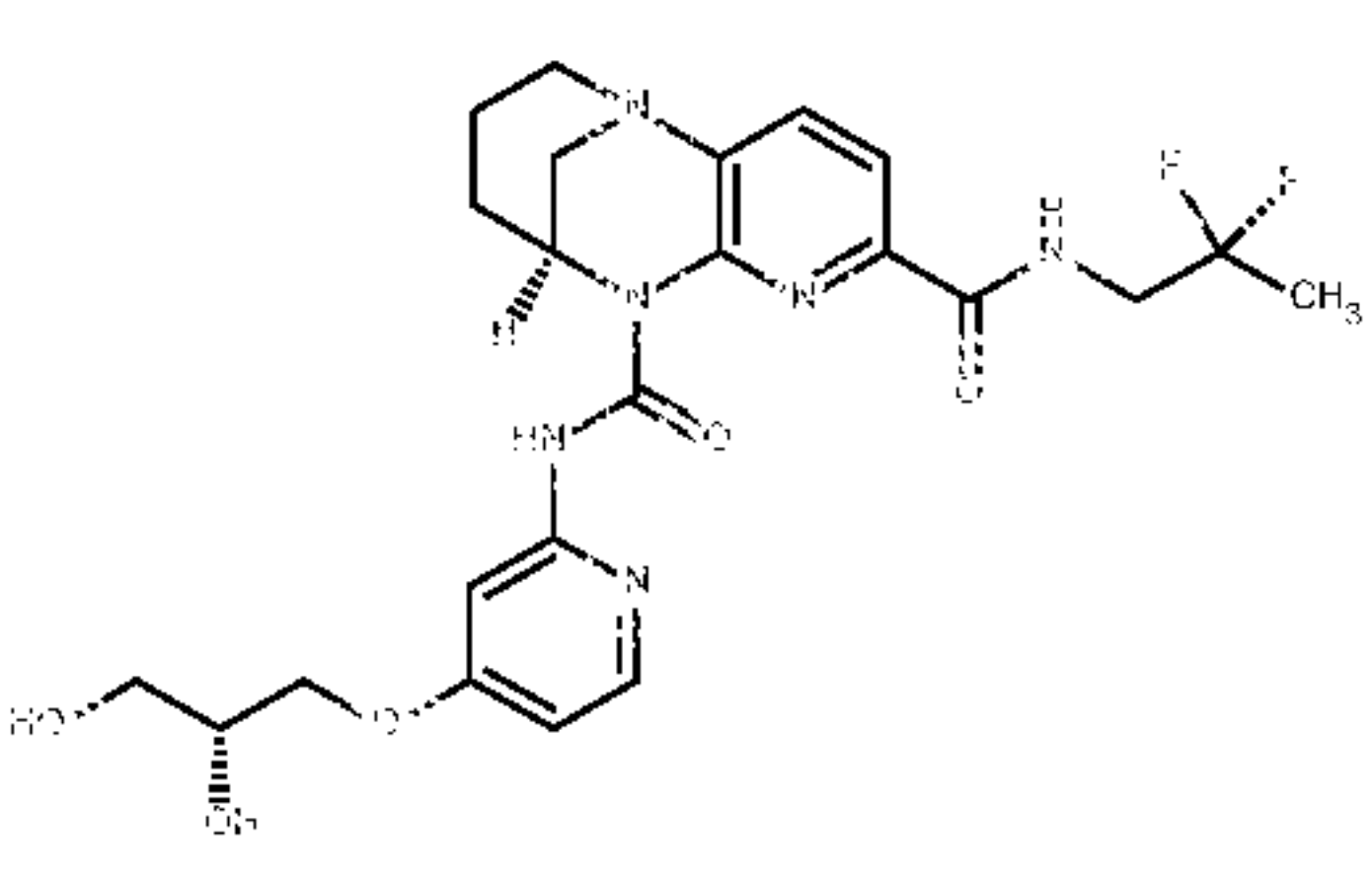
Example number	Structure	Chemical Name: Generated by CHemAxon	TRP Acti ty	TRP MAX RESP
6		(9S)-N-(pyridazin-3-yl)-5-[2-(trifluoromethyl)pyridin-4-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
7		(9S)-N-(pyridin-2-yl)-5-[2-(trifluoromethyl)pyridin-4-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
8		(9S)-N-(pyridin-2-yl)-5-[(3S)-3-(trifluoromethyl)pyrrolidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	B	A
9		(9S)-N-(pyrimidin-4-yl)-5-[2-(trifluoromethyl)pyridin-4-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
10		(9S)-N-(pyrimidin-5-yl)-5-[2-(trifluoromethyl)pyridin-4-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A

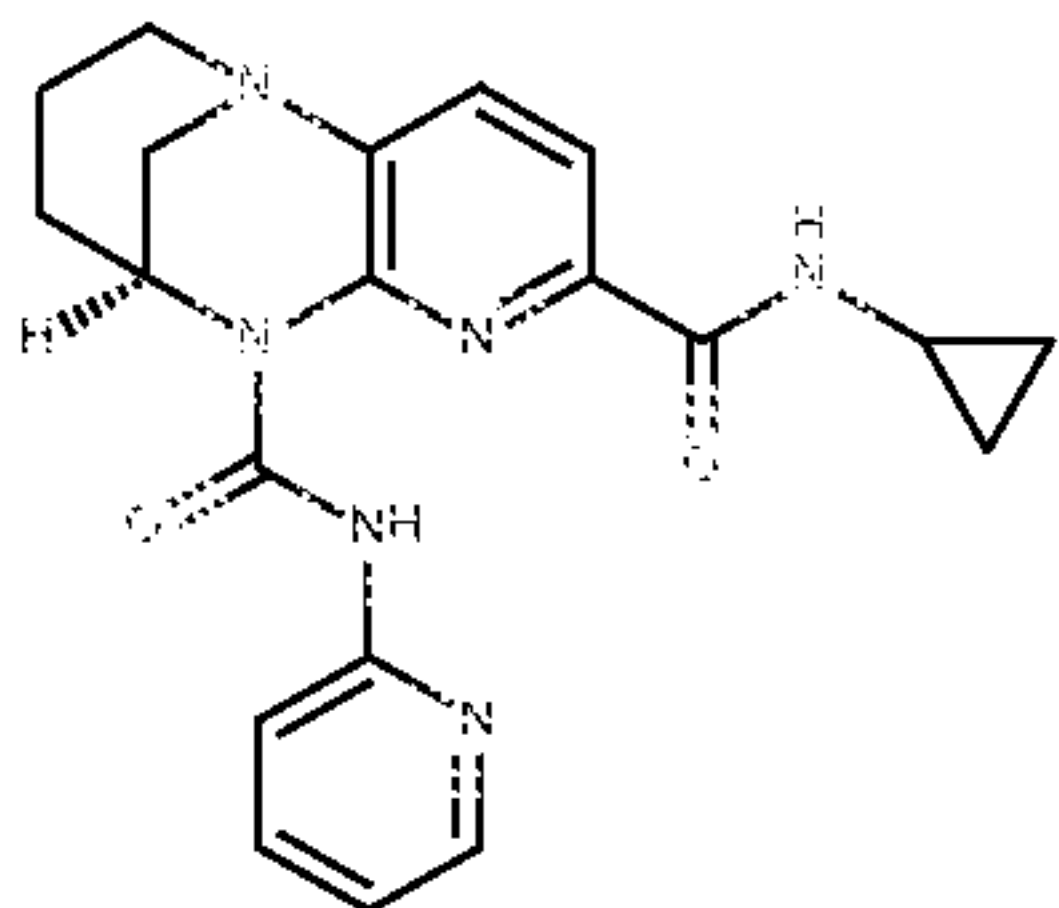
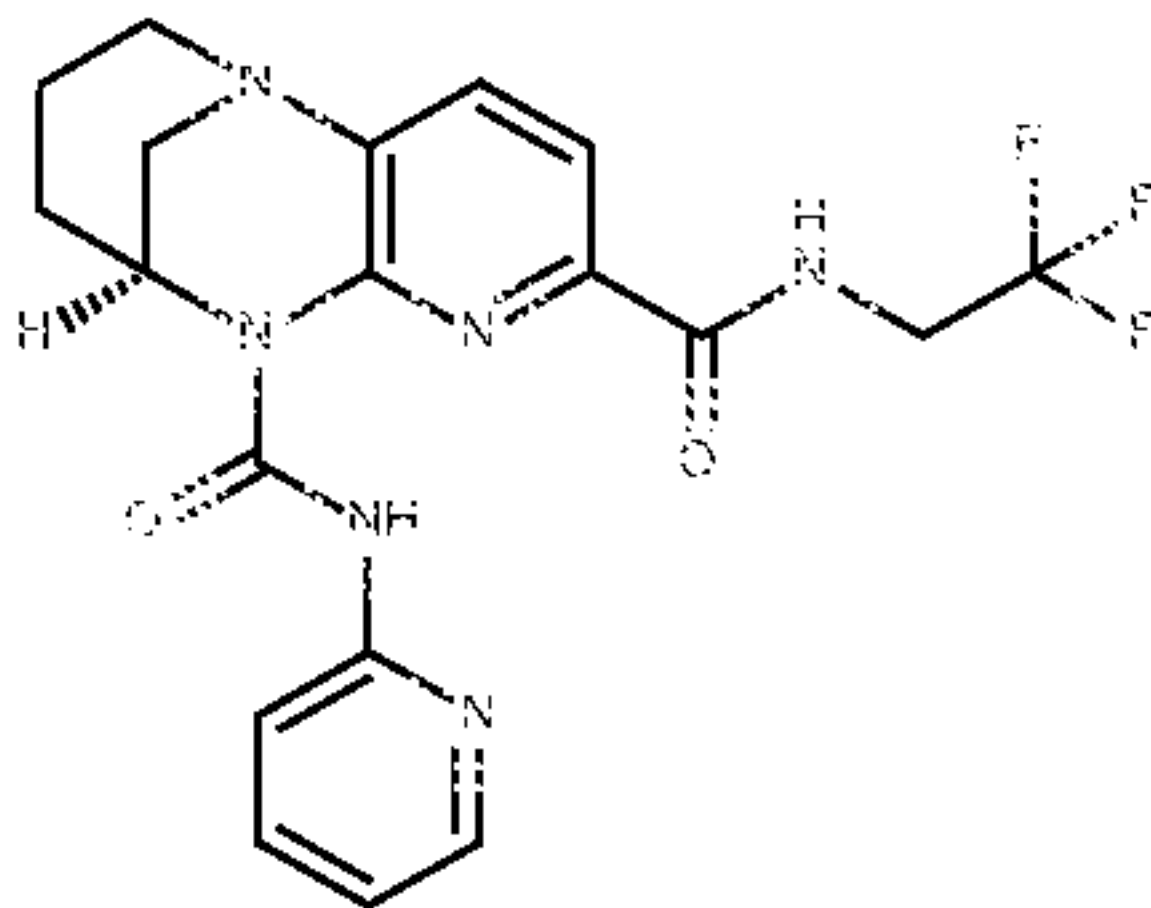
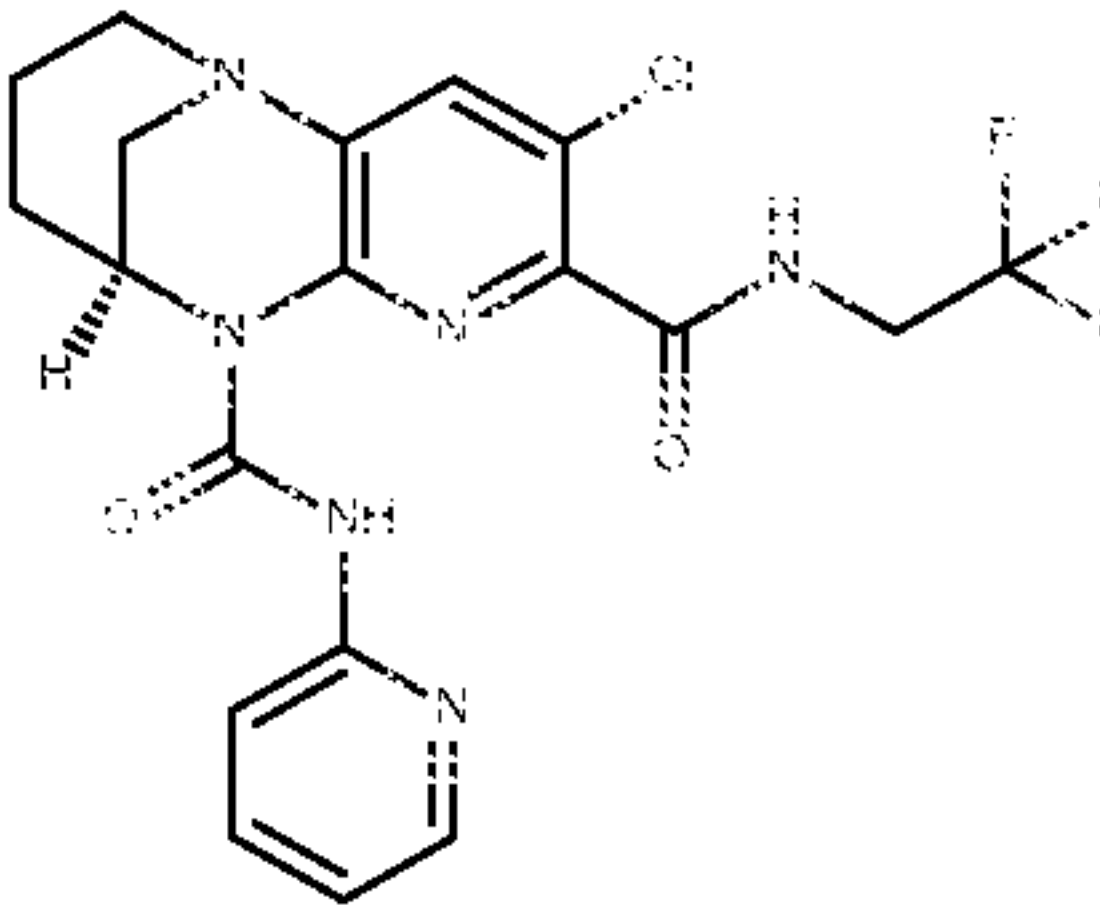
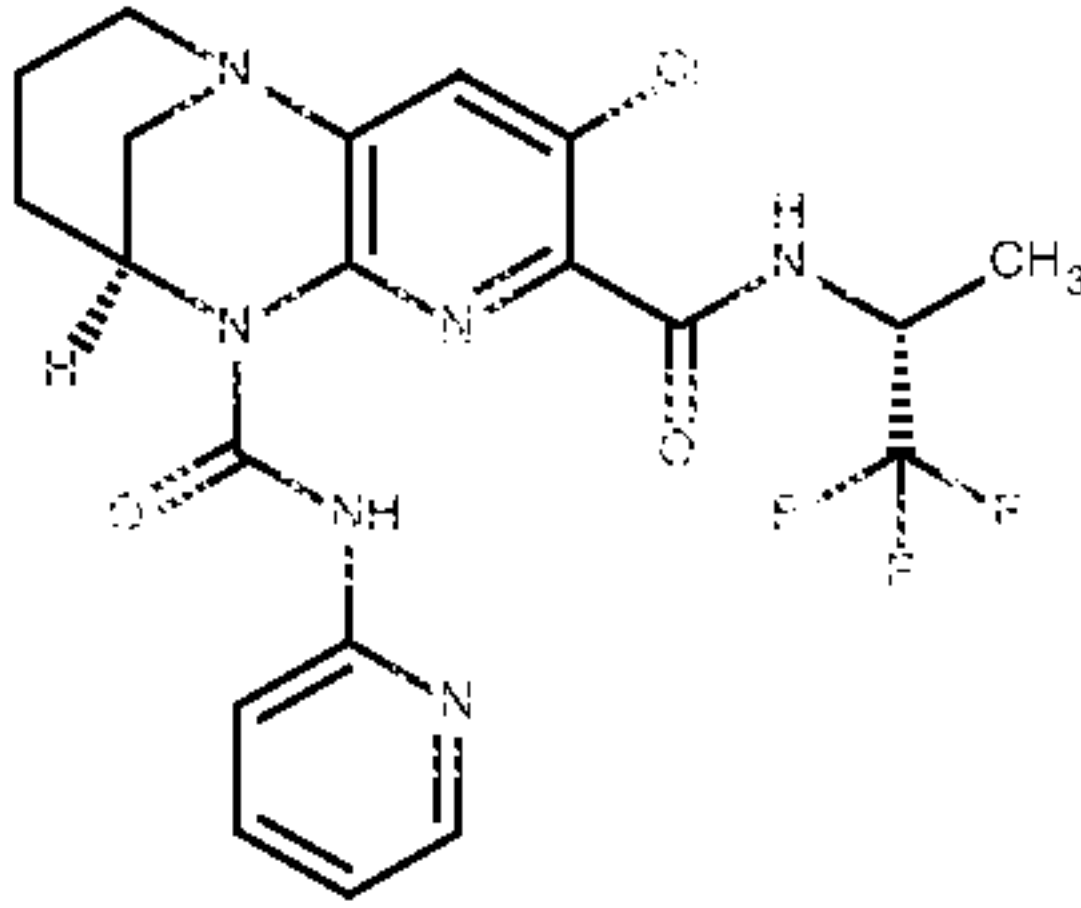
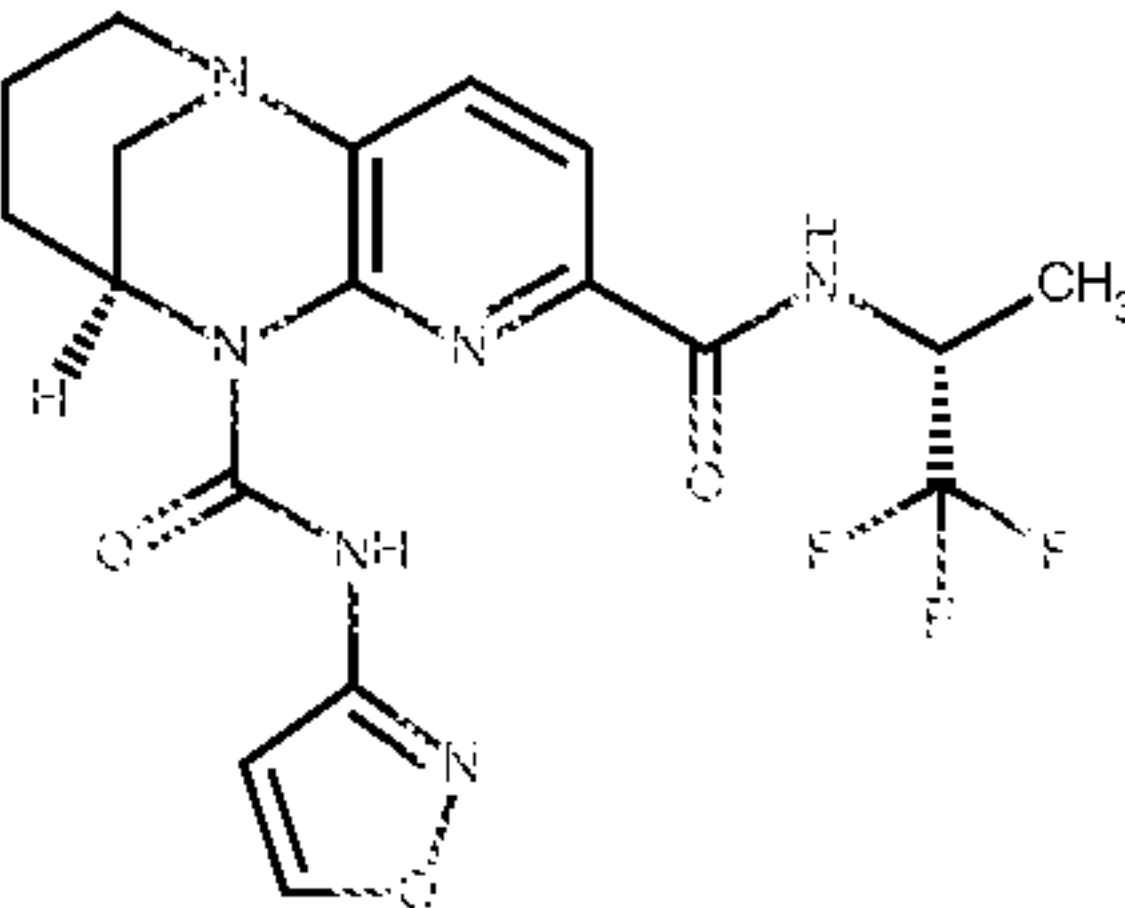
Example number	Structure	Chemical Name: Generated by CHemAxon	TRP Acti ty	TRP MAX RESP
11		(9S)-N-(pyridin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
12		(9S)-5-(2-methylpyridin-4-yl)-N-(pyrimidin-4-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
13		(9S)-5-(2-methylpyridin-4-yl)-N-(pyrazin-2-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
14		(9S)-5-(2-methylpyridin-4-yl)-N-(pyridazin-3-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
15		(9S)-N-(2-methyl-2H-indazol-5-yl)-5-(2-methylpyridin-4-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2,4,6-triene-8-carboxamide	A	A

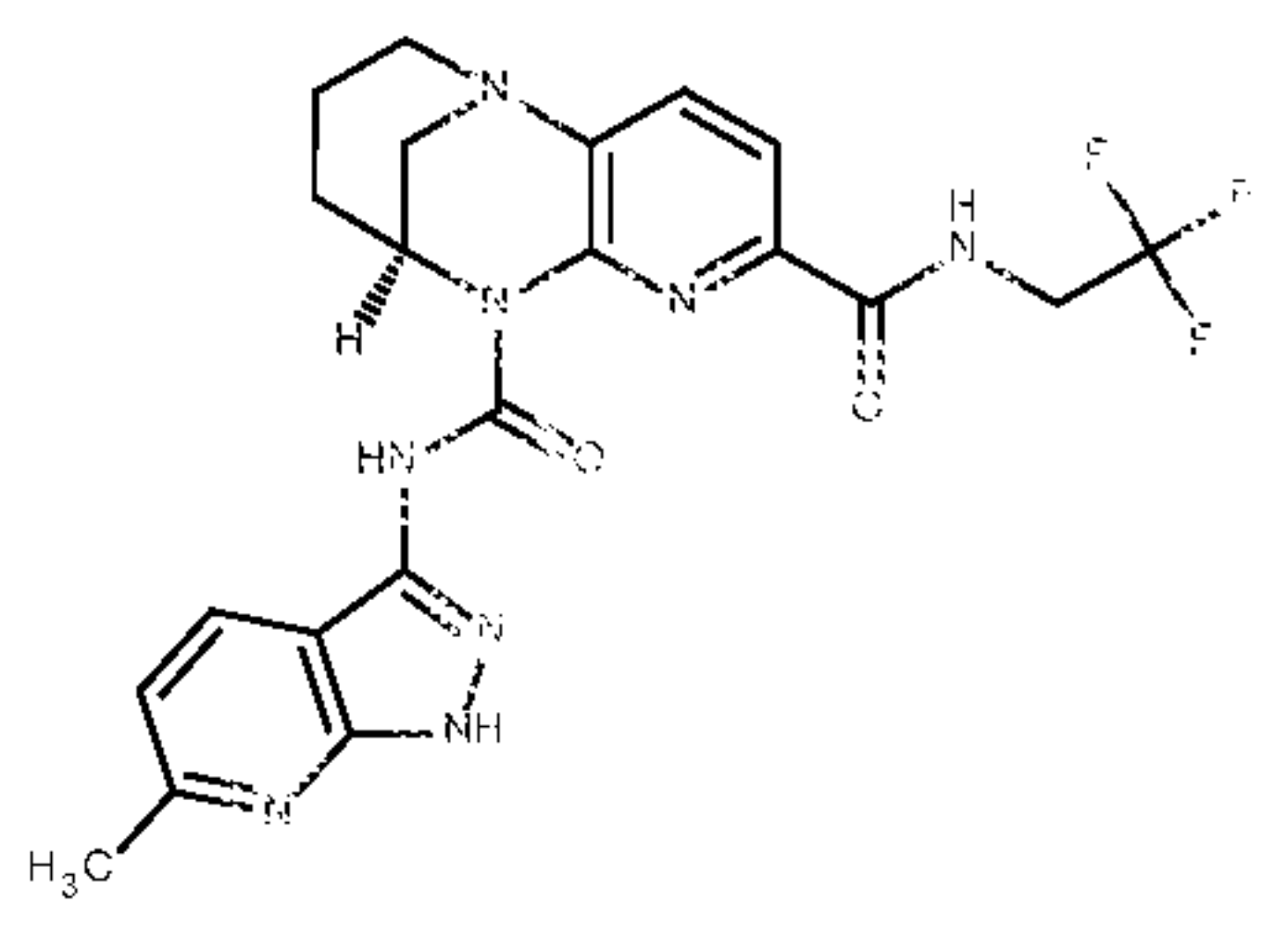
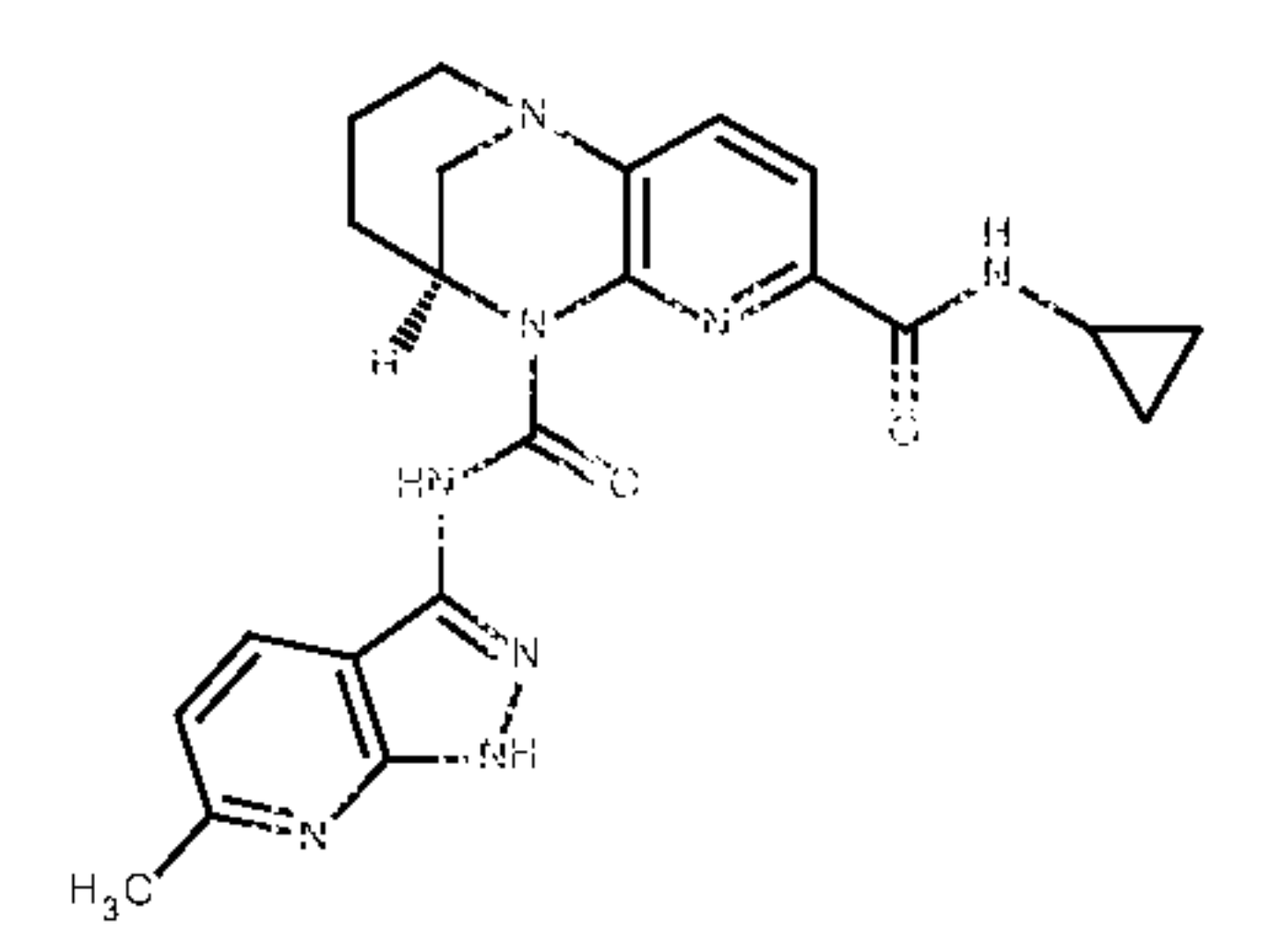
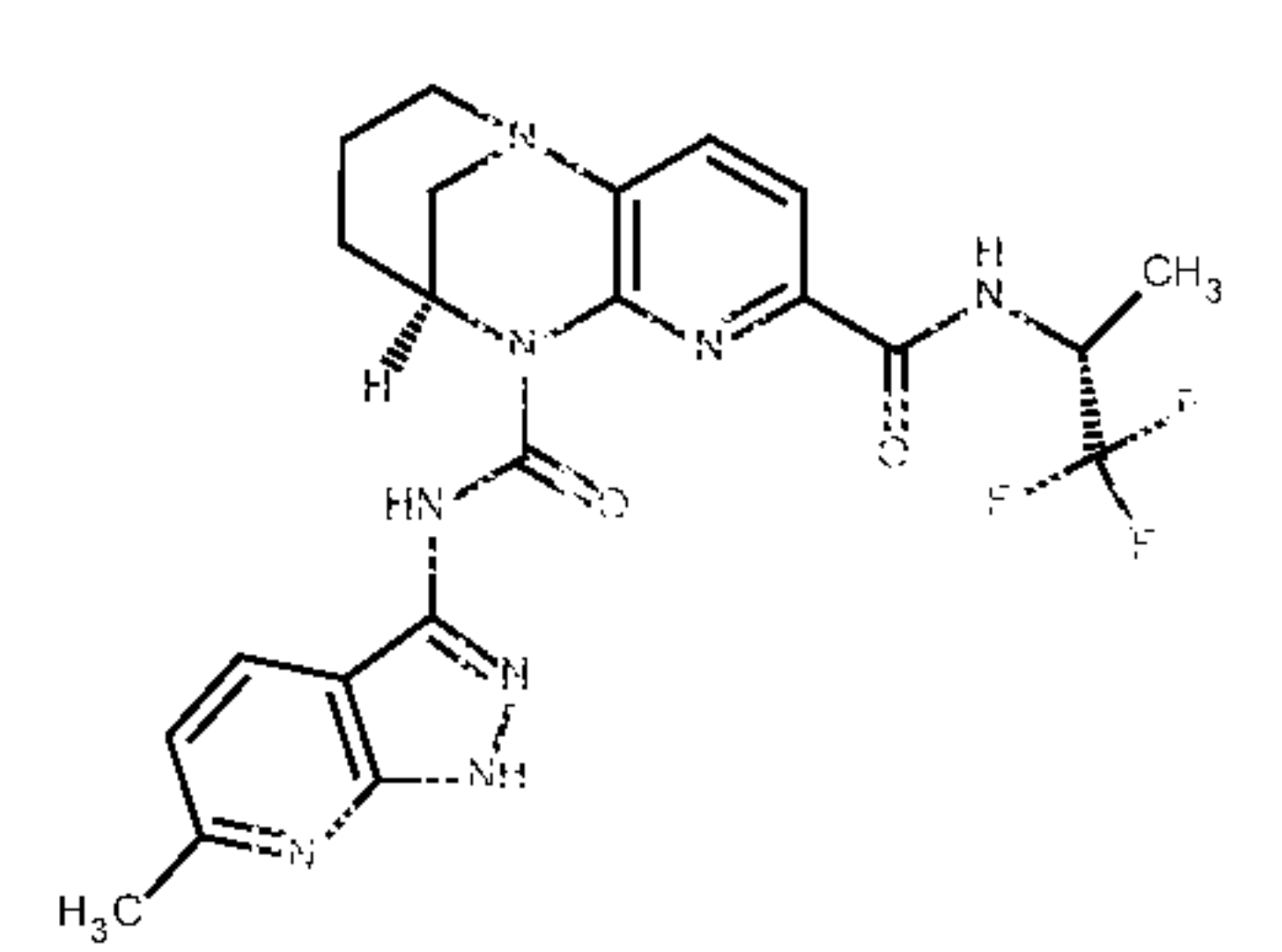
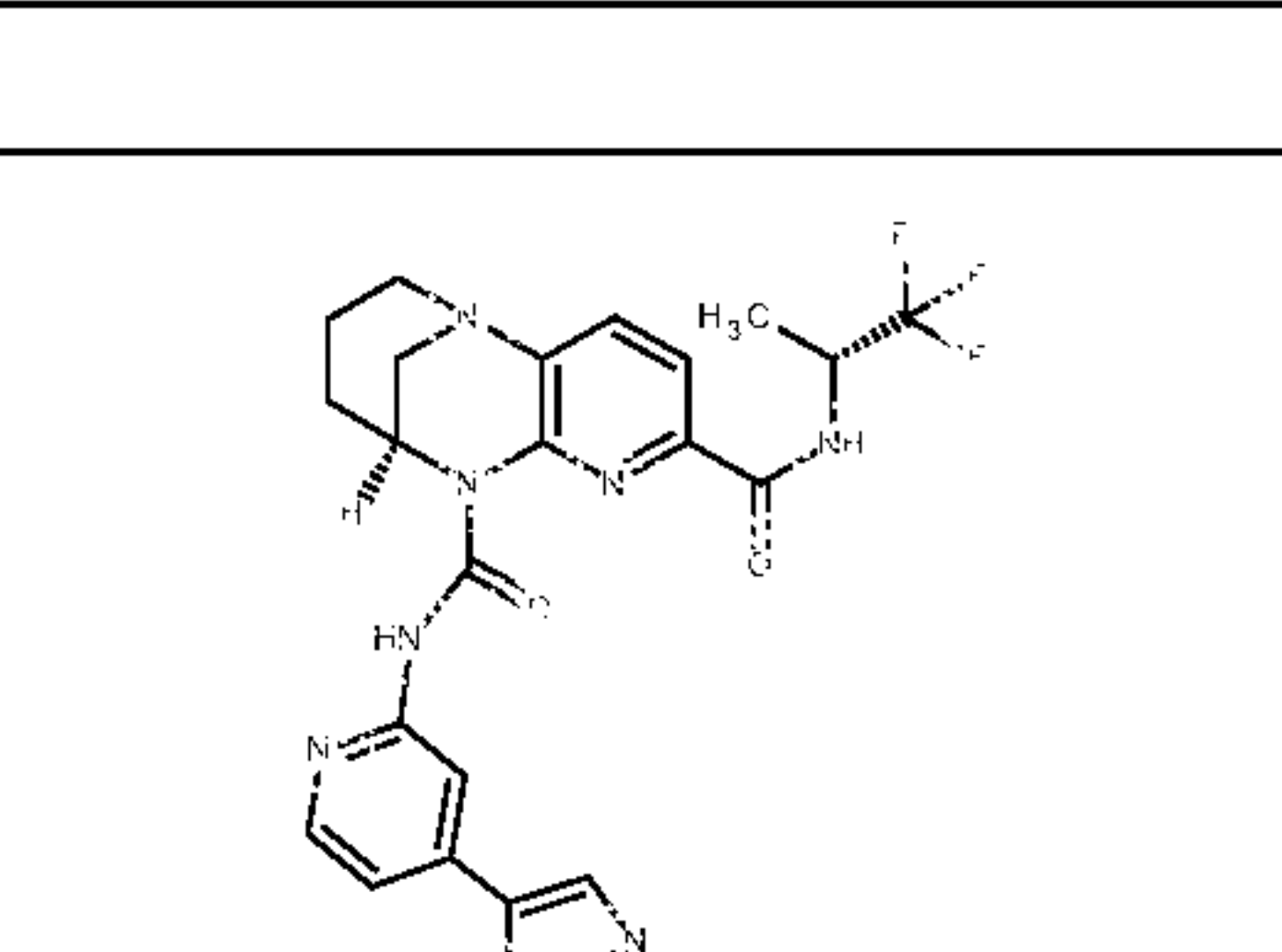
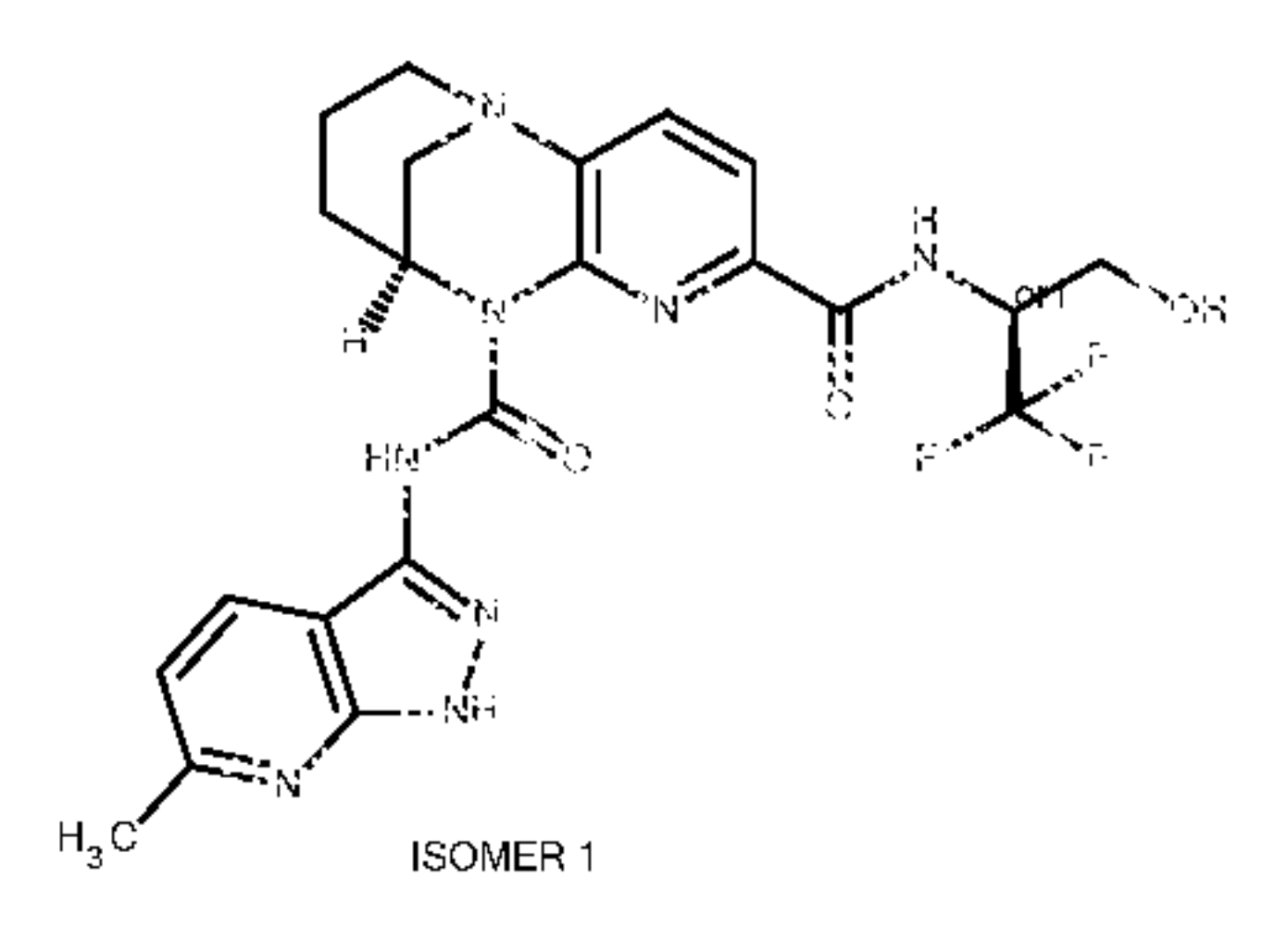
Example number	Structure	Chemical Name: Generated by CHemAxon	TRP Acti ty	TRP MAX RESP
16		(9S)-5-(2-methylpyridin-4-yl)-N-(pyrimidin-5-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	B	A
17		(9S)-N-(pyrazin-2-yl)-5-[2-(trifluoromethyl)pyridin-4-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
18		(9S)-N-(pyrimidin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
19		(9S)-N-(dimethyl-1,3-thiazol-2-yl)-5-(2-methylpyridin-4-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
20		(9S)-N-(6-methoxypyrazin-2-yl)-5-(2-methylpyridin-4-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A

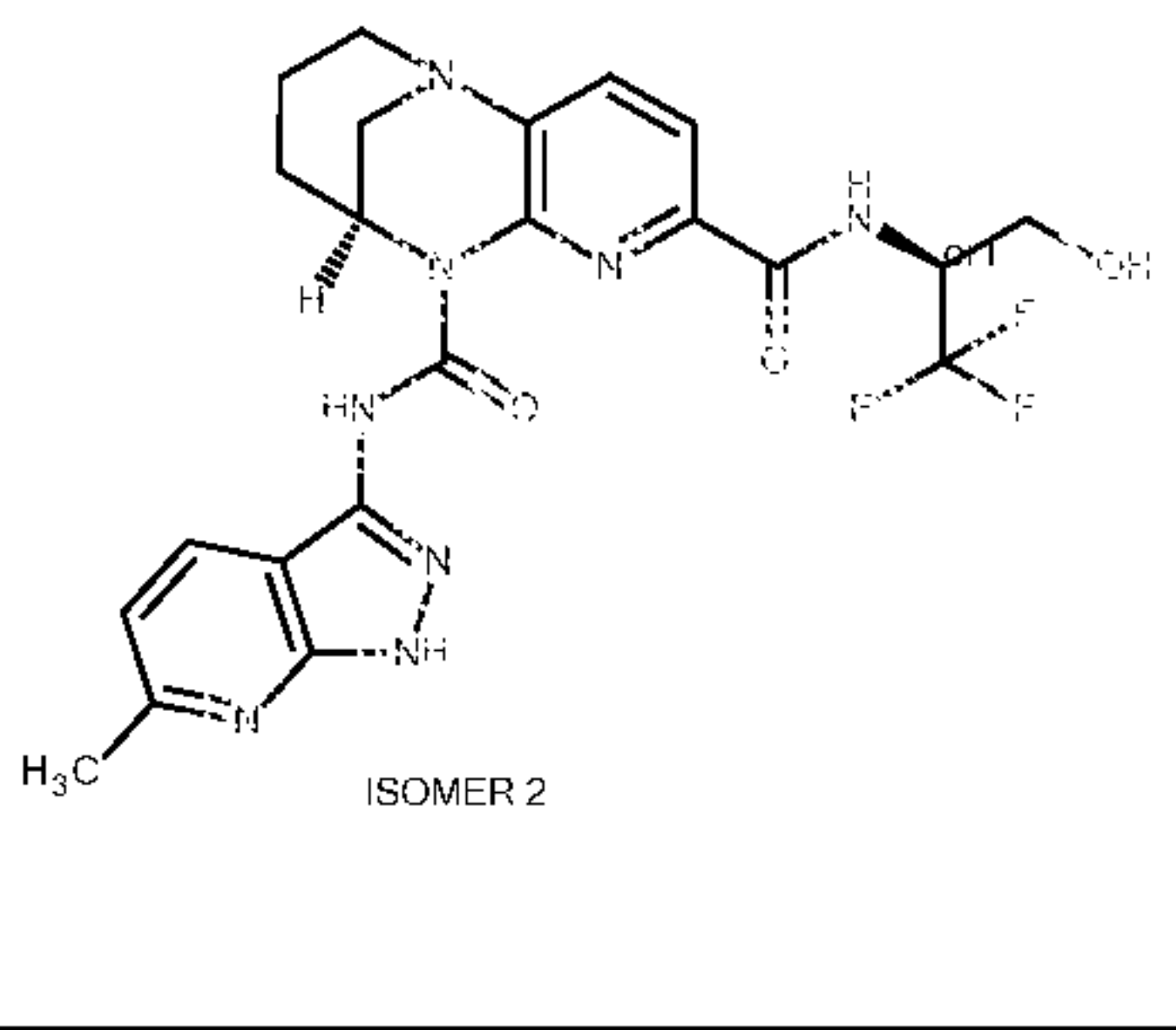
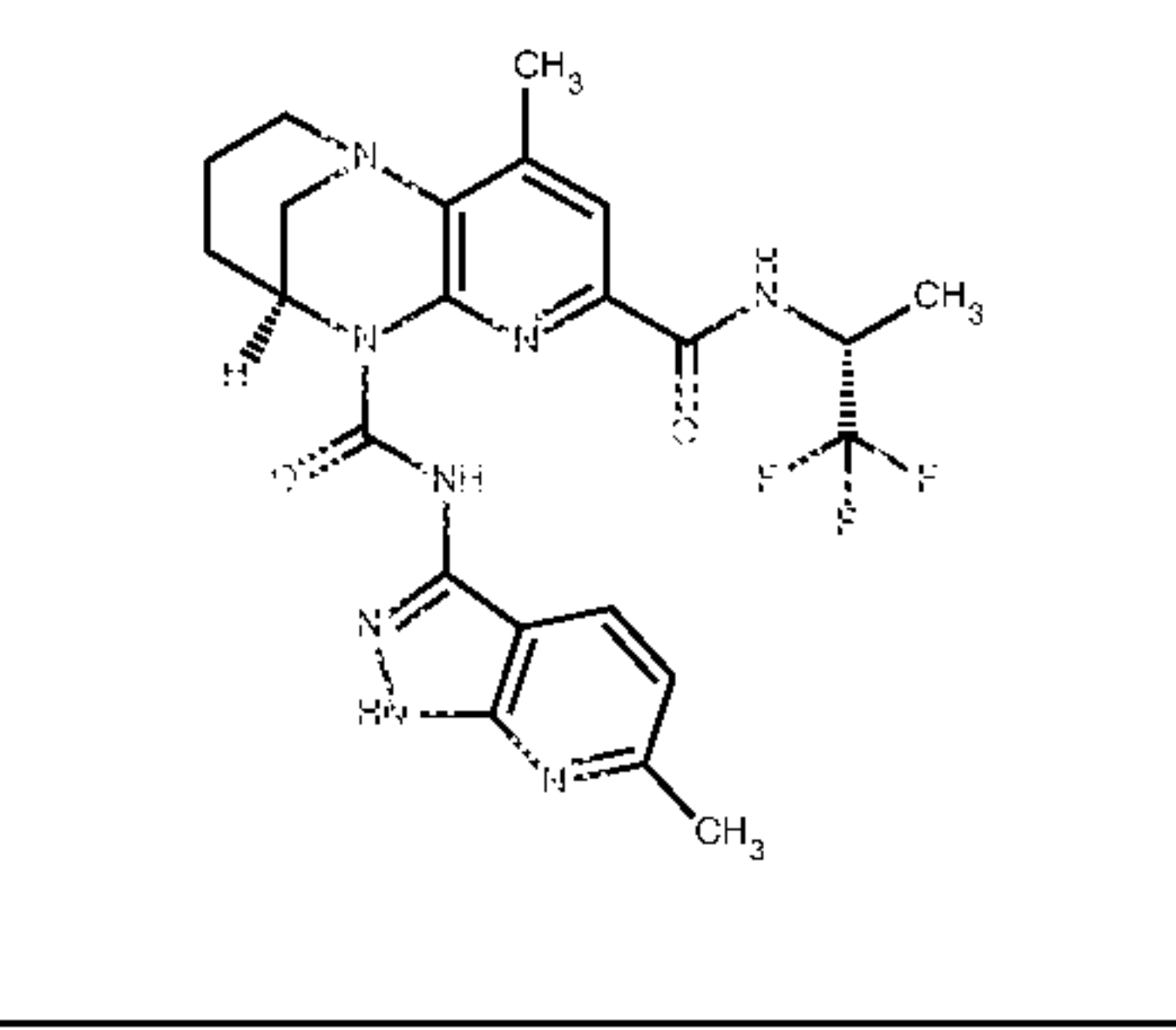
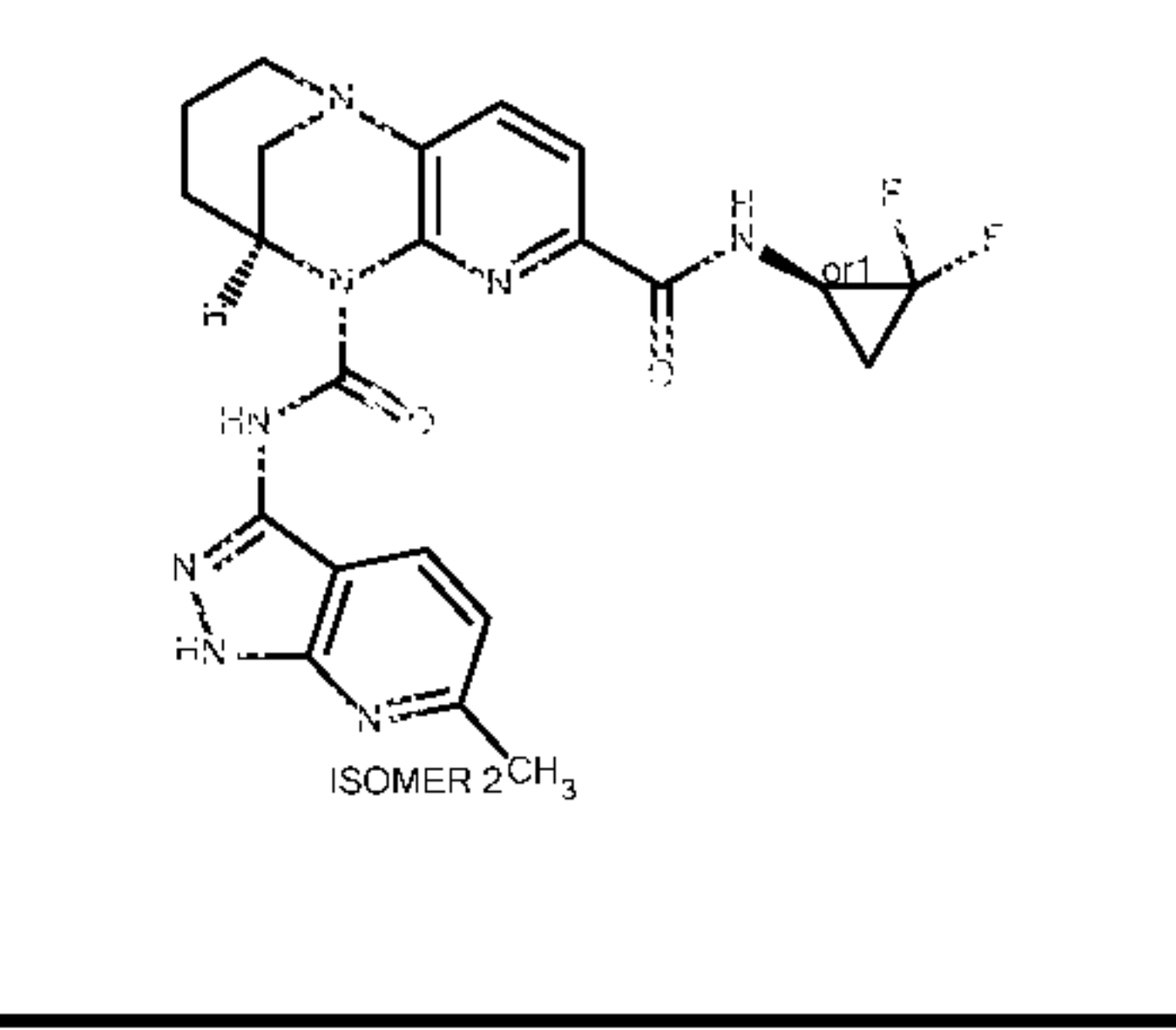
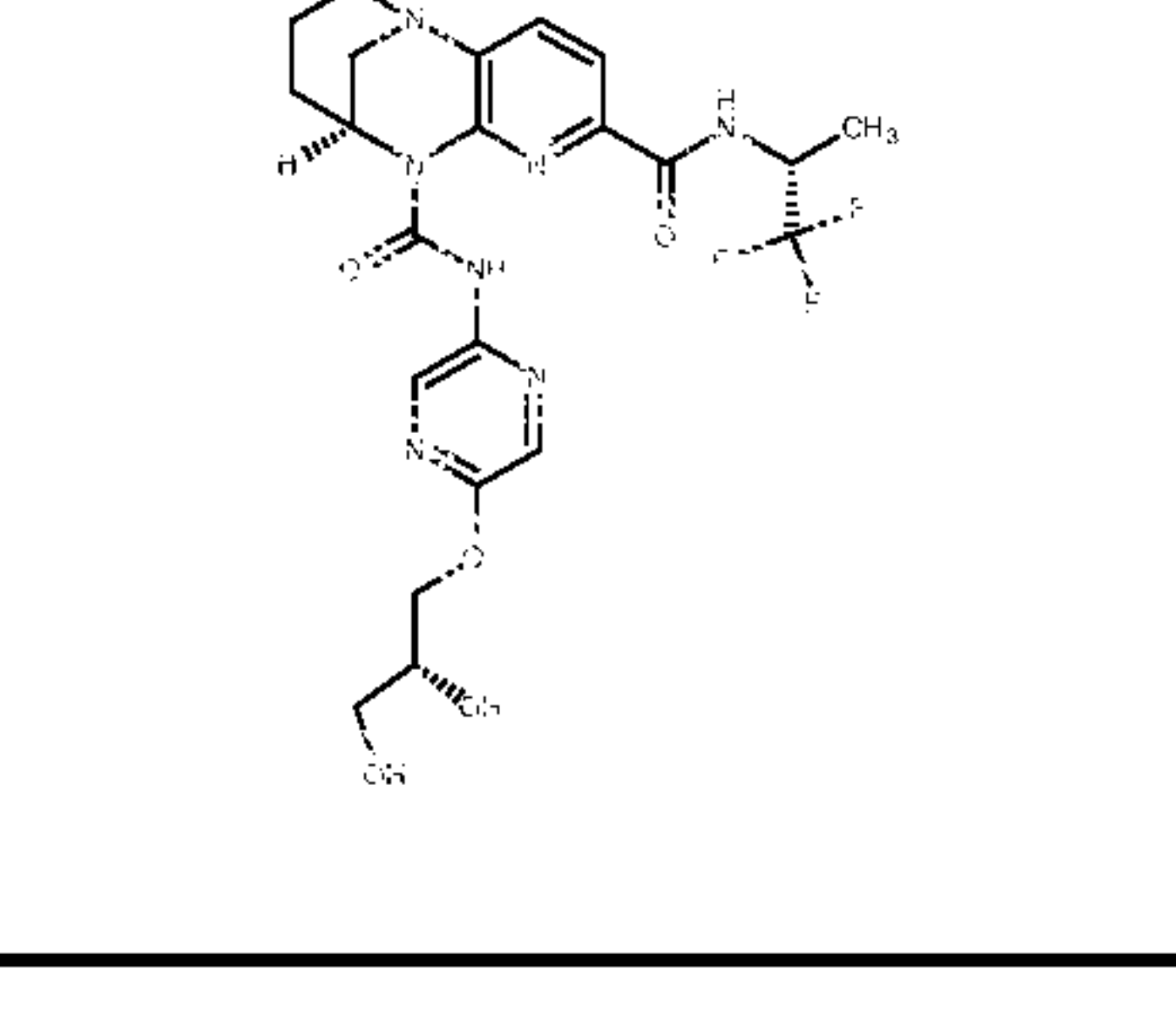
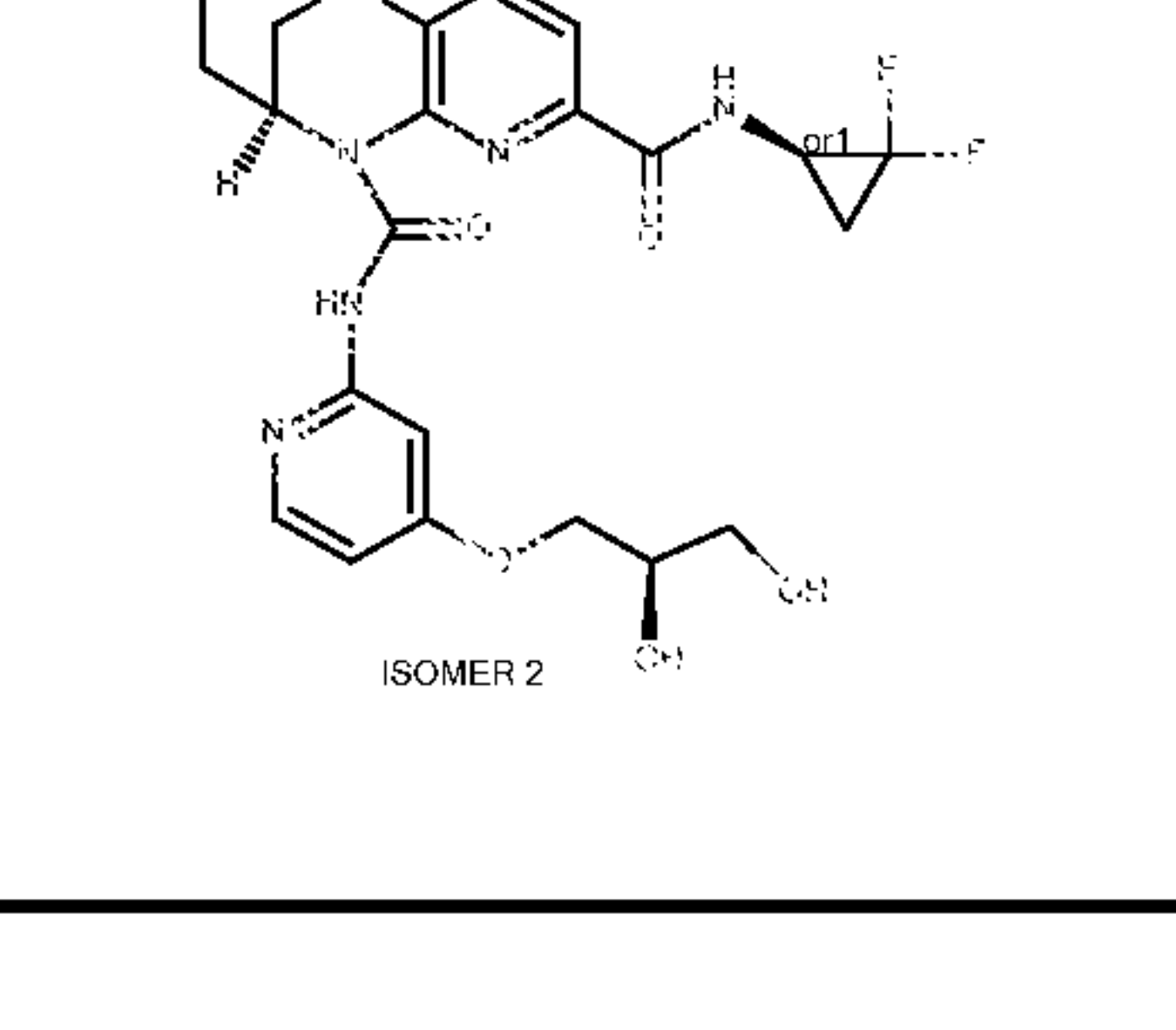
Example number	Structure	Chemical Name: Generated by CHemAxon	TRP Activi ty	TRP MAX RESP
21		(9S)-N-(6-methylpyrazin-2-yl)-5-(2-methylpyridin-4-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	B	A
22		(9S)-N-(pyrimidin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
23		(9S)-N-(pyridin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	B	A
24		(9S)-N-(5-fluoropyridin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
25		(9S)-N-(pyridin-3-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A

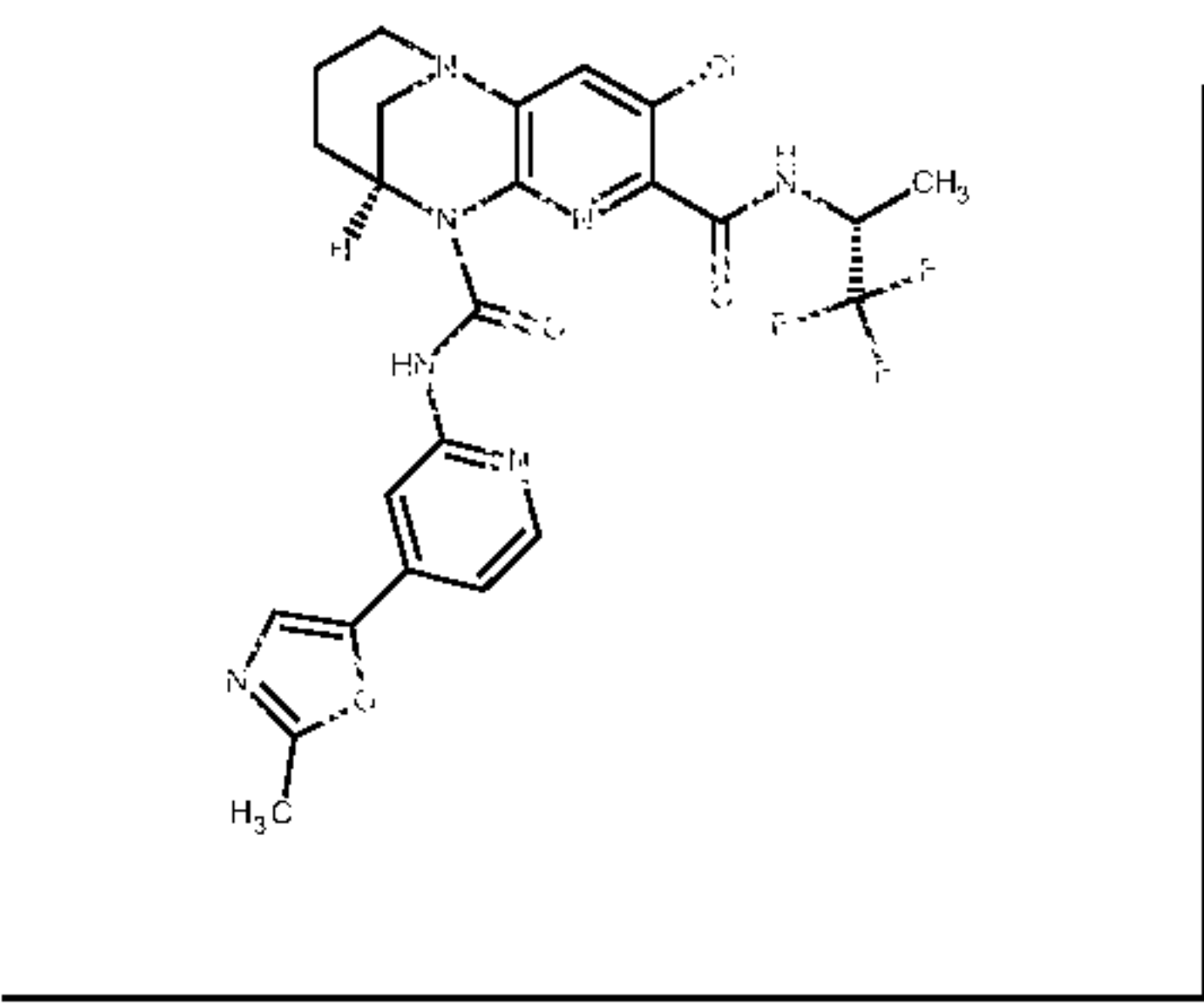
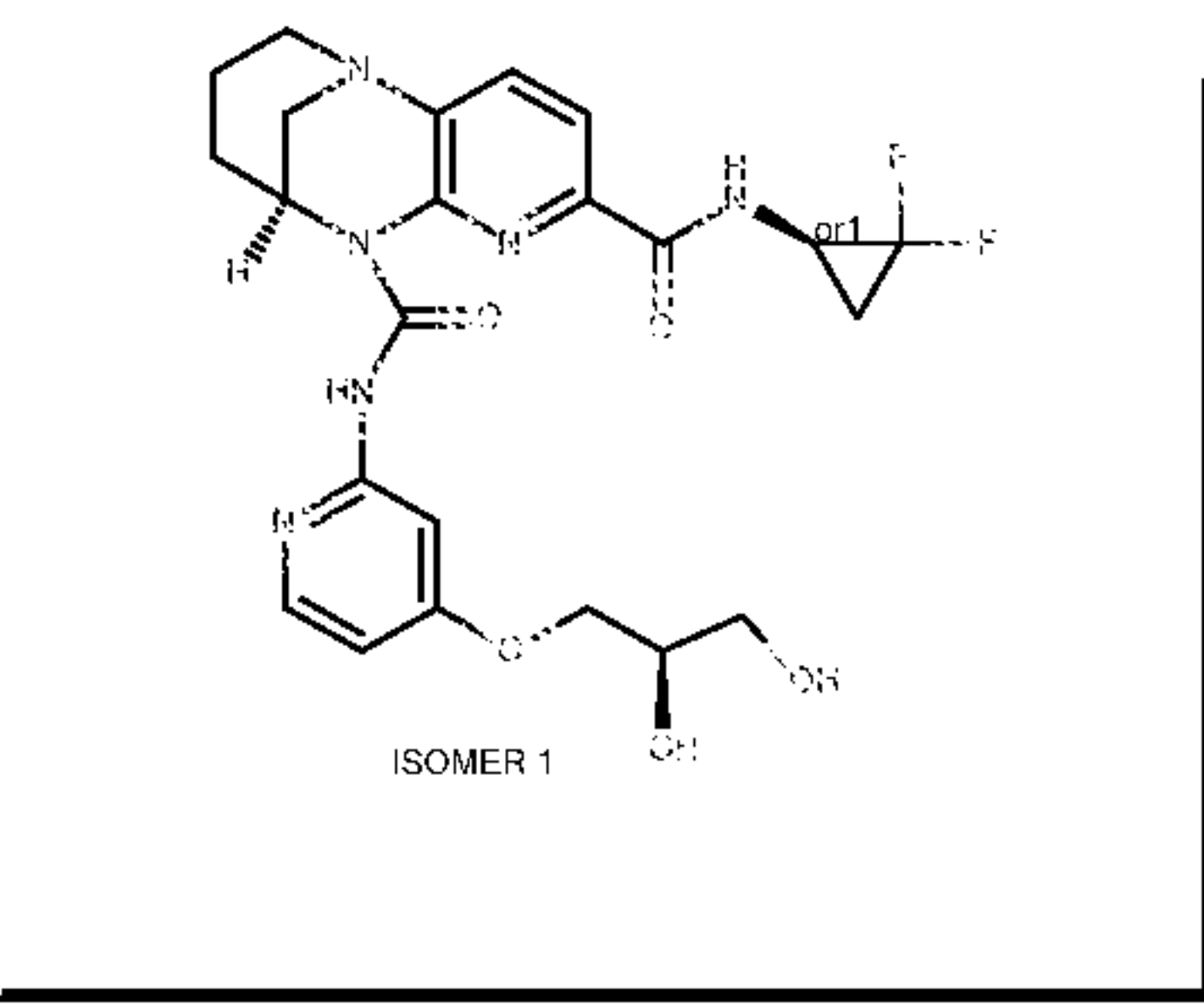
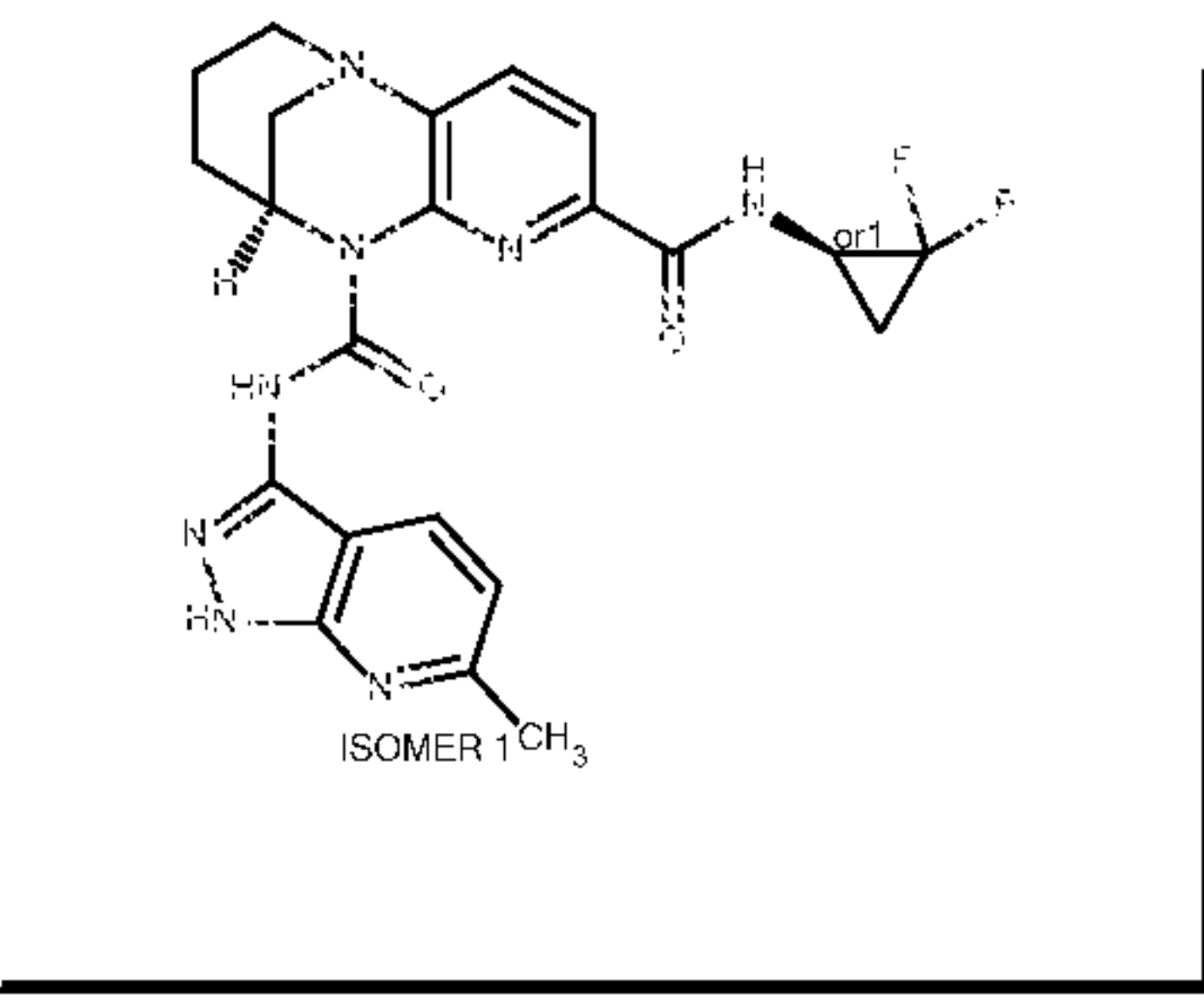
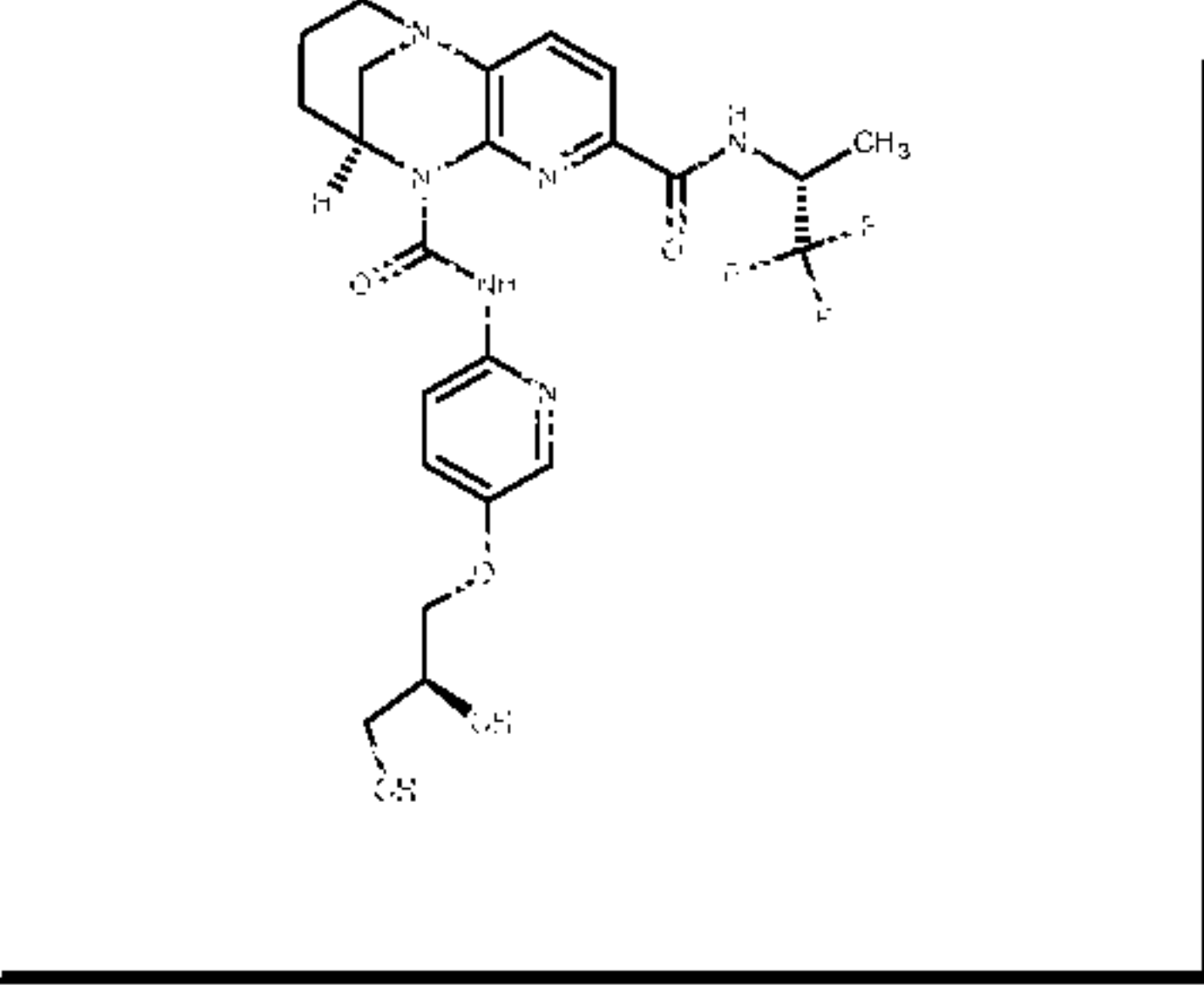
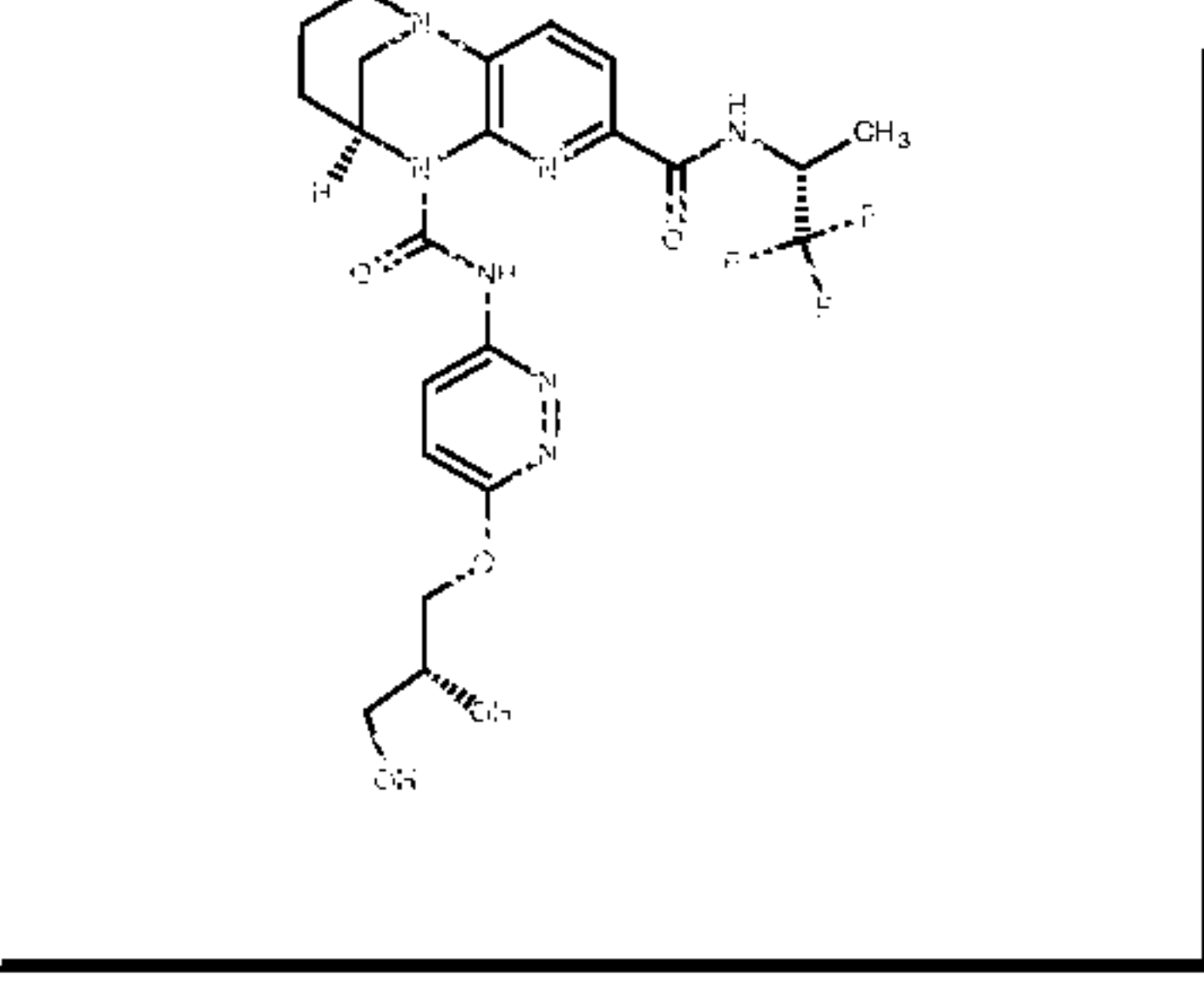
Example number	Structure	Chemical Name: Generated by CHemAxon	TRP Activi ty	TRP MAX RESP
26		(9S)-N-(1-methyl-1H-pyrazol-4-yl)-5-(2-methylpyridin-4-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	B	A
27		(9S)-N-(pyrazin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
28		(9S)-N-(pyridin-3-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
29		(9S)-N-(5-fluoropyridin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	B	A
30		(9S)-N-(pyrazin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A

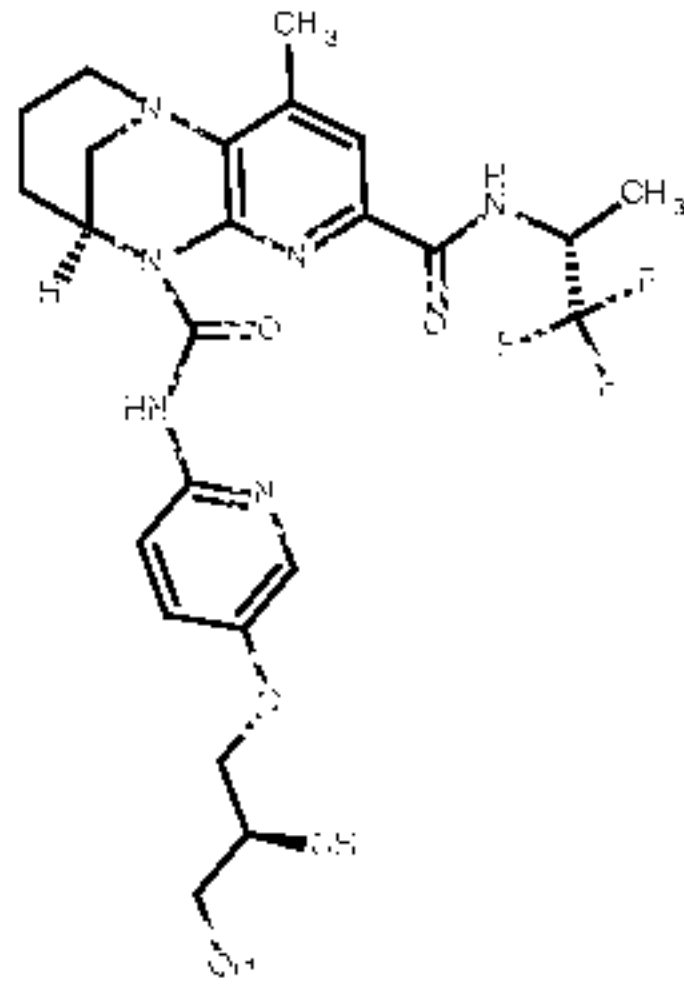
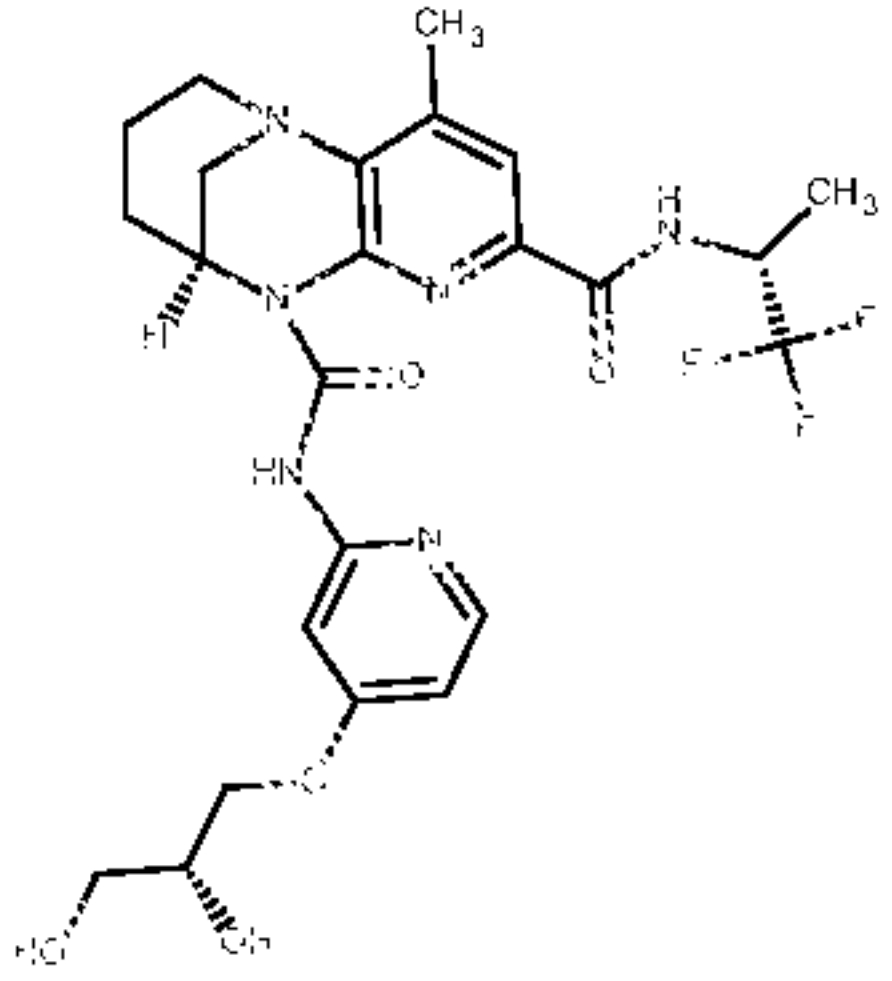
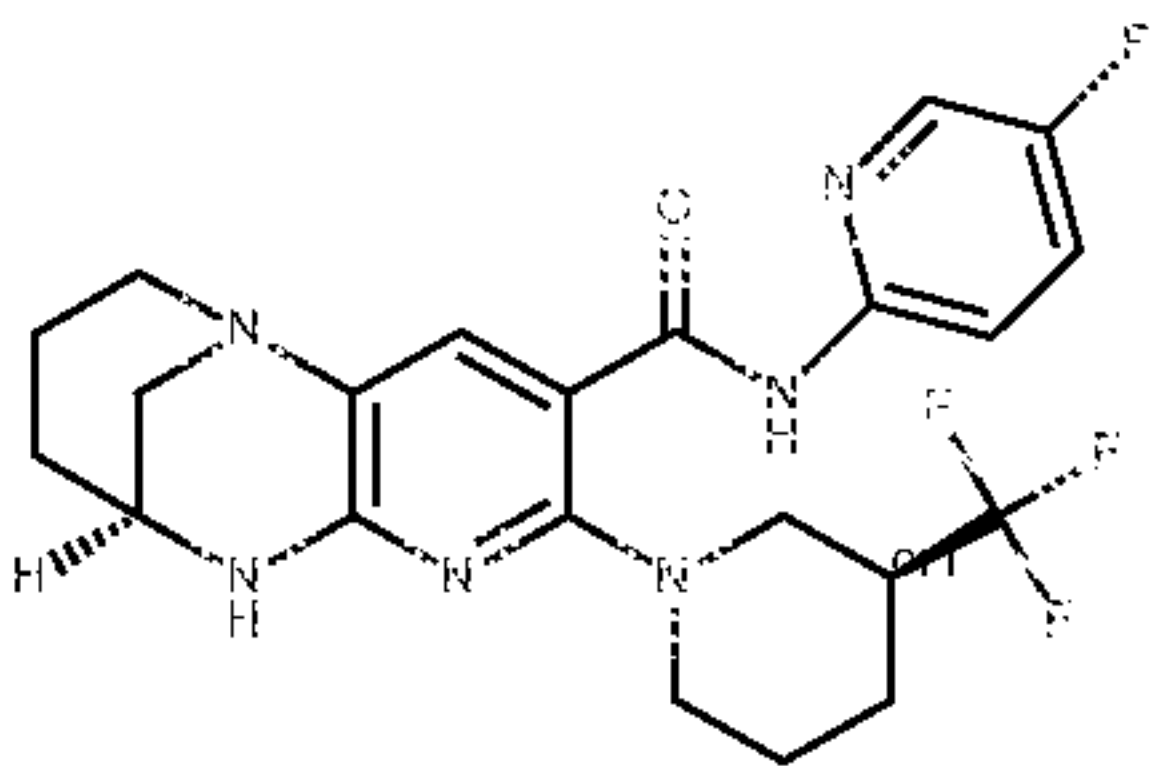
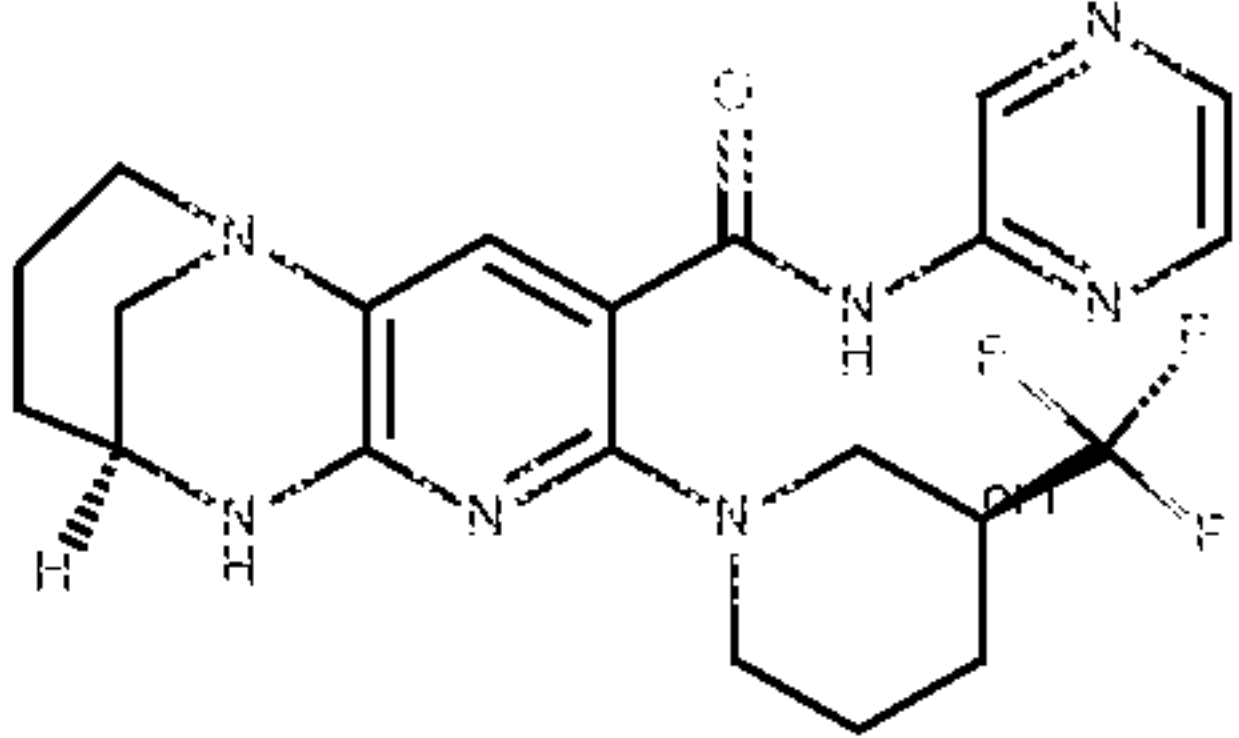
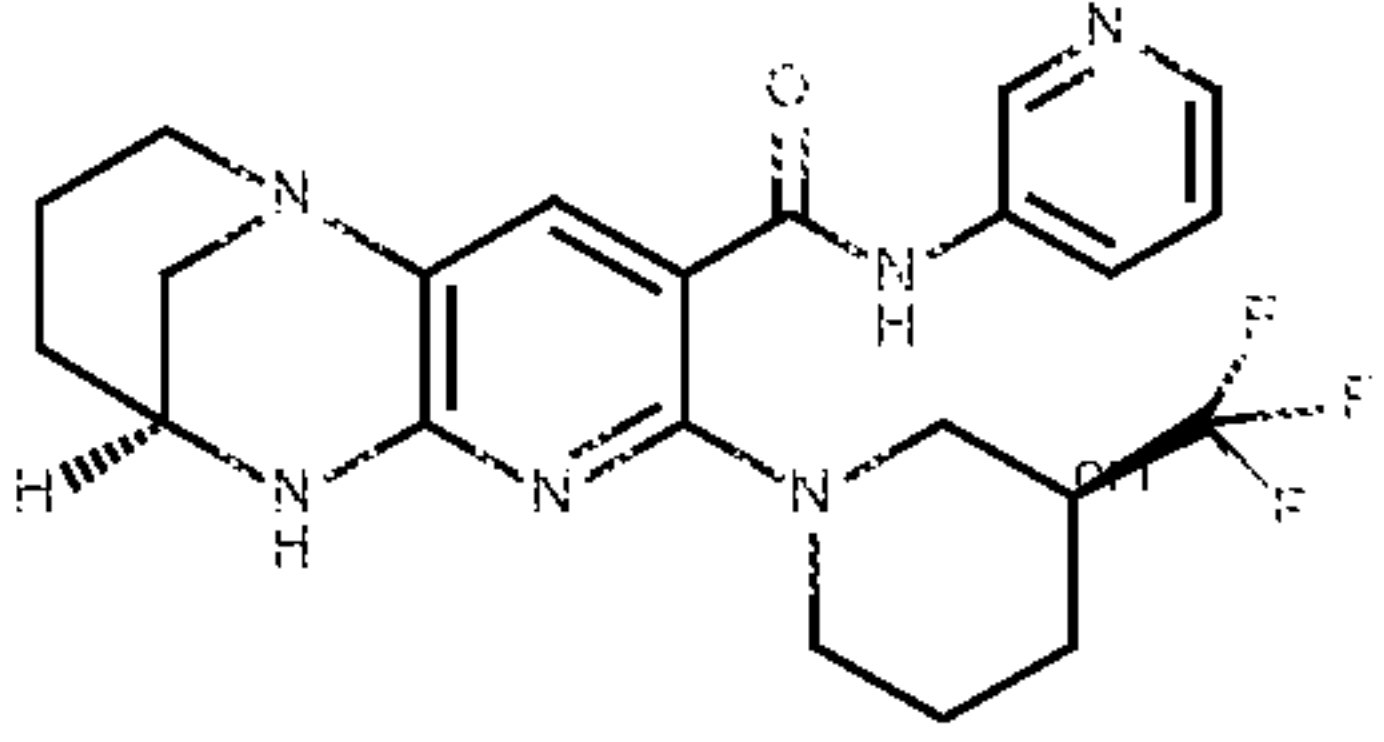
Example number	Structure	Chemical Name: Generated by CHemAxon	TRP Acti- ty	TRP MAX RESP
31		(9S)-N-(pyridin-2-yl)-5-[(3S)-3-(trifluoromethyl)pyrrolidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide hydrochloride	A	A
32		(9S)-5-(2-methylpyridin-4-yl)-N-(pyridin-3-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
33		(9S)-4-chloro-5-N-cyclopropyl-8-N-(pyridin-2-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	B	A
34		(9S)-8-N-{4-[(2R)-2,3-dihydroxypropoxy]pyridin-2-yl}-5-N-[(2R)-1,1,1-trifluoropropan-2-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
35		(9S)-5-N-(2,2-difluoropropyl)-8-N-{4-[(2R)-2,3-dihydroxypropoxy]pyridin-2-yl}-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A

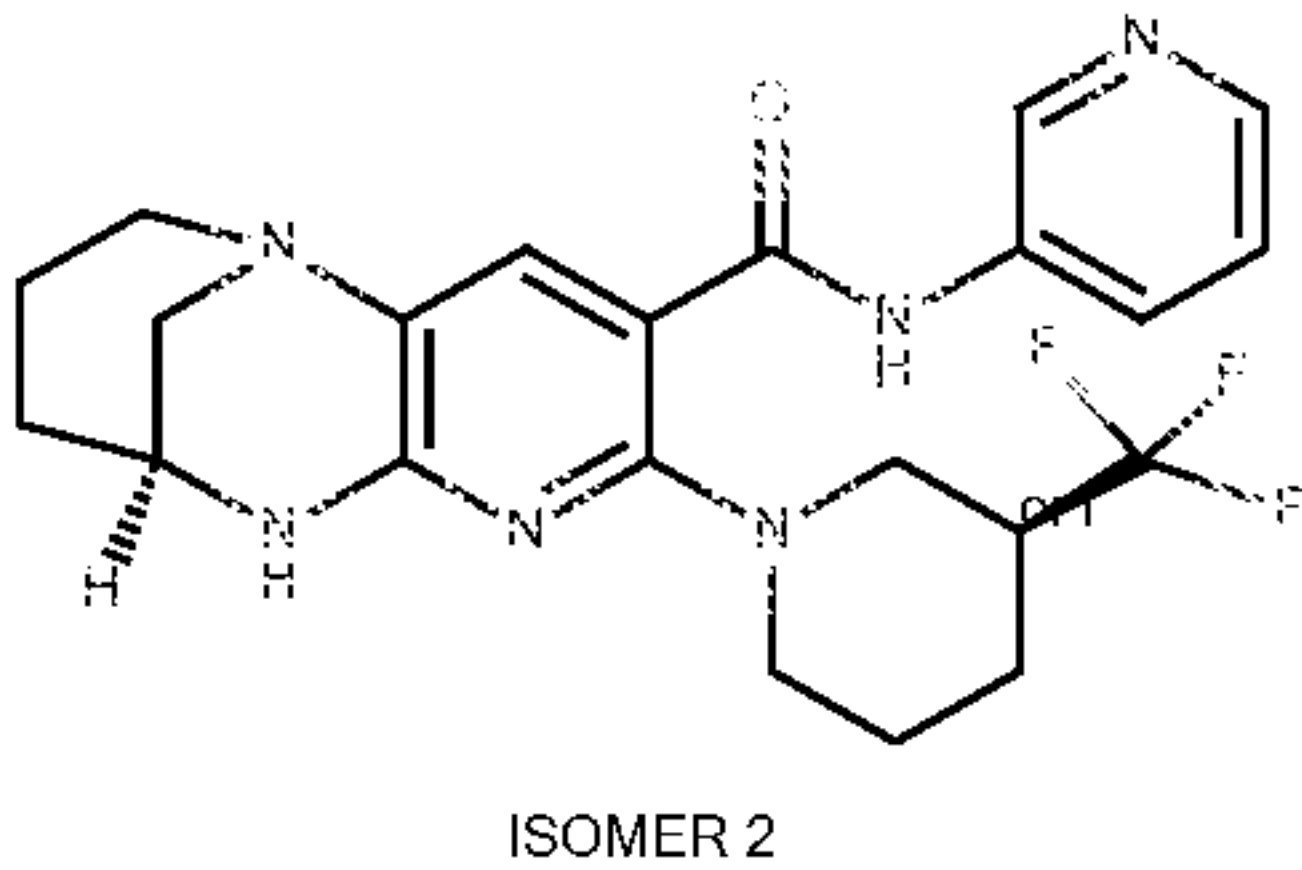
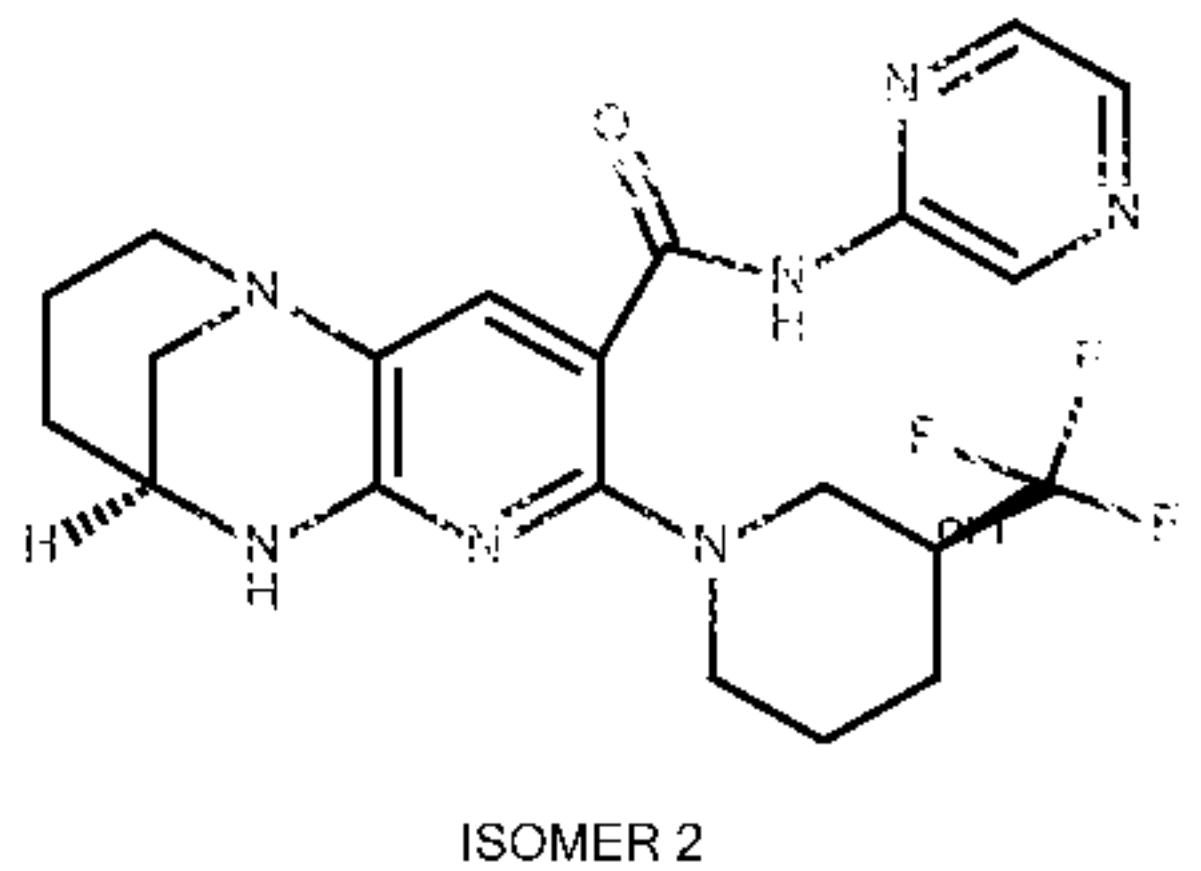
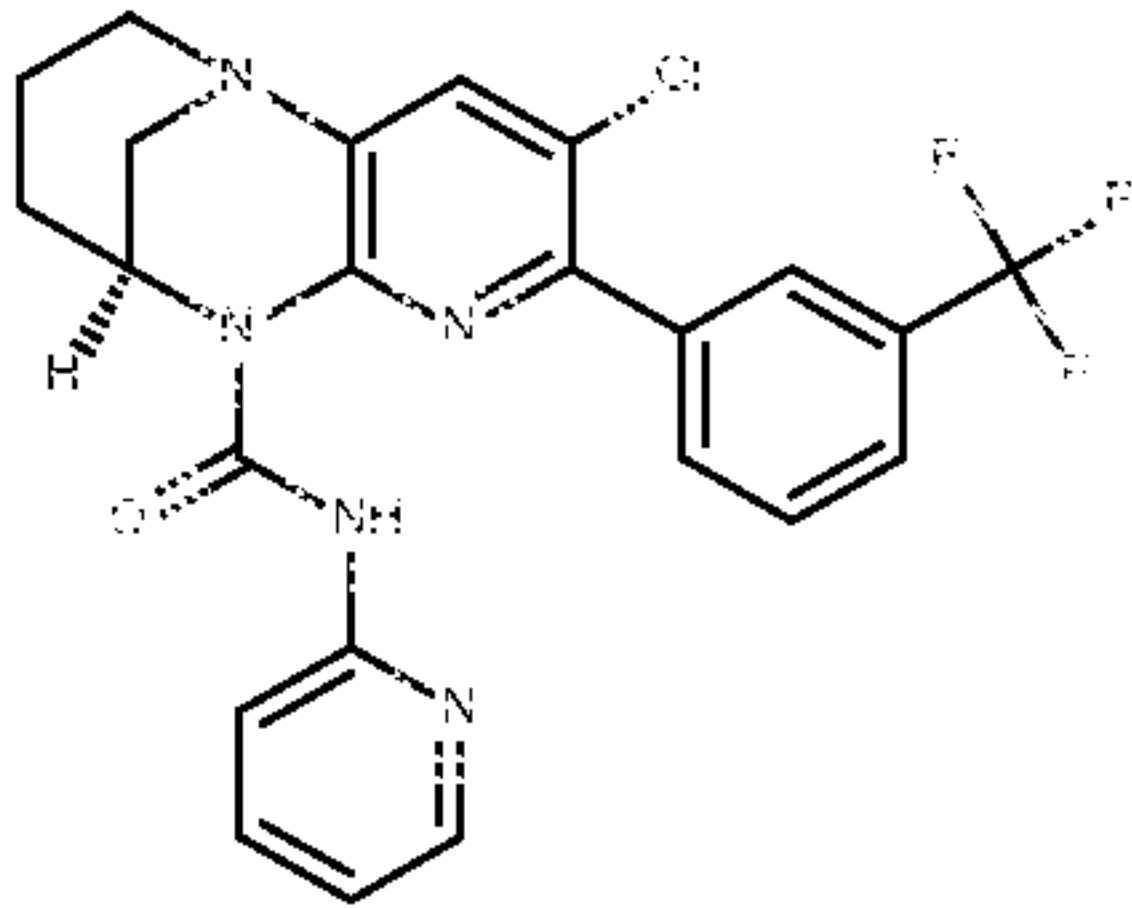
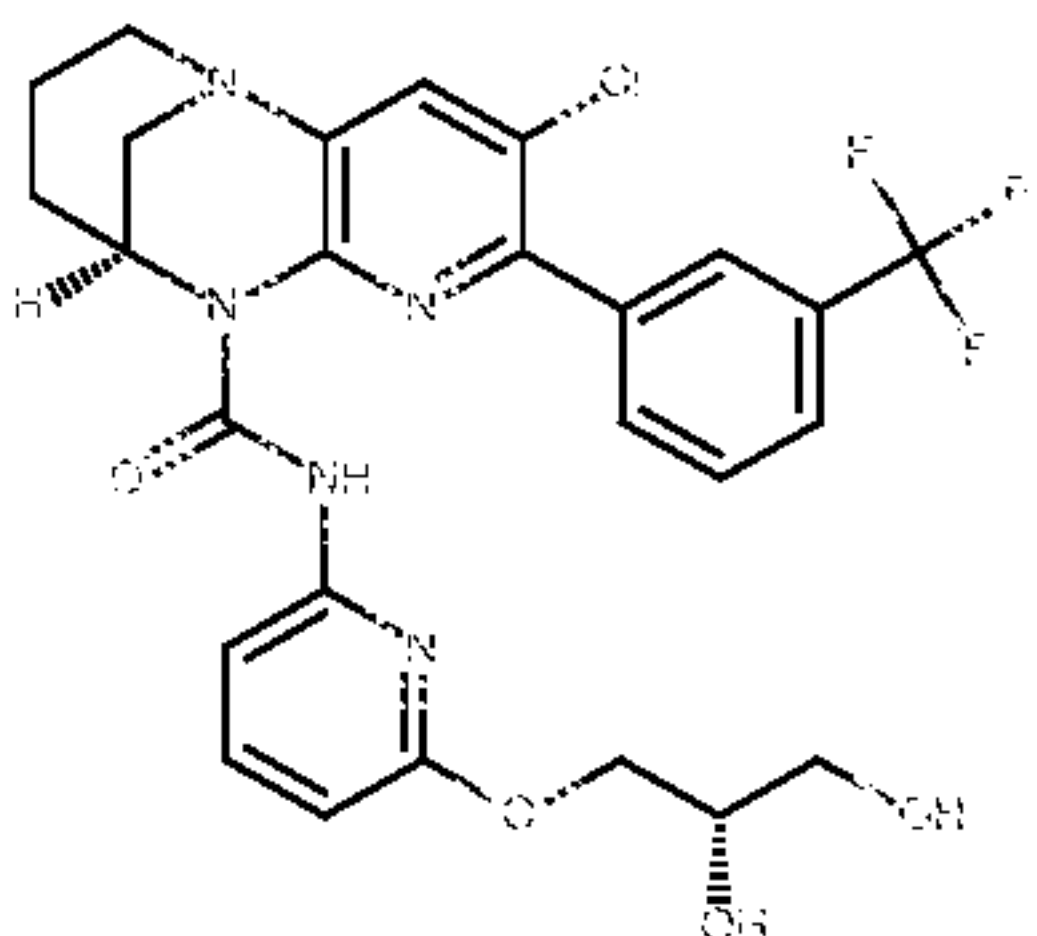
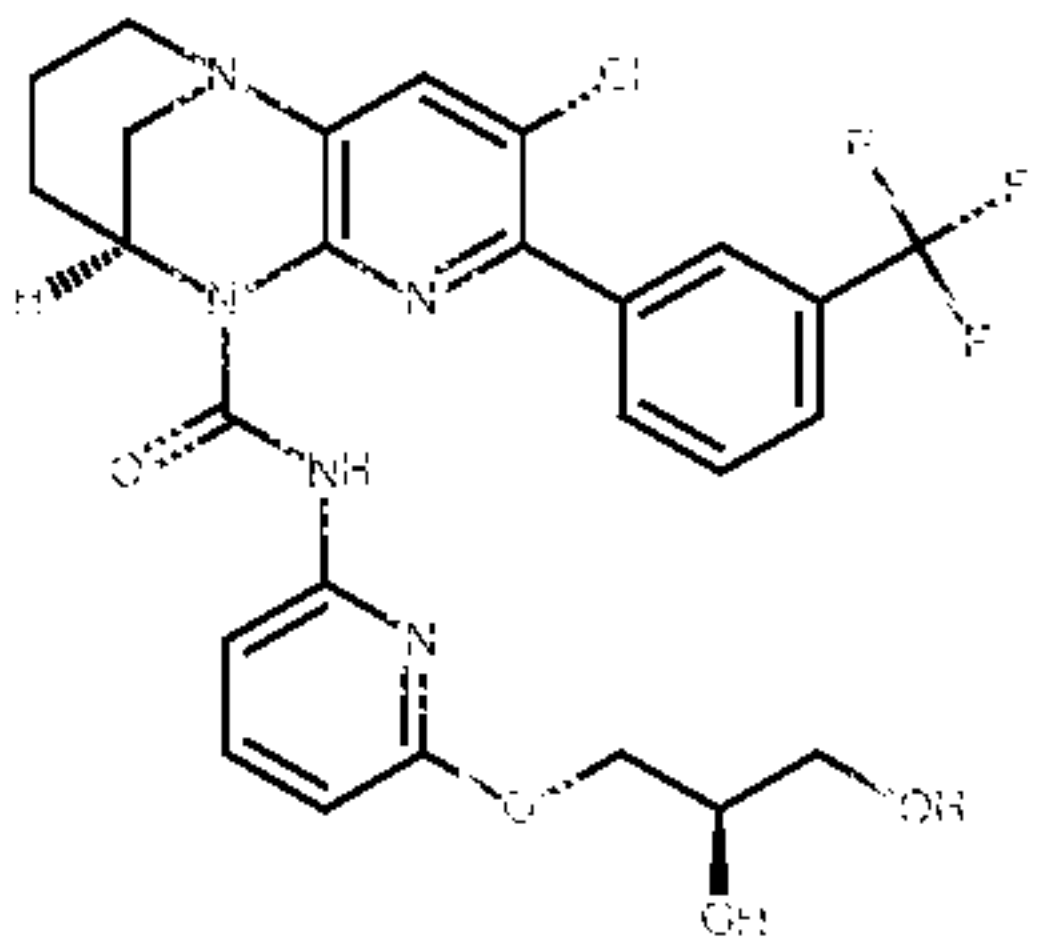
Example number	Structure	Chemical Name: Generated by CHemAxon	TRP Activi ty	TRP MAX RESP
36		(9S)-5-N-cyclopropyl-8-N-(pyridin-2-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
37		(9S)-8-N-(pyridin-2-yl)-5-N-(2,2,2-trifluoroethyl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
38		(9S)-4-chloro-8-N-(pyridin-2-yl)-5-N-(2,2,2-trifluoroethyl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
39		(9S)-4-chloro-8-N-(pyridin-2-yl)-5-N-[(2R)-1,1,1-trifluoropropan-2-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
40		(9S)-8-N-(1,2-oxazol-3-yl)-5-N-[(2R)-1,1,1-trifluoropropan-2-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A

Example number	Structure	Chemical Name: Generated by CHemAxon	TRP Activity	TRP MAX RESP
41		(9S)-8-N-{6-methyl-1H-pyrazolo[3,4-b]pyridin-3-yl}-5-N-(2,2,2-trifluoroethyl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
42		(9S)-5-N-cyclopropyl-8-N-{6-methyl-1H-pyrazolo[3,4-b]pyridin-3-yl}-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
43		(9S)-8-N-{6-methyl-1H-pyrazolo[3,4-b]pyridin-3-yl}-5-N-[(2R)-1,1,1-trifluoropropan-2-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
44		(9S)-8-N-[4-(2-methyl-1,3-thiazol-5-yl)pyridin-2-yl]-5-N-[(2R)-1,1,1-trifluoropropan-2-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
45		(9S)-8-N-{6-methyl-1H-pyrazolo[3,4-b]pyridin-3-yl}-5-N-[(2S)-1,1,1-trifluoro-3-hydroxypropan-2-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	B	A

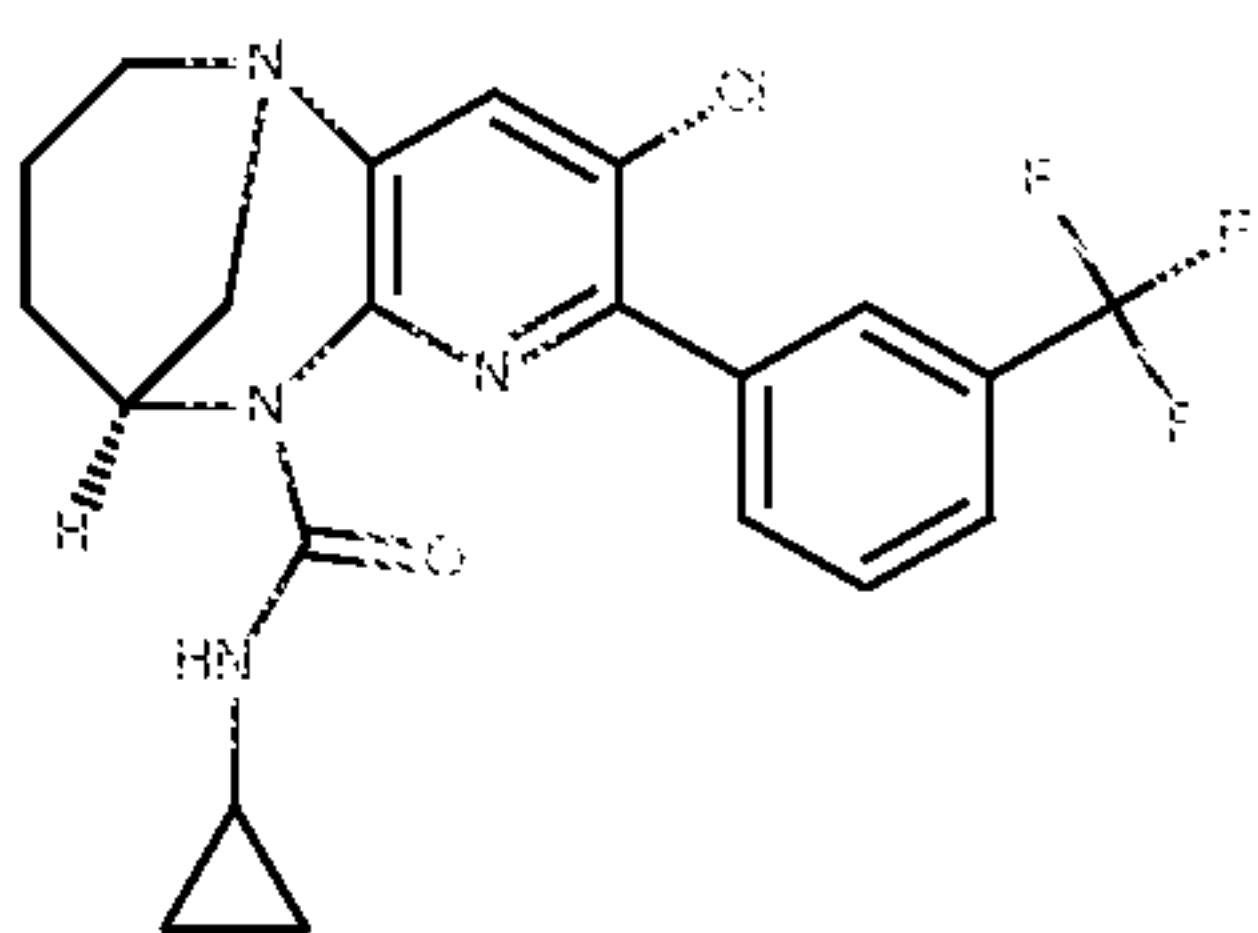
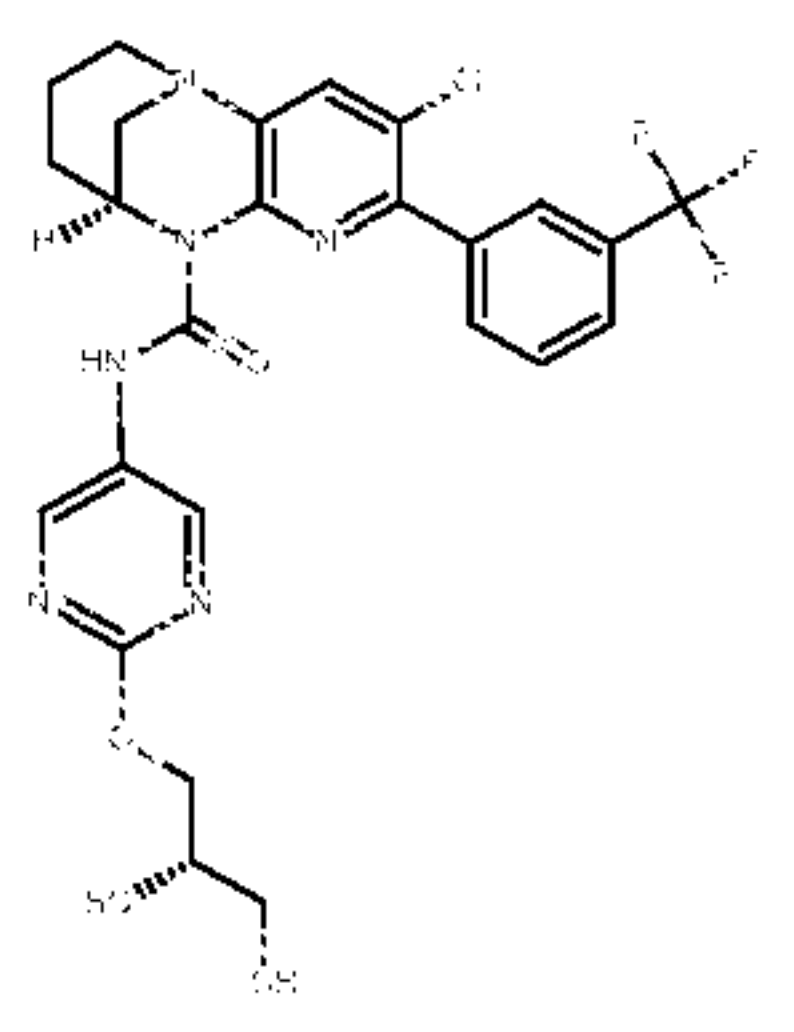
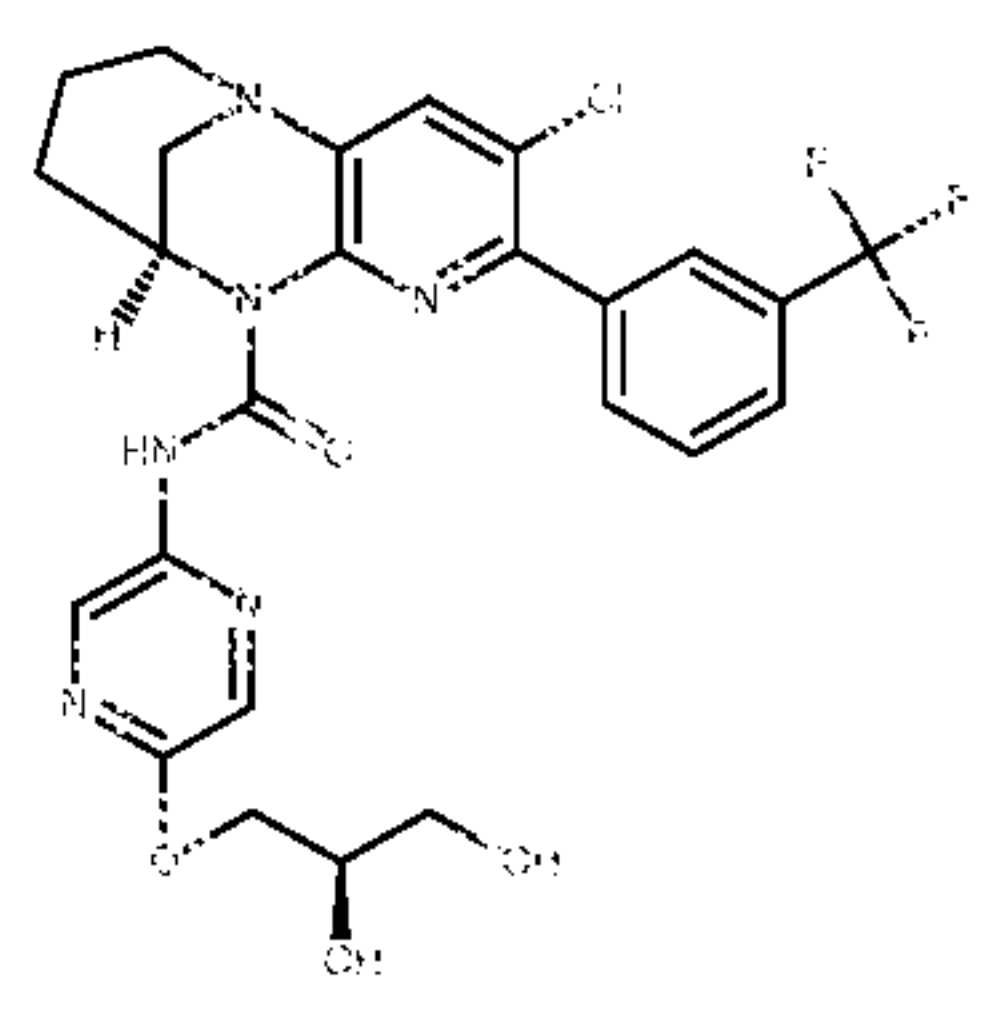
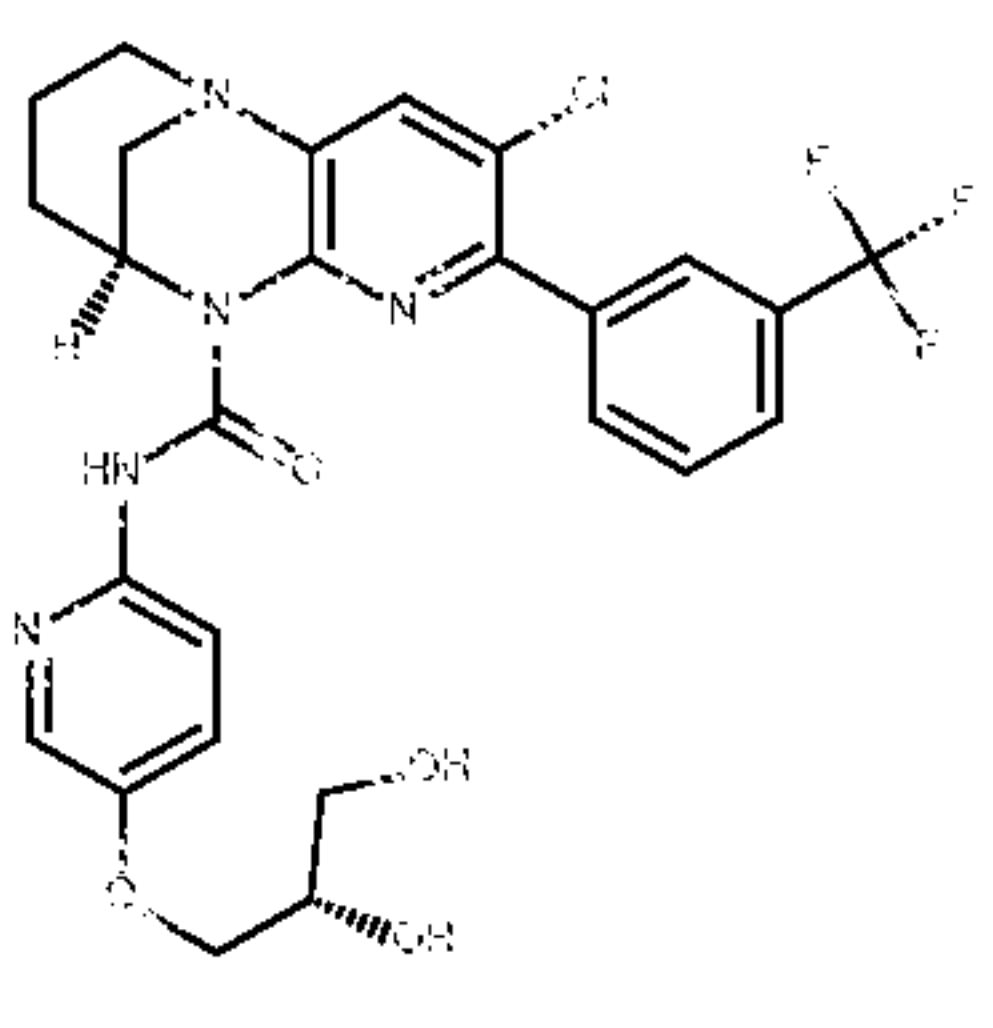
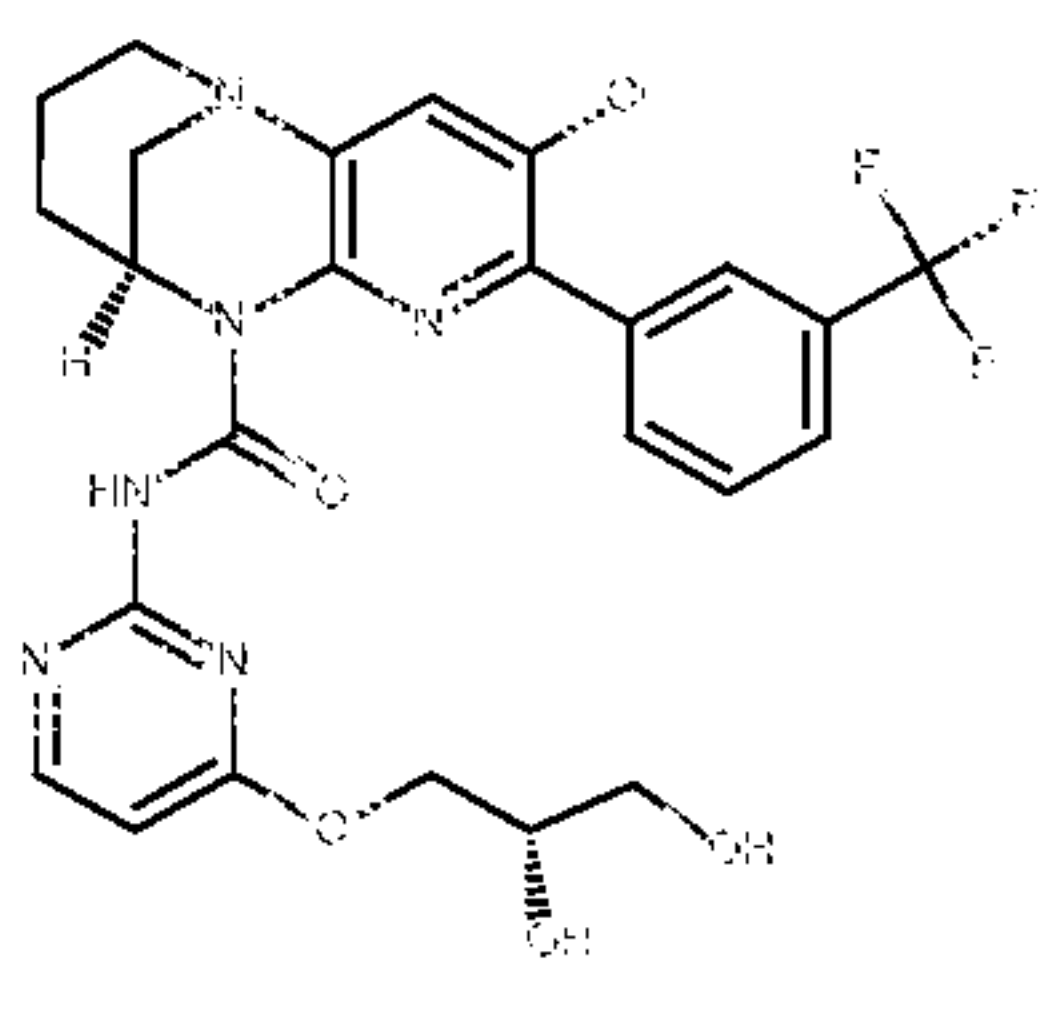
Example number	Structure	Chemical Name: Generated by CHemAxon	TRP Activi ty	TRP MAX RESP
46		(9S)-8-N-{6-methyl-1H-pyrazolo[3,4-b]pyridin-3-yl}-5-N-[(2S)-1,1,1-trifluoro-3-hydroxypropan-2-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
47		(9S)-3-methyl-8-N-{6-methyl-1H-pyrazolo[3,4-b]pyridin-3-yl}-5-N-[(2R)-1,1,1-trifluoropropan-2-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
48		(9S)-5-N-[(1R)-2,2-difluorocyclopropyl]-8-N-{6-methyl-1H-pyrazolo[3,4-b]pyridin-3-yl}-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
49		(9S)-8-N-{5-[(2R)-2,3-dihydroxypropoxy]pyrazin-2-yl}-5-N-[(2R)-1,1,1-trifluoropropan-2-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
50		(9S)-5-N-[(1R)-2,2-difluorocyclopropyl]-8-N-{4-[(2R)-2,3-dihydroxypropoxy]pyridin-2-yl}-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A

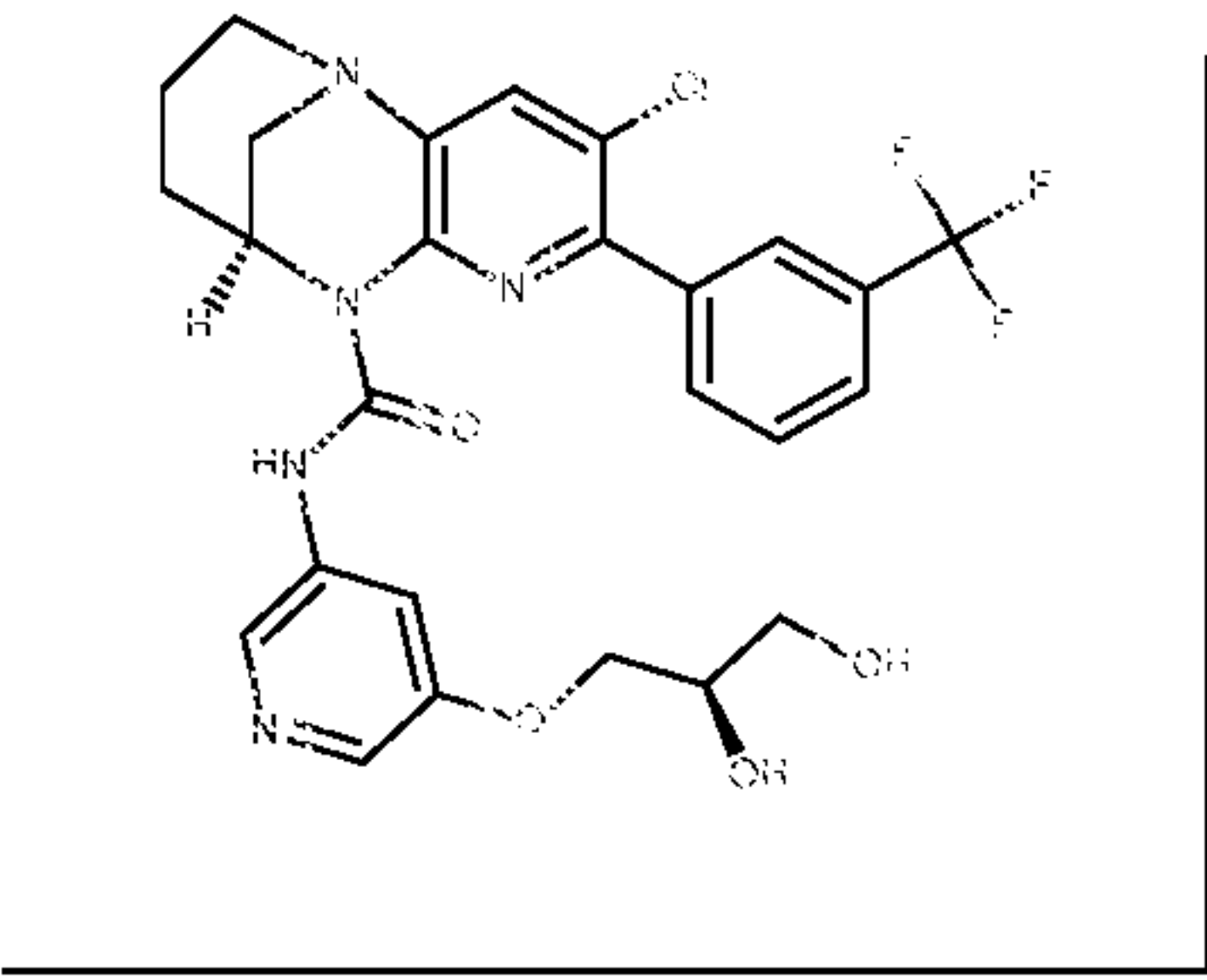
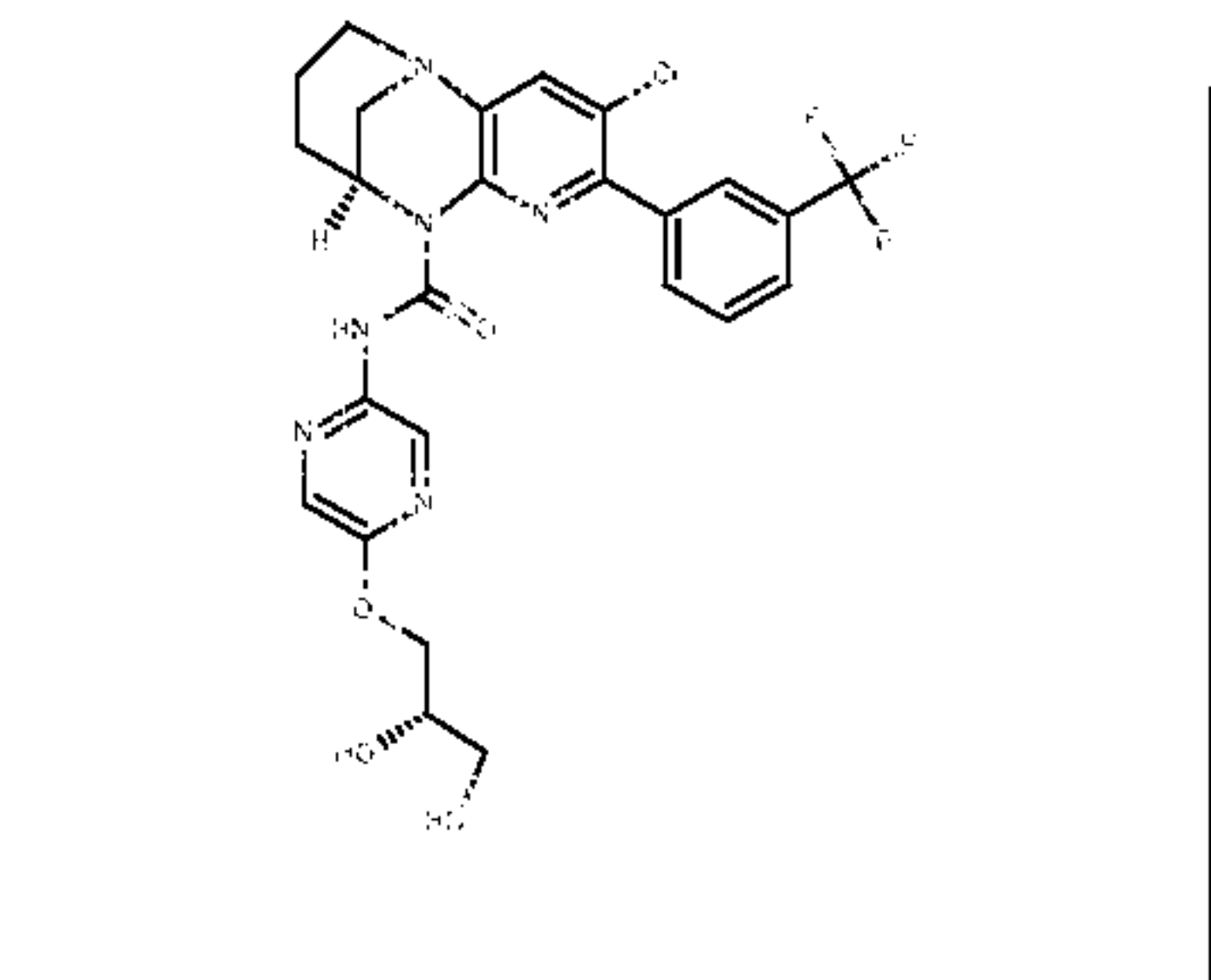
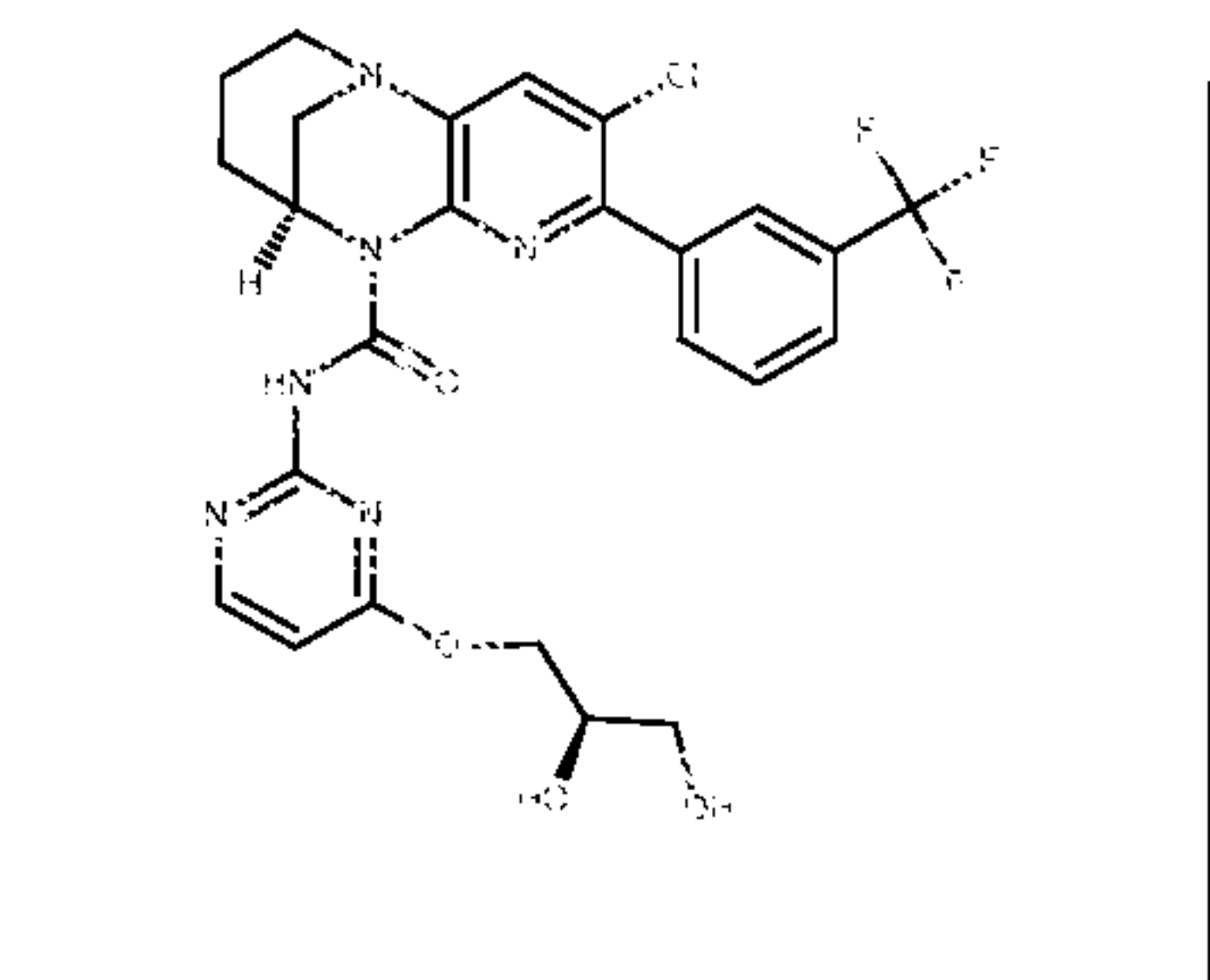
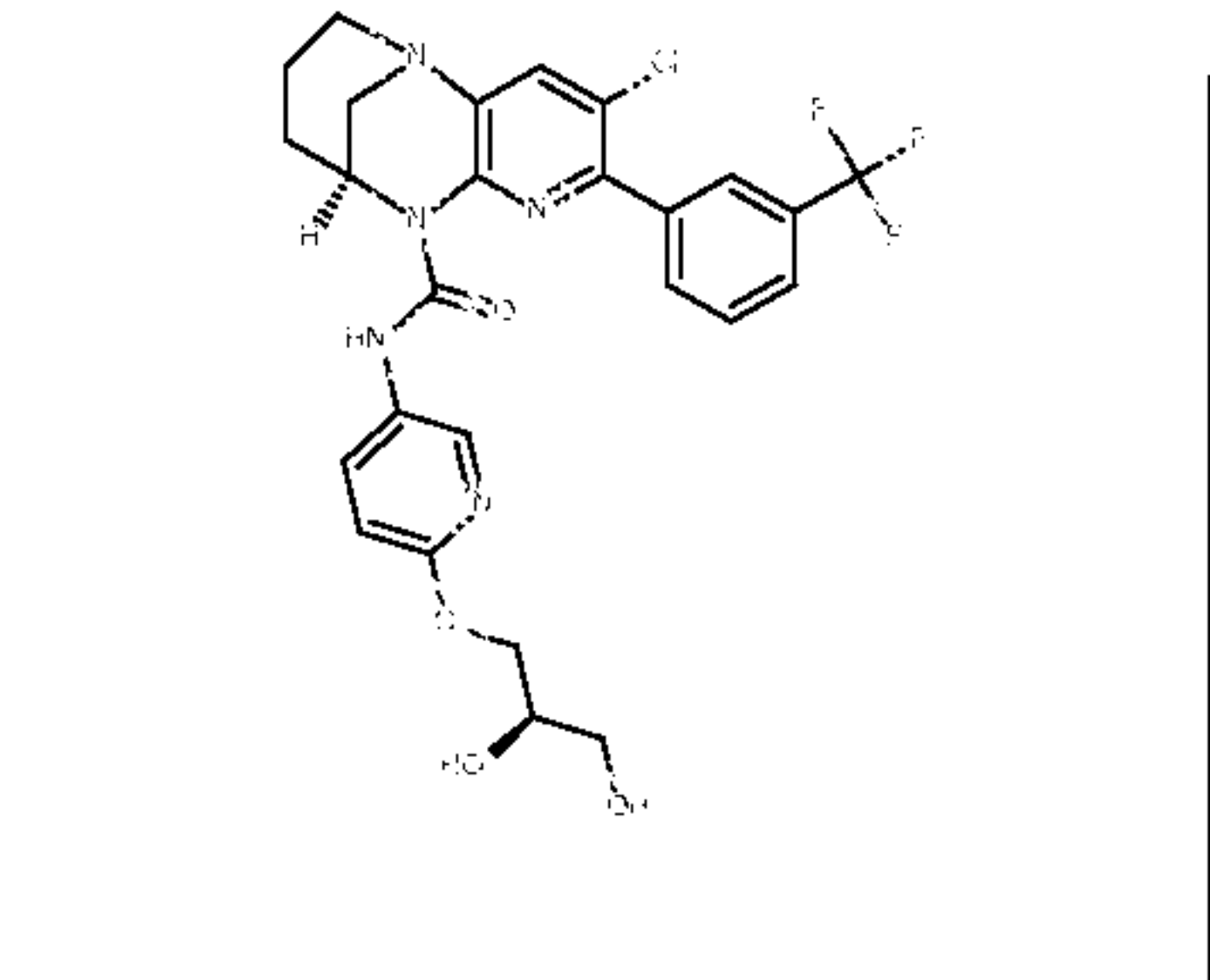
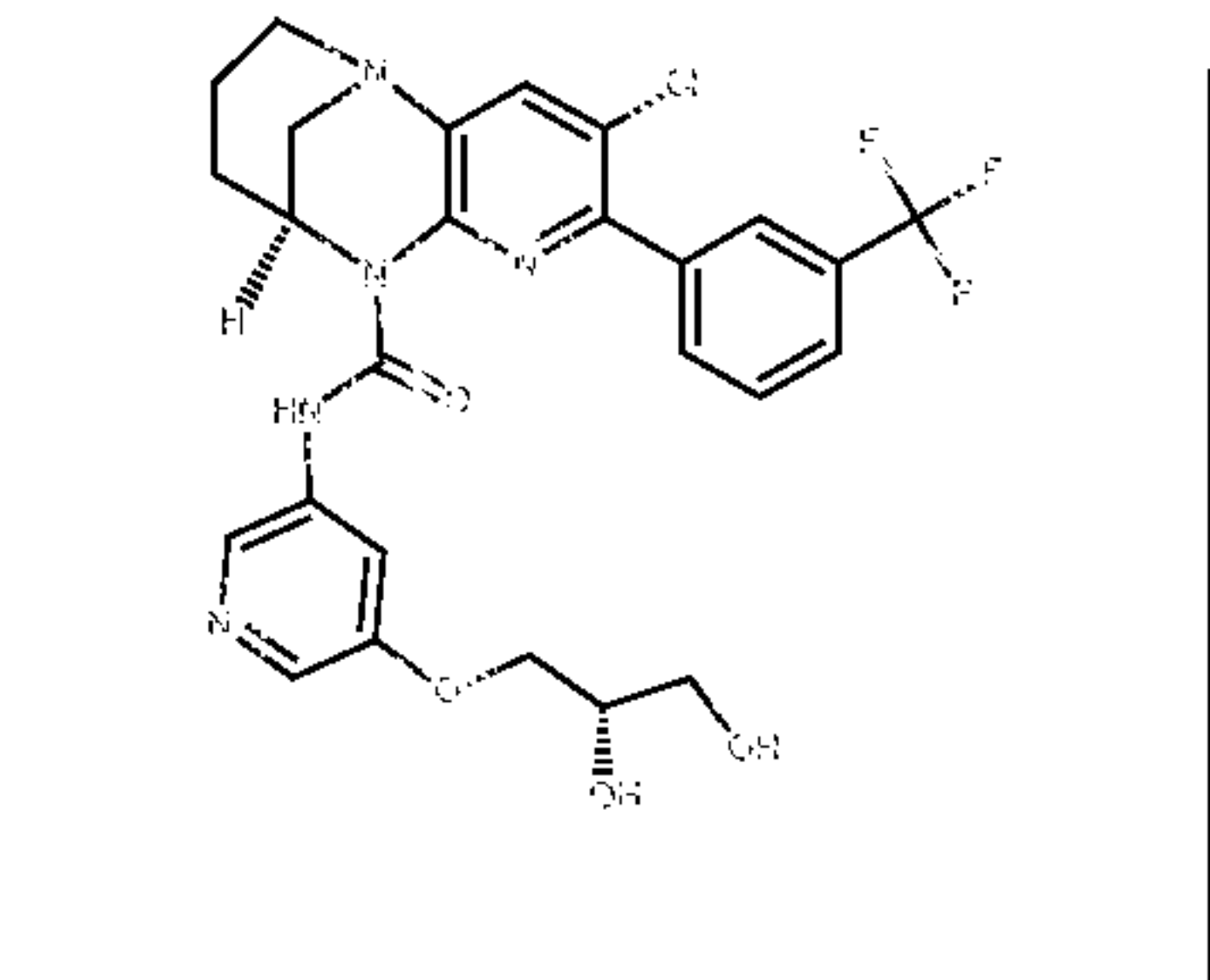
Example number	Structure	Chemical Name: Generated by CHemAxon	TRP Activi ty	TRP MAX RESP
51		(9S)-4-chloro-8-N-[4-(2-methyl-1,3-oxazol-5-yl)pyridin-2-yl]-5-N-[(2R)-1,1,1-trifluoropropan-2-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
52		(9S)-5-N-[(1R)-2,2-difluorocyclopropyl]-8-N-{4-[(2R)-2,3-dihydroxypropoxy]pyridin-2-yl}-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
53		(9S)-5-N-[(1R)-2,2-difluorocyclopropyl]-8-N-{6-methyl-1H-pyrazolo[3,4-b]pyridin-3-yl}-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
54		(9S)-8-N-{5-[(2S)-2,3-dihydroxypropoxy]pyridin-2-yl}-5-N-[(2R)-1,1,1-trifluoropropan-2-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
55		(9S)-8-N-{6-[(2R)-2,3-dihydroxypropoxy]pyridazin-3-yl}-5-N-[(2R)-1,1,1-trifluoropropan-2-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A

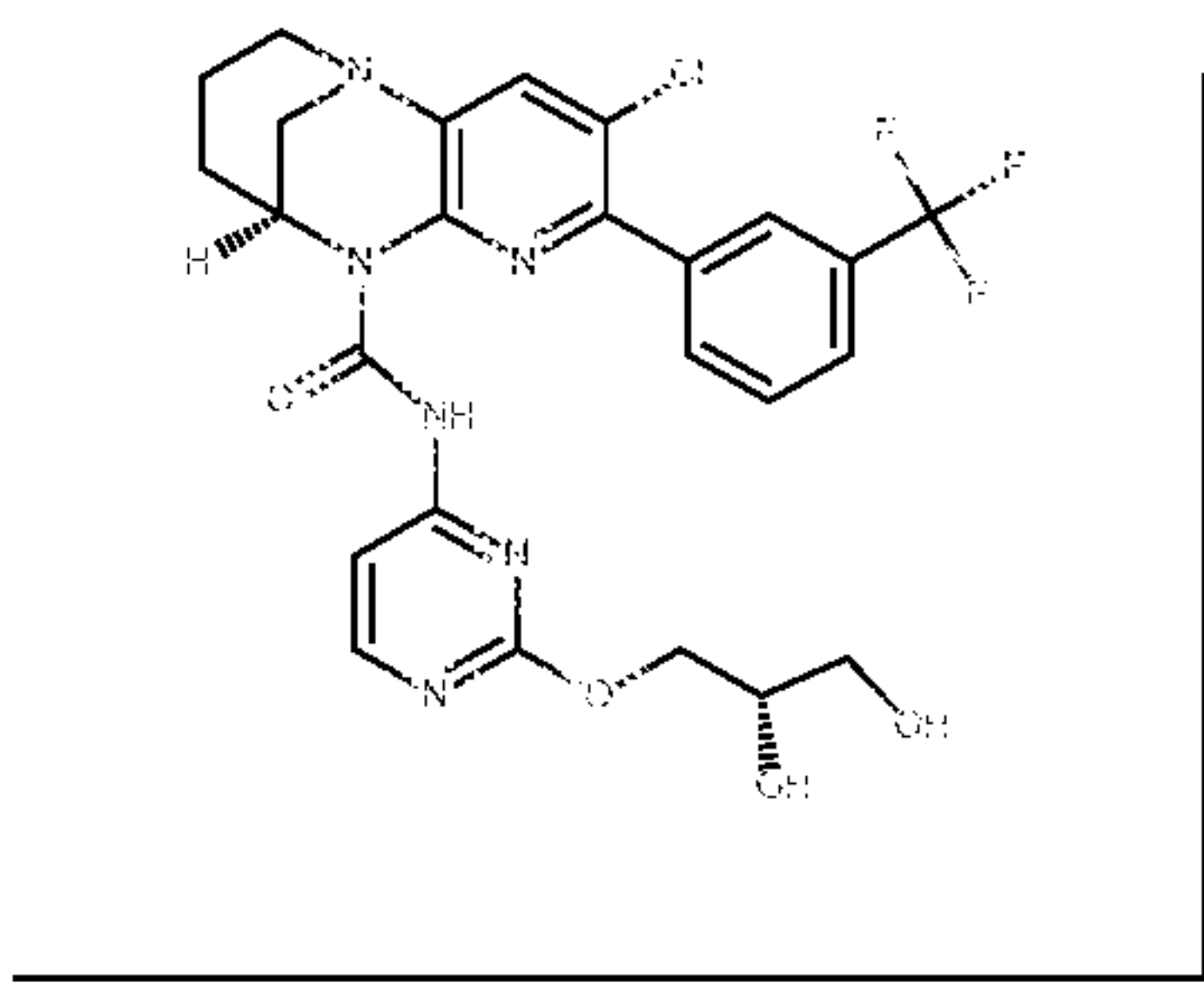
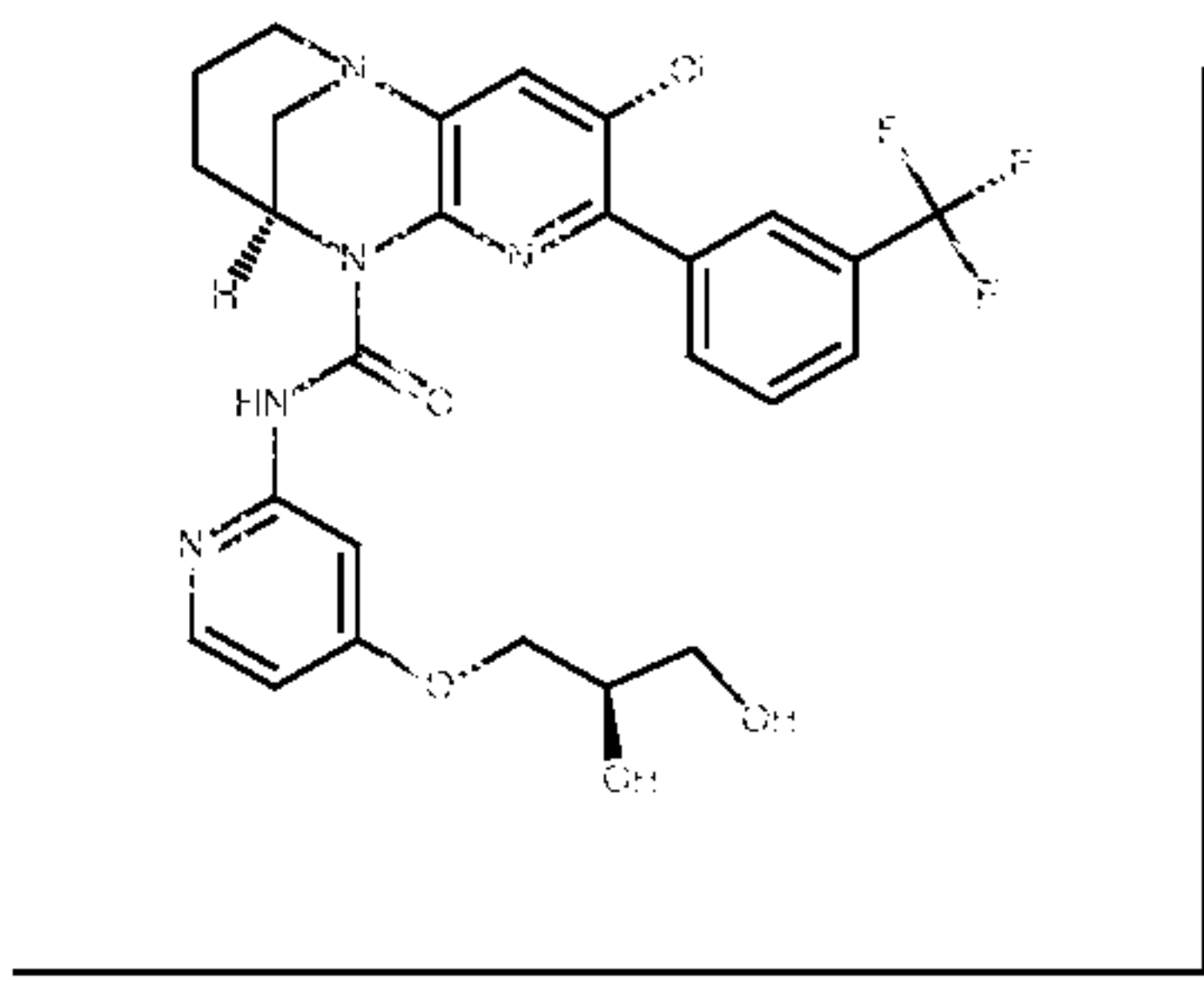
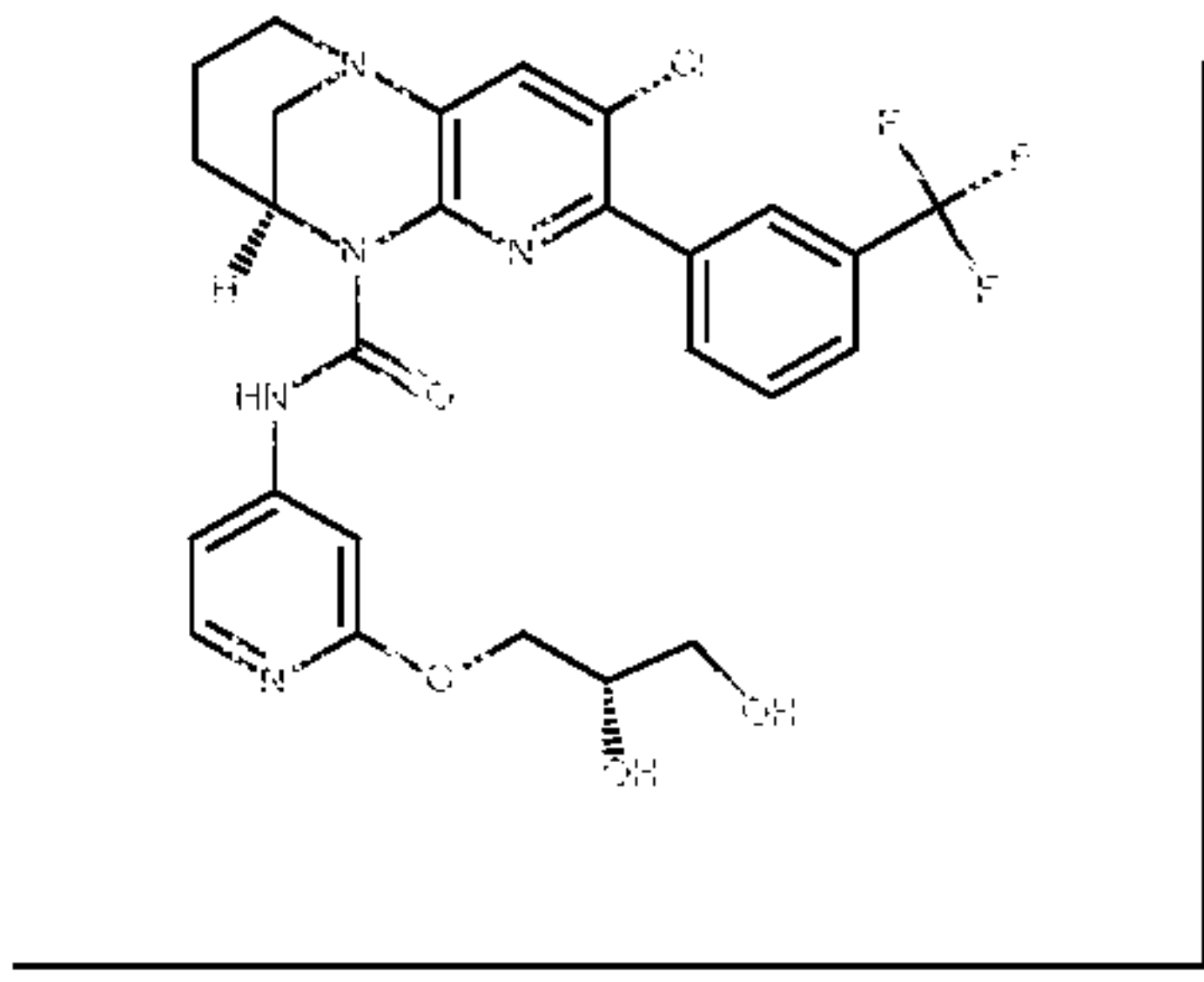
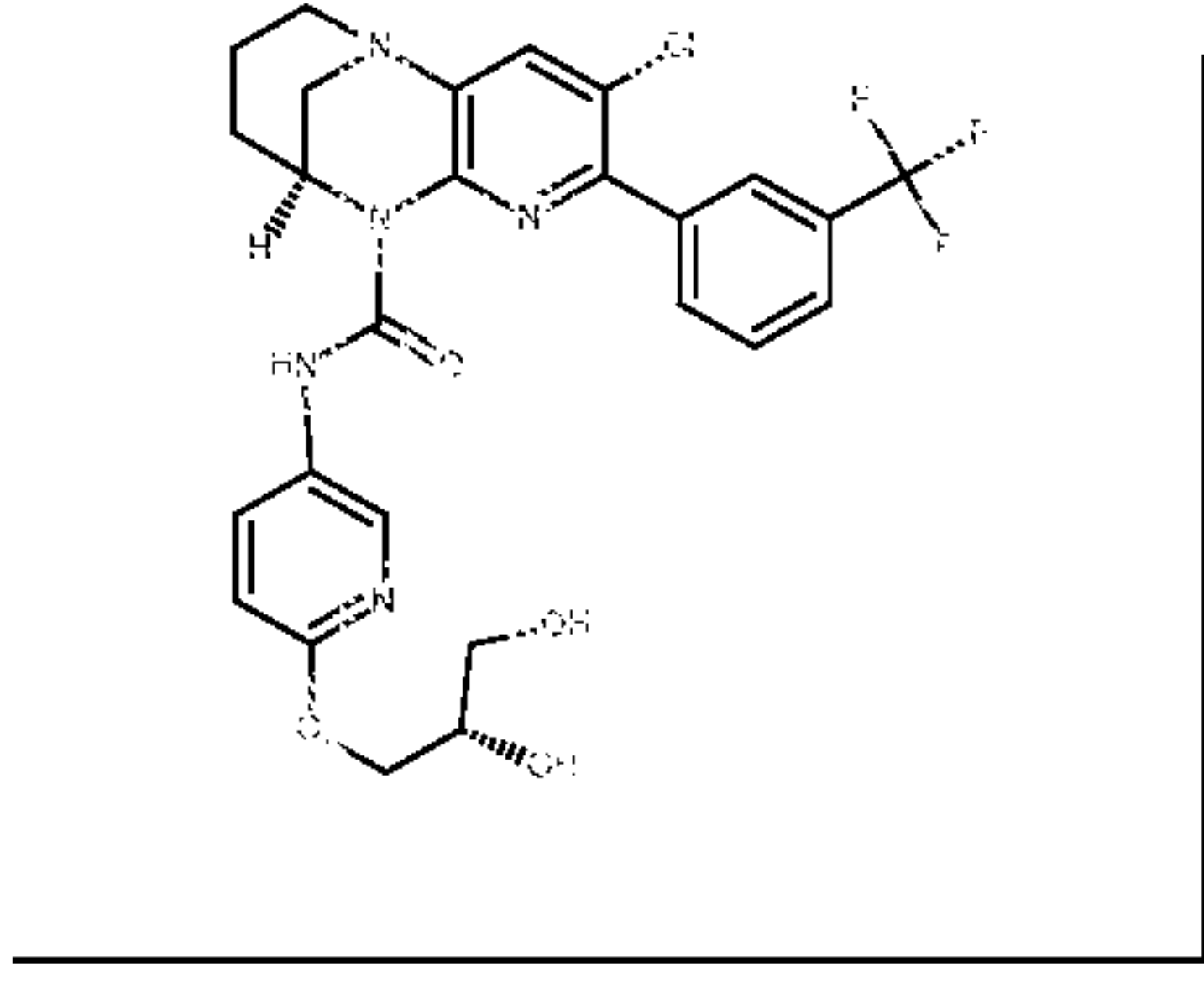
Example number	Structure	Chemical Name: Generated by CHemAxon	TRP Acti ty	TRP MAX RESP
56		(9S)-8-N-{5-[(2S)-2,3-dihydroxypropoxy]pyridin-2-yl}-3-methyl-5-N-[(2R)-1,1,1-trifluoropropan-2-yl]-1,6,8-triazatricyclo[7.3.1.0²,⁷]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
57		(9S)-8-N-{4-[(2R)-2,3-dihydroxypropoxy]pyridin-2-yl}-3-methyl-5-N-[(2R)-1,1,1-trifluoropropan-2-yl]-1,6,8-triazatricyclo[7.3.1.0²,⁷]trideca-2(7),3,5-triene-5,8-dicarboxamide	A	A
58		(9S)-N-(5-fluoropyridin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0²,⁷]trideca-2(7),3,5-triene-4-carboxamide		B
59		(9S)-N-(pyrazin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0²,⁷]trideca-2(7),3,5-triene-4-carboxamide		B
60		(9S)-N-(pyridin-3-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0²,⁷]trideca-2(7),3,5-triene-4-carboxamide		B

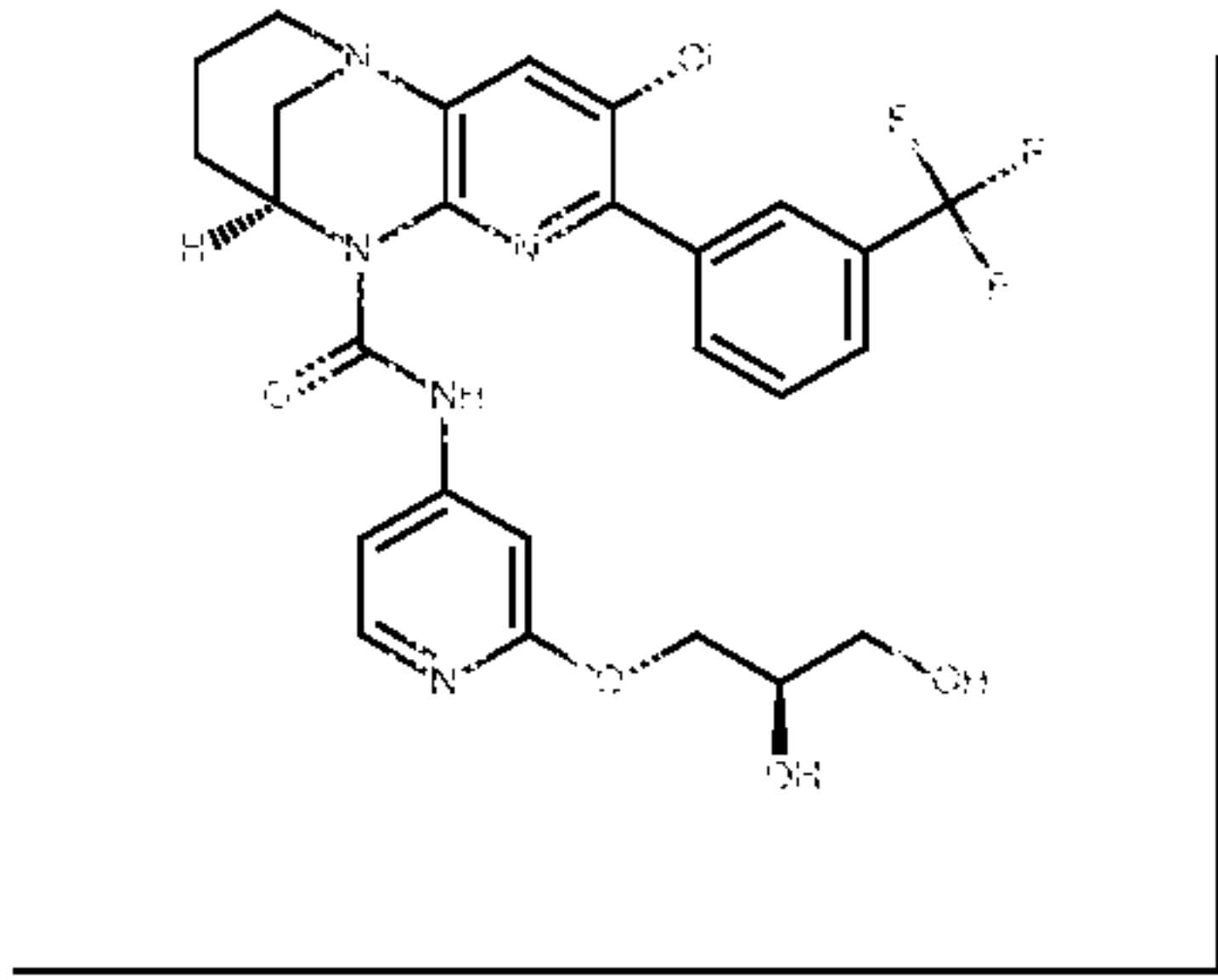
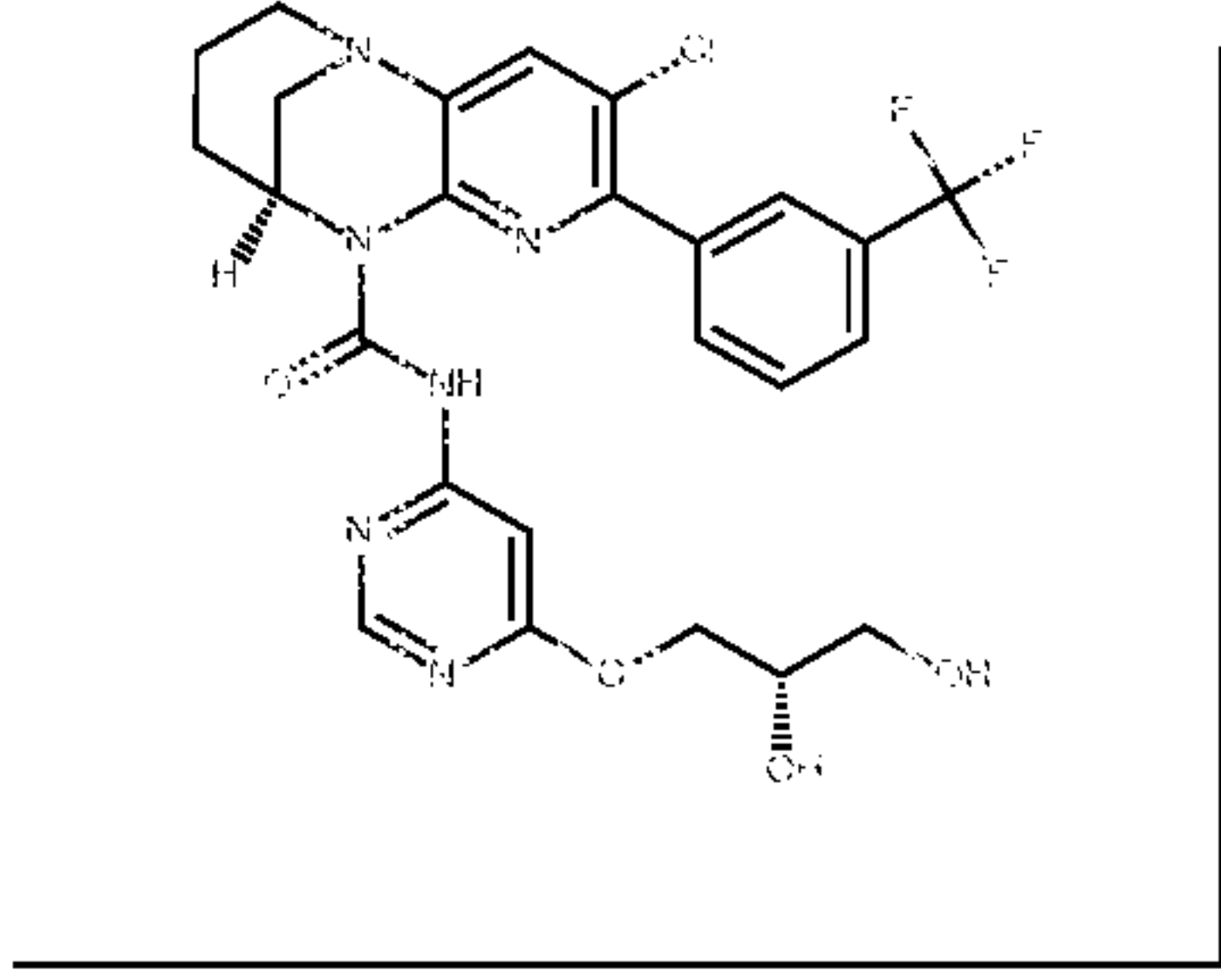
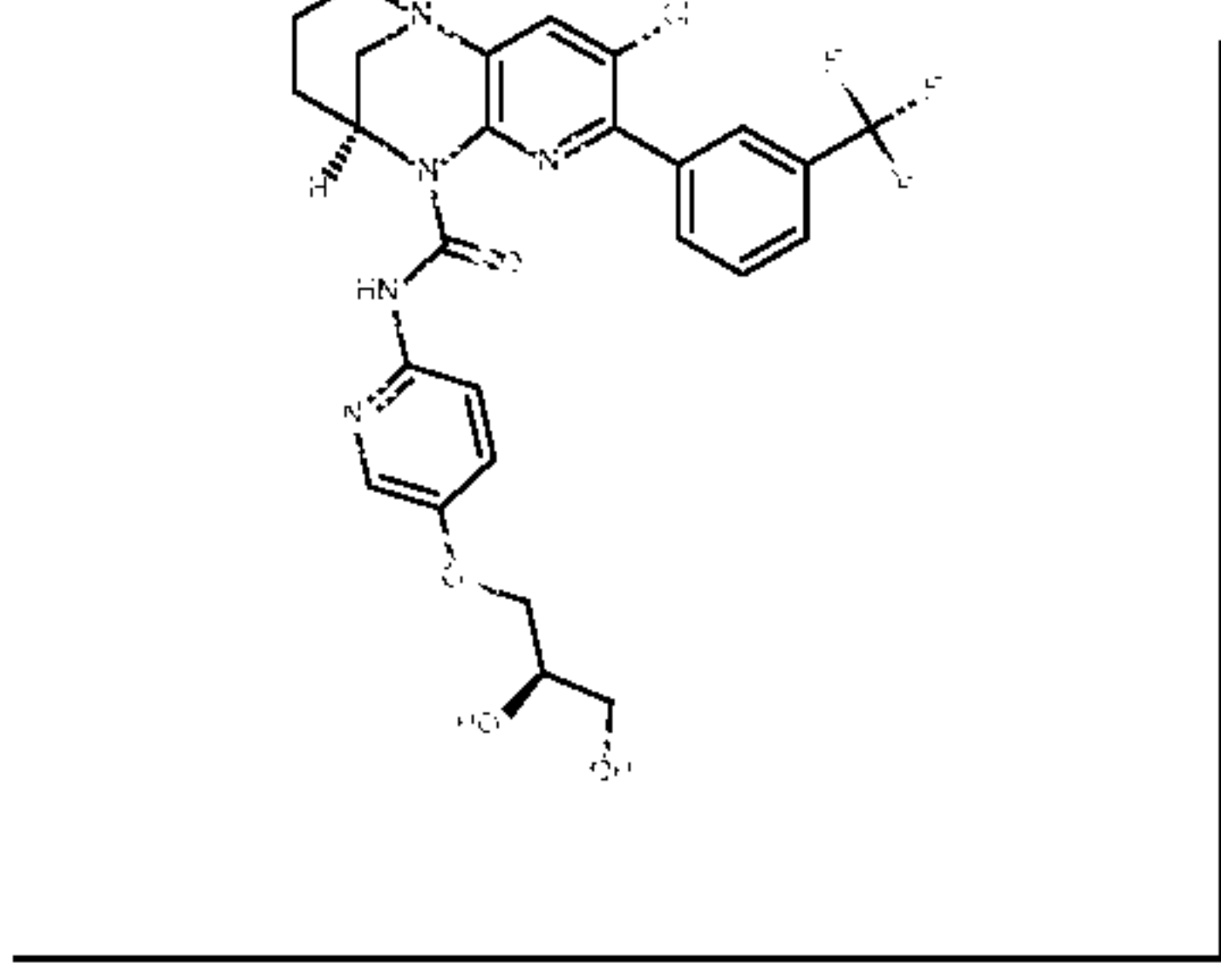
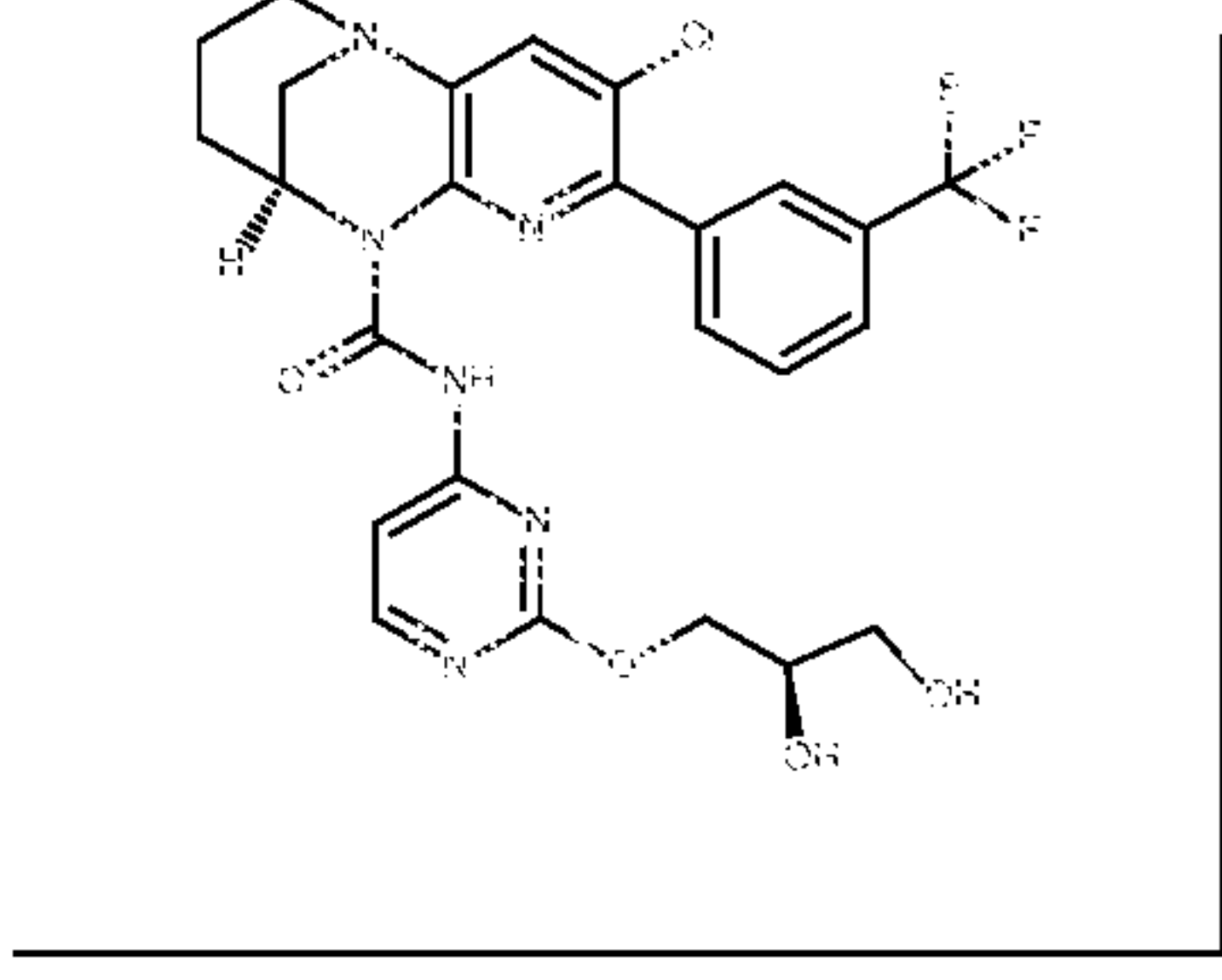
Example number	Structure	Chemical Name: Generated by CHemAxon	TRP Acti- ty	TRP MAX RESP
61	 ISOMER 2	(9S)-N-(pyridin-3-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-4-carboxamide		B
62	 ISOMER 2	(9S)-N-(pyrazin-2-yl)-5-[(3S)-3-(trifluoromethyl)piperidin-1-yl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-4-carboxamide		B
63		(9S)-4-chloro-N-(pyridin-2-yl)-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
64		(9S)-4-chloro-N-{6-[(2S)-2,3-dihydroxypropoxy]pyridin-2-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
65		(9S)-4-chloro-N-{6-[(2R)-2,3-dihydroxypropoxy]pyridin-2-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A

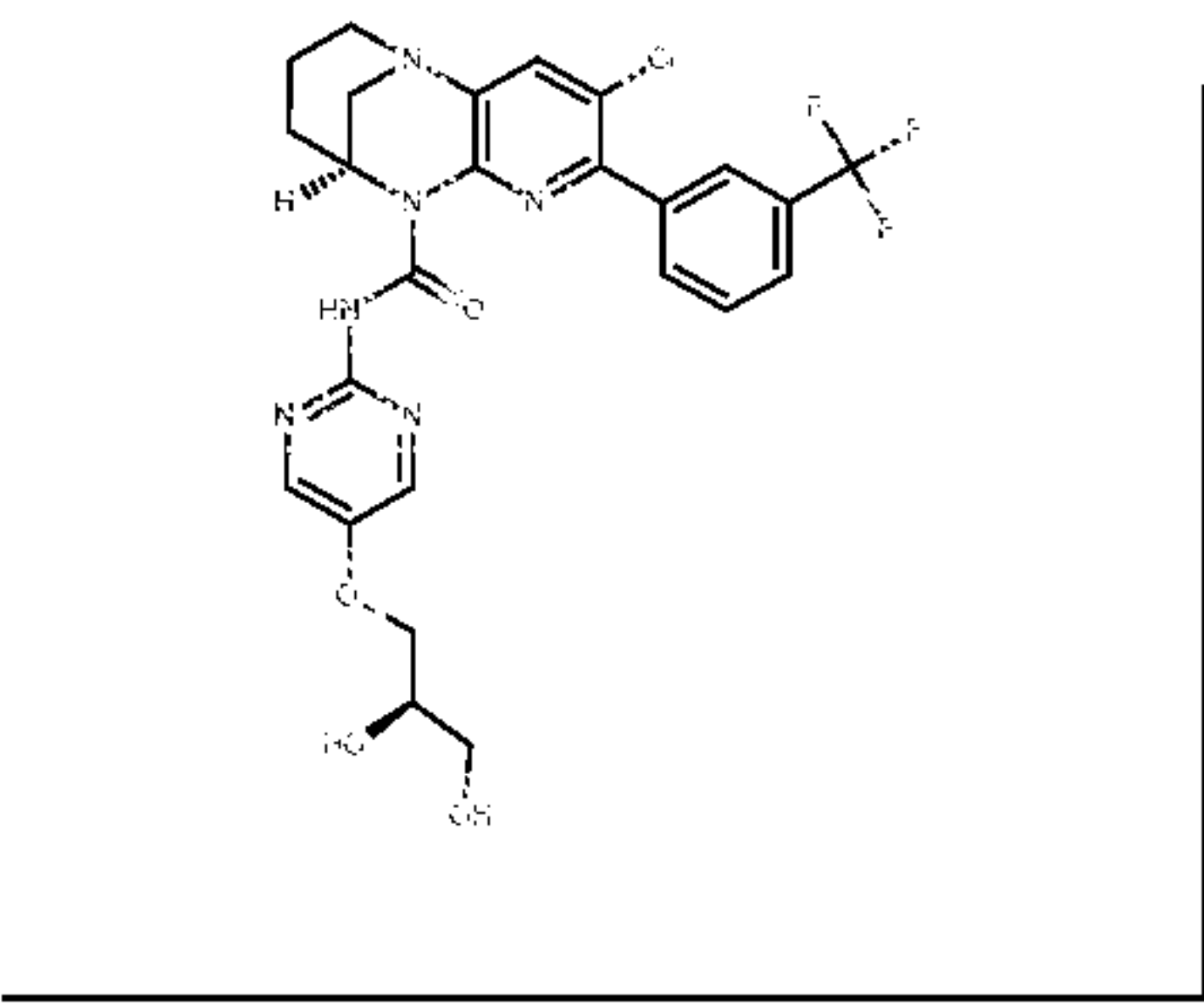
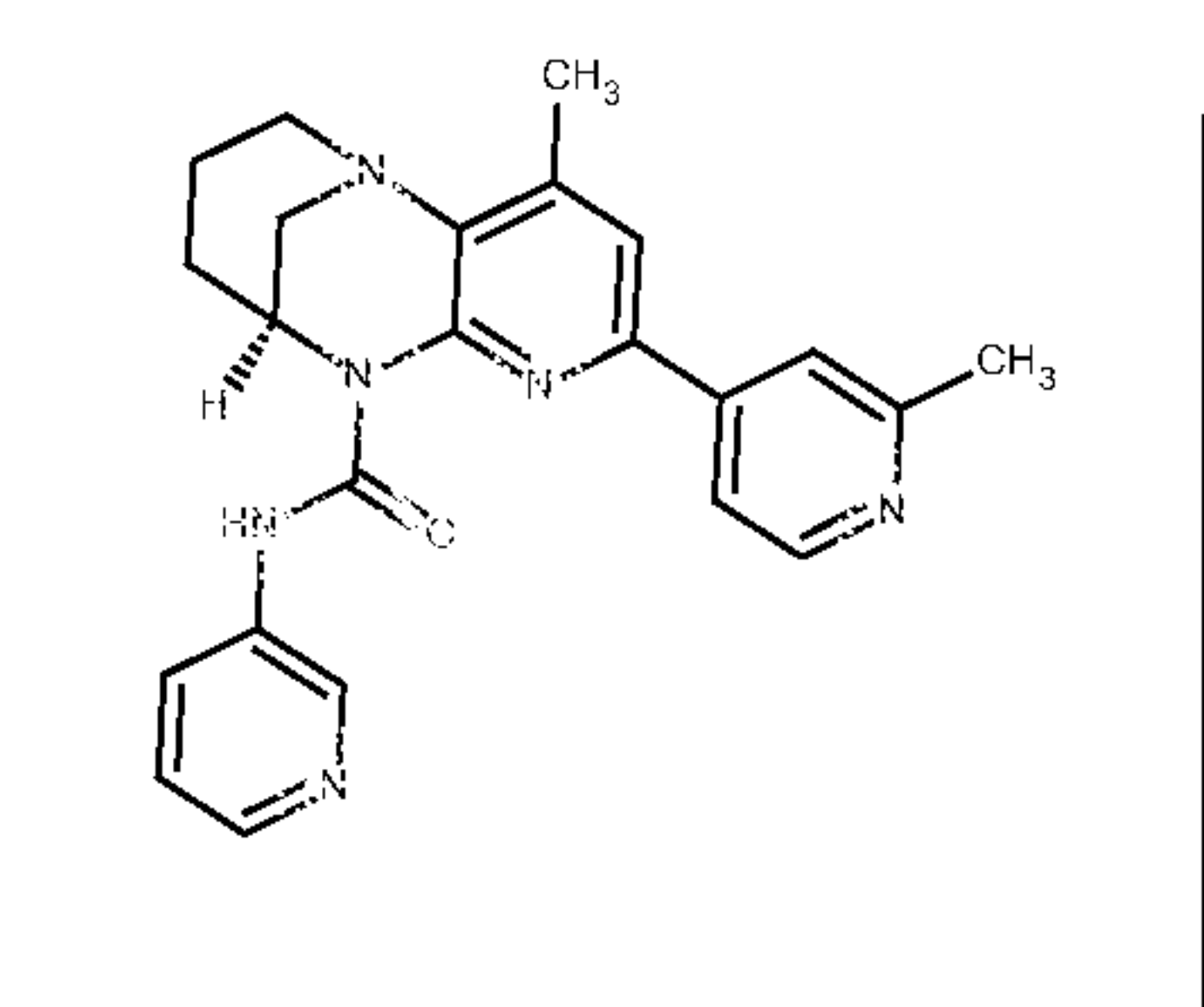
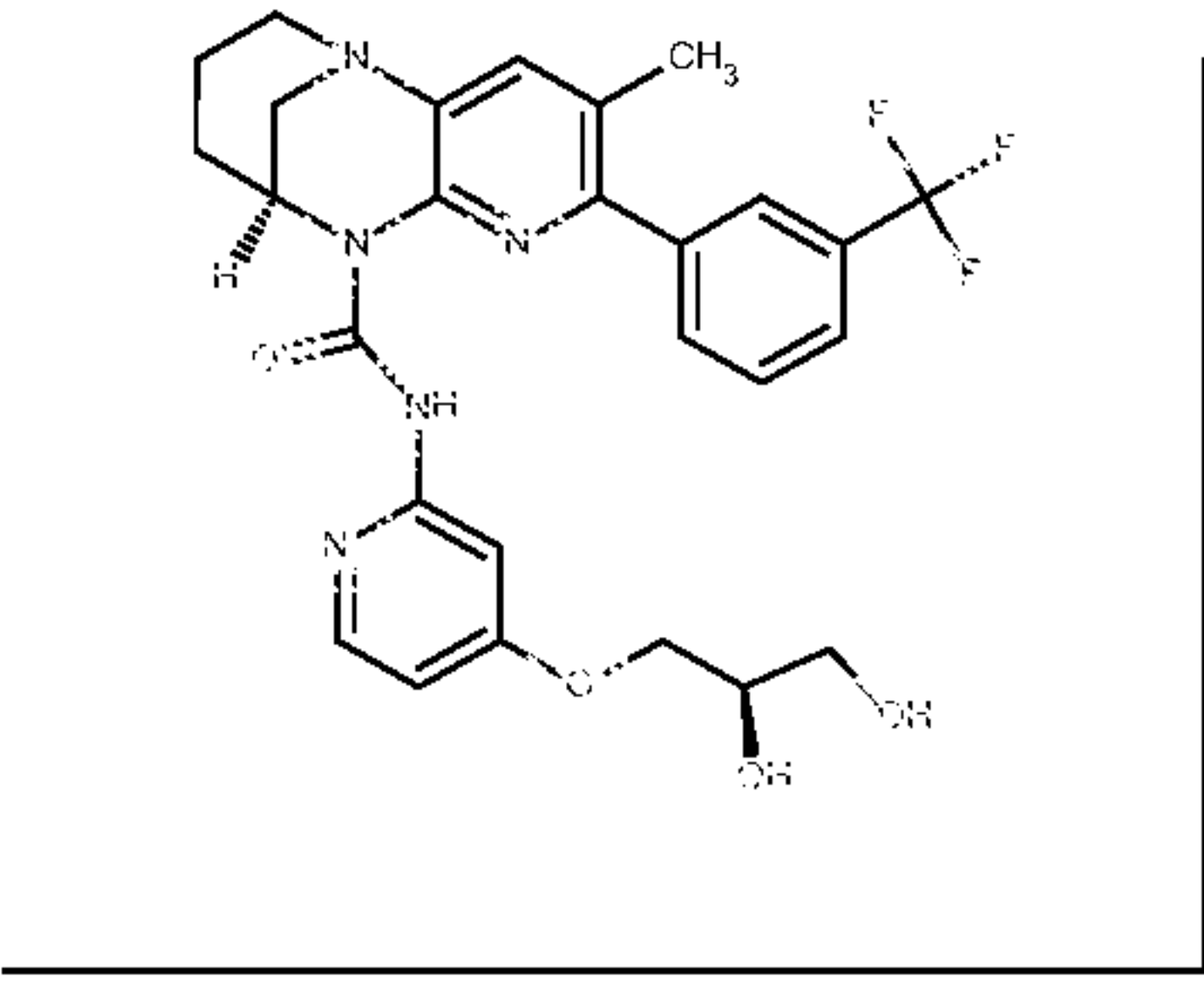
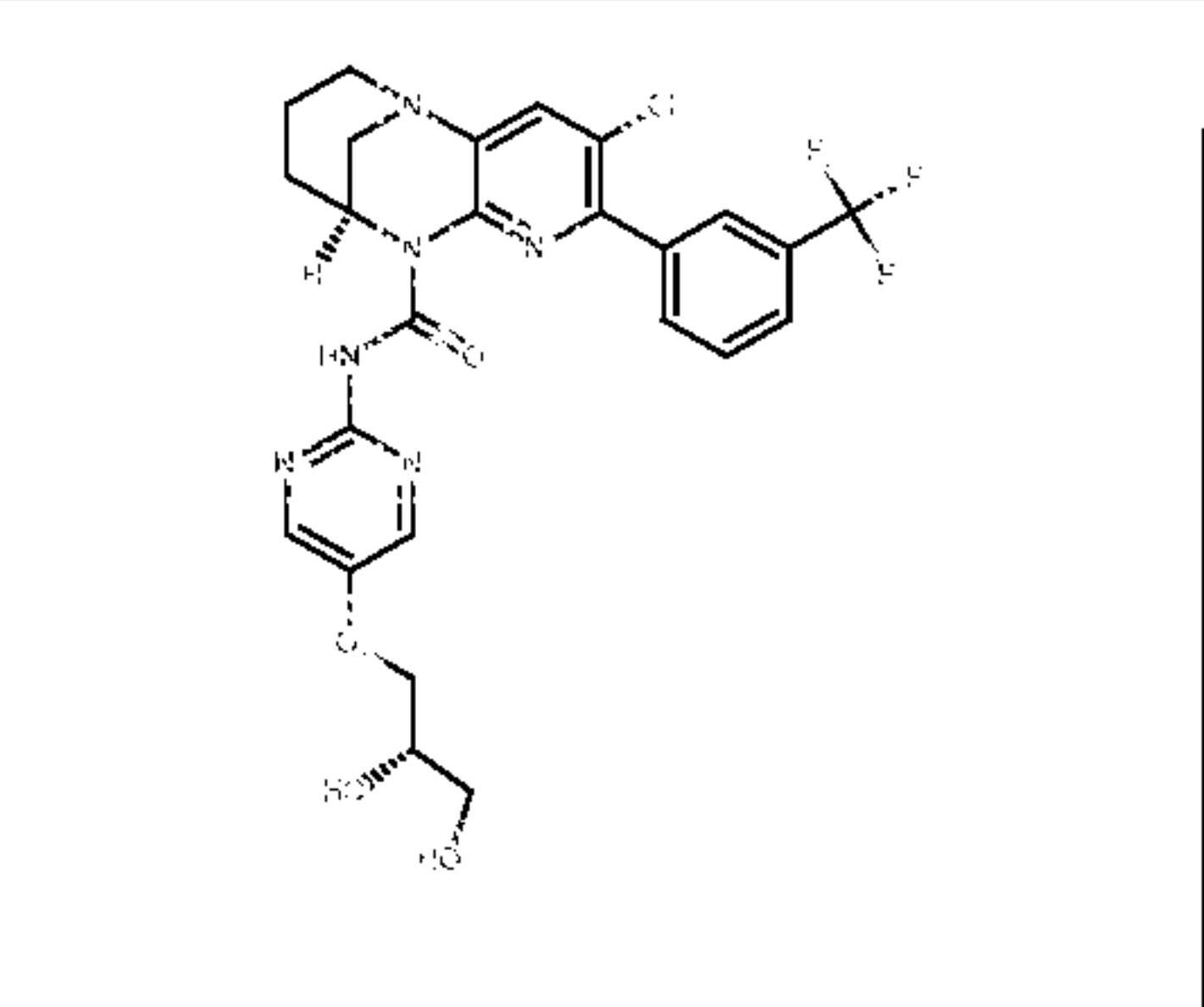
Example number	Structure	Chemical Name: Generated by CHemAxon	TRP Acti- ty	TRP MAX RESP
66		(9S)-4-chloro-5-(2-methylpyridin-4-yl)-N-(pyridin-3-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	B	A
67		(9S)-4-chloro-N-{6-[(2S)-2,3-dihydroxypropoxy]pyrazin-2-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
68		(9S)-4-methyl-5-(2-methylpyridin-4-yl)-N-(pyridin-3-yl)-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
69		(9S)-4-chloro-N-{2-[(2R)-2,3-dihydroxypropoxy]pyrimidin-5-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
70		(9S)-4-chloro-N-{6-[(2R)-2,3-dihydroxypropoxy]pyrimidin-4-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2,4,6-triene-8-carboxamide	B	A

Example number	Structure	Chemical Name: Generated by CHemAxon	TRP Activi ty	TRP MAX RESP
71		(9S)-4-chloro-N-cyclopropyl-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
72		(9S)-4-chloro-N-{2-[(2S)-2,3-dihydroxypropoxy]pyrimidin-5-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
73		(9S)-4-chloro-N-{5-[(2R)-2,3-dihydroxypropoxy]pyrazin-2-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
74		(9S)-4-chloro-N-{5-[(2S)-2,3-dihydroxypropoxy]pyridin-2-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
75		(9S)-4-chloro-N-{4-[(2S)-2,3-dihydroxypropoxy]pyrimidin-2-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A

Example number	Structure	Chemical Name: Generated by CHemAxon	TRP Activi ty	TRP MAX RESP
76		(9S)-4-chloro-N-{5-[(2R)-2,3-dihydroxypropoxy]pyridin-3-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
77		(9S)-4-chloro-N-{5-[(2S)-2,3-dihydroxypropoxy]pyrazin-2-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
78		(9S)-4-chloro-N-{4-[(2R)-2,3-dihydroxypropoxy]pyrimidin-2-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
79		(9S)-4-chloro-N-{6-[(2R)-2,3-dihydroxypropoxy]pyridin-3-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
80		(9S)-4-chloro-N-{5-[(2S)-2,3-dihydroxypropoxy]pyridin-3-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-	A	A

Example number	Structure	Chemical Name: Generated by CHemAxon	TRP Acti- ty	TRP MAX RESP
		8-carboxamide		
81		(9S)-4-chloro-N-{2-[(2S)-2,3-dihydroxypropoxy]pyrimidin-4-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
82		(9S)-4-chloro-N-{4-[(2R)-2,3-dihydroxypropoxy]pyridin-2-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
83		(9S)-4-chloro-N-{2-[(2S)-2,3-dihydroxypropoxy]pyridin-4-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
84		(9S)-4-chloro-N-{6-[(2S)-2,3-dihydroxypropoxy]pyridin-3-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A

Example number	Structure	Chemical Name: Generated by CHemAxon	TRP Activi ty	TRP MAX RESP
85		(9S)-4-chloro-N-{2-[(2R)-2,3-dihydroxypropoxy]pyridin-4-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
86		(9S)-4-chloro-N-{6-[(2S)-2,3-dihydroxypropoxy]pyrimidin-4-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2,4,6-triene-8-carboxamide	A	A
87		(9S)-4-chloro-N-{5-[(2R)-2,3-dihydroxypropoxy]pyridin-2-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A
88		(9S)-4-chloro-N-{2-[(2R)-2,3-dihydroxypropoxy]pyrimidin-4-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0 ^{2,7}]trideca-2(7),3,5-triene-8-carboxamide	A	A

Example number	Structure	Chemical Name: Generated by CHemAxon	TRP Activity	TRP MAX RESP
89		(9S)-4-chloro-N-{5-[(2R)-2,3-dihydroxypropoxy]pyrimidin-2-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0²,⁷]trideca-2(7),3,5-triene-8-carboxamide	A	A
90		(9S)-3-methyl-5-(2-methylpyridin-4-yl)-N-(pyridin-3-yl)-1,6,8-triazatricyclo[7.3.1.0²,⁷]trideca-2,4,6-triene-8-carboxamide	A	A
91		(9S)-N-{4-[(2R)-2,3-dihydroxypropoxy]pyridin-2-yl}-4-methyl-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0²,⁷]trideca-2,4,6-triene-8-carboxamide	A	A
92		(9S)-4-chloro-N-{5-[(2S)-2,3-dihydroxypropoxy]pyrimidin-2-yl}-5-[3-(trifluoromethyl)phenyl]-1,6,8-triazatricyclo[7.3.1.0²,⁷]trideca-2,4,6-triene-8-carboxamide	A	A

Example 96

5 The present invention relates to Sirtuin Modulators, which are known in the scientific literature for being useful for increasing lifespan of a cell, and in treating

and/or preventing a wide variety of diseases and disorders, which include, but are not limited to, for example, diseases or disorders related to aging or stress, diabetes, obesity, neurodegenerative diseases, cardiovascular disease, blood clotting disorders, inflammation, cancer, and/or flushing as well as diseases or disorders that would benefit
 5 from increased mitochondrial activity.

In addition to therapeutic potential, structural and biophysical studies of SIRT1 activity and activation by small molecule sirtuin modulators would be useful in advancing understanding of the biological function of sirtuins, mechanism of action of sirtuin activation and to aid in development of assays that identify novel sirtuin modulators.

10 Based on the foregoing, the following literature references, respectively, are cited to demonstrate the utility of compounds of the present invention as Sirtuin Modulators and its interconnection with various diseases as exemplified or disclosed in the following references:

- 15 1. Marcia C. Haigis and David A. Sinclair, Mammalian Sirtuins: Biological Insights and Disease Relevance, *Annu Rev Pathol.* 2010 ; 5: 253–295.

Haigis and Sinclair teach:

20 “Aging is accompanied by a decline in the healthy function of multiple organ systems, leading to increased incidence and mortality from diseases such as type II diabetes mellitus, neurodegenerative diseases, cancer, and cardiovascular disease. Historically, researchers have focused on investigating individual pathways in isolated organs as a strategy to identify the root cause of a disease, with hopes of designing better drugs. Studies of aging in yeast led to the discovery of a family of conserved
 25 enzymes known as the sirtuins, which affect multiple pathways that increase the life span and the overall health of organisms. Since the discovery of the first known mammalian sirtuin, SIRT1, 10 years ago, there have been major advances in our understanding of the enzymology of sirtuins, their regulation, and their ability to broadly improve mammalian physiology and health span. This review summarizes
 30 and discusses the discovery advances of the past decade and the challenges that will confront the field in the coming years (see, ABSTRACT, therein and reference).”

- 35 2. Gizem Donmez et al., SIRT1 and SIRT2: emerging targets in neurodegeneration, *EMBO Mol Med* (2013) 5, 344–352.

Gizem Donmez et al., teaches:

40 “Sirtuins are NAD-dependent protein deacetylases known to have protective effects against age-related diseases such as cancer, diabetes, cardiovascular and neurodegenerative diseases. In mammals, there are seven sirtuins (SIRT1-7), which

display diversity in subcellular localization and function. While SIRT1 has been extensively investigated due to its initial connection with lifespan extension and involvement in calorie restriction, important biological and therapeutic roles of other sirtuins have only recently been recognized. Here, we review the potential roles and effects of SIRT1 and SIRT2 in neurodegenerative diseases. We discuss different functions and targets of SIRT1 and SIRT2 in a variety of neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's Disease (HD). We also cover the role of SIRT1 in neuronal differentiation due to the possible implications in neurodegenerative conditions, and conclude with an outlook on the potential therapeutic value of SIRT1 and SIRT2 in these disorders (see, ABSTRACT, therein and reference)."

3. Bracke et al., Targeted silencing of DEFB4 in a bioengineered skin-humanized mouse model for psoriasis: development of siRNA SECosome-based novel therapies; Exp Dermatol. 2014 Mar;23(3):199-201. doi: 10.1111/exd.12321.

In particular, Bracke et al. teaches

"Psoriasis is a complex inflammatory skin disease that presents a wide variety of clinical manifestations. Human β defensin-2 (hBD-2) is highly up-regulated in psoriatic lesions and has been defined as a biomarker for disease activity. We explored the potential benefits of targeting hBD-2 by topical application of DEFB4-siRNA-containing SECosomes in a bioengineered skin-humanized mouse model for psoriasis. A significant improvement in the psoriatic phenotype was observed by histological examination, with a normalization of the skin architecture and a reduction in the number and size of blood vessels in the dermal compartment. Treatment leads to the recovery of transglutaminase activity, filaggrin expression and stratum corneum appearance to the levels similar to those found in normal regenerated human skin. The availability of a reliable skin-humanized mouse model for psoriasis in conjunction with the use of the SECosome technology may provide a valuable preclinical tool for identifying potential therapeutic targets for this disease."

4. Karline Guilloteau et al., Skin Inflammation Induced by the Synergistic Action of IL-17A, IL-22 Recapitulates Some Features of Psoriasis Oncostatin M, IL-1a, and TNF-a, *J Immunol* 2010; 184:5263-5270.

Guilloteau et al. teaches:

"Keratinocytes play a crucial role in the regulation of skin inflammation, responding to environmental and immune cells stimuli. They produce soluble factors that can act in an autocrine or paracrine manner on immune cells or directly on aggressors. A screening of the activities of 36 cytokines on keratinocyte gene expression identified IL-17A, IL-22, oncostatin M, TNF-a, and IL-1a as potent cytokines in inducing cutaneous inflammation. These five proinflammatory cytokines synergistically increased production of CXCL8 and b-defensin 2 (BD2). In addition, ex vivo studies on human skin explants demonstrated upregulation of BD2, S100A7, and CXCL8 expression in response to the same combination of

cytokines. In vivo intradermal injection of these five cytokines in mouse increased CXCL1, CXCL2, CXCL3, S100A9, and BD3 expression, associated with neutrophil infiltration. We confirmed and extended this synergistic effect using quantitative real-time PCR analysis and observed increased expression of nine chemokines and 12 antimicrobial peptides. Production of CXCL, CXCL5, and CXCL8 by keratinocytes stimulated in the presence of this cytokine combination was associated with increased neutrophil chemotactic activity. Similarly, high production of BD2, BD3, and S100A7 was associated with an increased antimicrobial activity. Finally, the transcriptional profile observed in this in vitro model of inflammatory keratinocytes correlated with the one of lesional psoriatic skin. Our results demonstrate the important potentiating activities of IL-17A, IL-22, oncostatin M, TNF- α , and IL-1 α on keratinocytes. This is particularly interesting in the context of psoriasis where these cytokines are overexpressed and could synergize to play an important role in upregulation of chemokines and antimicrobial peptides production. The Journal of Immunology, 2010, 184: 5263–5270 (see, ABSTRACT, therein and reference)”.
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 10
 15

Example 97

Description of assays:

20 PBMC ASSAY

Sirtuin 1 (Sirt1) is a homolog of silent information regulator 2 (Sir2) and a member of the NAD dependent class III histone deacetylase. Sirt1 deacetylates lysine residues on histones, transcription factors and nonhistone proteins. Sirt1 has been shown to be involved in aging, cell cycle regulation, apoptosis, metabolic modulation and inflammation. The activation of Sirt1 causes deacetylation at lysine 310 of RelA/p65 subunit of nuclear factor κ B (NF- κ B) transcriptional factor which inhibits NF- κ B transcription and down-regulates levels of TNF α . TNF α is a pleiotropic cytokine that is mainly produced by macrophages and monocytes. TNF α is closely involved in immune defense and chronic inflammation including Psoriasis. The expression of type-1 cytokines such as TNF α was known to be increased in psoriatic skin and it plays important role in the etiology of psoriasis (Uyemura K et al, 1993, J. Invest Dermatol, 101, p701). Importantly, anti-TNF agent has been in clinical use for psoriasis. Therefore, Sirt1 activators that induce a reduction in TNF α expression in inflammatory cells should have therapeutic effect in moderate to severe psoriatic patients.
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A PBMC/TNF α cell based assay was developed to identify activators of Sirt1 that inhibit the release of TNF α in response to lipopolysaccharide (LPS) stimulation of peripheral blood mononuclear cells (PBMC's). Briefly, PBMC's were stimulated by LPS, leading to an increase in the production of TNF α secretion. TNF α protein level

was measured by TNFalpha HTRF (homogeneous time resolved fluorescence) kit (CisBio, Inc). Cell lysis and TNFalpha detection were performed according to manufacturer's instructions. Sirt1 activators were tested in the presence of LPS to evaluate their inhibitory effect on TNFa release and IC50 were determined in a dose-response experiment.

5

Beta-defensin 2 (bD2) ASSAY

Sirtuin is a family of NAD-dependent deacetylases which have broad physiological functions and have been implicated in a number of autoimmune and metabolic disorders including rheumatoid arthritis and type I diabetes. Substrates of SIRT1 are diverse and
10 include inflammatory components with well established roles in innate and adaptive immune response such as NF- κ B, AP-1, FOXO, and p53.

Psoriasis is a chronic inflammatory skin disorder induced by genetic, autoimmune, and environmental factors. Lesions are characterized by hyperproliferation of keratinocytes in the epidermis and infiltration of inflammatory cells resulting in chronic
15 erythematous plaques covered by white scales. Previous studies have shown that SIRT1 can impede the effects of IL-22, a key cytokine in psoriasis, through direct inhibition of STAT3 acetylation (Sestito et al, 2011). In addition, both SIRT1 overexpression and resveratrol treatment (SIRT1 activation) can induce keratinocyte differentiation (Blander et al, 2009).

20 Beta-defensin 2 (bD2) is an antimicrobial peptide that can be secreted from the epithelia where it acts as a chemoattractant for memory T-cells, immature dendritic cells, and neutrophils. As such, bD2 is a major part of the inflammatory response in the skin. Not only is bD2 induced in lesional epidermal cells of psoriasis patients compared to normal skin, but it is also a serum biomarker for disease severity in psoriasis patients
25 (Jansen et al, 2009; Kamsteeg et al 2009). In addition, bD2 may be genetically linked to psoriasis as a recent study uncovered a significant association between increased beta-defensin gene copy number and psoriasis risk (Hollox et al, 2008). Of note, topical delivery of bD2 siRNA resulted in recovery of normal skin architecture and protein expression in a bioengineered skin-humanized mouse model for psoriasis (Bracke et al,
30 2014).

An *in vitro* keratinocyte inflammation assay generated to mimic psoriatic inflammation was previously described (Guilloteau et al, 2010; Teng et al 2014). In these studies, a cytokine cocktail of IL-1alpha, IL-17A, IL-22, OSM, and TNFalpha (referred to

as “M5”) was found to synergize to produce a “psoriasiform” transcriptional profile in primary human keratinocytes *in vitro*. In these studies, bD2 was one of the strongest responders to the induction of keratinocyte inflammation.

Therefore, this assay was further developed in order to assess the efficacy of SIRT1 activator compounds for the topical psoriasis program. Specifically, conditions were optimized for an immortalized human keratinocyte cell line (HaCaT) treated *in vitro* with the M5 cytokine combination to induce psoriatic inflammation (as in reference above). In a 48 hour time frame, bD2 secretion, as measured by a bD2 ELISA assay (Alpha Diagnostics), is significantly increased compared to unstimulated keratinocytes. This bD2 induction can be suppressed with treatment of compounds known to suppress psoriatic inflammation or, importantly, with a subset of SIRT1 activators. In parallel, cytotoxicity over the length of the 48 hour assay is ascertained by a CellTiter-Glo Luminescent Cell Viability Assay (Promega) to determine whether toxicity might play a role in bD2 response.

References:

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Psoriasis & IL-17

- 15 Psoriasis is a chronic, relapsing, inflammatory autoimmune skin disorder with a multifactorial pathogenesis influenced by genetic, environmental, and immunopathologic factors (Griffiths CE et al., *Lancet* 2007;370:263-71) . Psoriasis is characterized by recurrent episodes of raised, well-demarcated erythematous oval plaques with adherent silvery scales. Histologically, the hallmark of psoriasis is the presence of a thickened nucleated keratinocyte layer, with exaggeration of the rete pegs, caused by
- 20 hyperproliferation of keratinocytes and dermal infiltration by activated T cells, neutrophils, and dendritic cells (Schon MP N. *Engl. J. Med.* 352: 1899–1912).

- 25 An accumulating body of evidence suggests psoriasis as a Th17-mediated disease, driven by its signature cytokines IL-17 A, IL-17 F and IL-22. IL-22 induces proliferation of keratinocytes, whereas IL-17A stimulates keratinocytes to secrete chemokines and other proinflammatory mediators that recruit additional inflammatory cells, including neutrophils, dendritic cells, and innate lymphoid cells (Martin DA et al, *J Invest Dermatol* 2013; 133:17-26).

- 30 The clinical validation of the IL-17 pathway in mediating psoriasis is demonstrated by successful Ph3 studies that show significant improvement of disease using monoclonal antibody therapy targeting IL-17 (Langley et al., *NEJM* 2014). In addition, global transcription profiling in psoriasis lesions following IL-17 inhibition suppressed multiple

inflammatory factors from keratinocytes and leukocyte subsets to similar levels as observed in non-lesional skin (Russell et al., J Immunol 2014, 192: 3828–3836). Taken together, these findings support the role of IL-17 in mediating psoriasis pathogenesis.

5 **Method (Ex Vivo Skin Assay)**

Stimulation of skin-resident immune cells in ex vivo human skin explants using a Th17 cytokine cocktail results in a dramatic upregulation of Th17 related cytokines (IL-17A, IL-17F and IL-22), which establishes this system as a human tissue-based model for psoriasis. The ability of test compounds to modulate the expression of IL-17A, IL-17 F and IL-22 was assessed using the ex vivo skin culture method post stimulation with Th17 cytokine cocktail.

Briefly, ex vivo human skin obtained from abdominoplasty surgery was processed to remove fat and the tissue was dermatomed to ~750 microns. Dermatomed skin was then cleaned in two serial rinses of 5-10 minutes each in room temperature PBS containing an antibiotic/antimycotic solution. The skin section was cut with disposable single-use biopsy punches to 10 mm diameter round sections, which were then placed in the upper chamber of a 0.4 µm PCF membrane transwell (Millicell #PIHP01250) containing 30 µl of a 64% bovine collagen solution (Organogenesis, #200-055) prepared with Cornification media. The skin samples were allowed to set on the collagen solution for 30 min at 37°C in a humidified chamber. The skin samples on transwells were transferred to 6-well plates (1 sample per well) and the lower chamber was filled with 1 ml complete media (Cornification Media).

On the first day following abdominoplasty surgery, skin explants were cultured in Cornification media and allowed to incubate overnight at 37°C. Specifically, human skin explants (N=3 per condition) were stimulated with the Th17 cocktail (CD3, 1 µg/ml, CD28, 2 µg/ml, IL-1b, 10 ng/ml, IL-6, 5 ng/ml, TGFb, 1 ng/ml, IL-21, 10 ng/ml, anti-IL-4, 1 µg/ml and anti-INFg, 1 µg/ml). Test compound at 1,3 and 10uM was added at the same time as Th17 cocktail. Tissue was harvested 24 hrs after Th17 activation and RNA was isolated for transcript quantification (IL-17A, IL-17F, IL-22) using qPCR.

Total RNA was isolated from ~40 mg of tissue using Qiagen's Mini RNA Isolation kit (Cat # 74106). Briefly, tissue was minced and homogenized in the Precellys-24 machine using 300 µl of RLT buffer supplemented with 1% 2-Beta-Mercapto-Ethanol at 6300 rpm

for 30 seconds for 10 cycles with a 2-minute ice break. 490 µl of water containing 10 µl Proteinase K was added to the homogenate and digested at 55 °C for 15 minutes.

Digested tissue was spun down for 3 minutes at 10,000 G to pellet cell debris and the

supernatant was used for RNA isolation using Qiagen's RNeasy mini columns according

5 to manufacturer's protocol. Total RNA was quantified using Nanodrop 2000 and analyzed on Agilent bioanalyser (files attached). 1.4 µg of RNA was used as template in a 20 µl

PCR volume using Invitrogen SuperScript VILO cDNA Synthesis kit (# 11754-050) to

create a cDNA template. Then cDNA was diluted 1:25 for the subsequent qPCR with the specific TaqMan probe for each gene to be quantified. RNA levels of gene of interest's

10 relative expression were calculated using the Delta Delta CT formula.

EQUIVALENTS

The present invention provides among other things sirtuin-modulating compounds and methods of use thereof. While specific embodiments of the subject invention have

15 been discussed, the above specification is illustrative and not restrictive. Many variations

of the invention will become apparent to those skilled in the art upon review of this

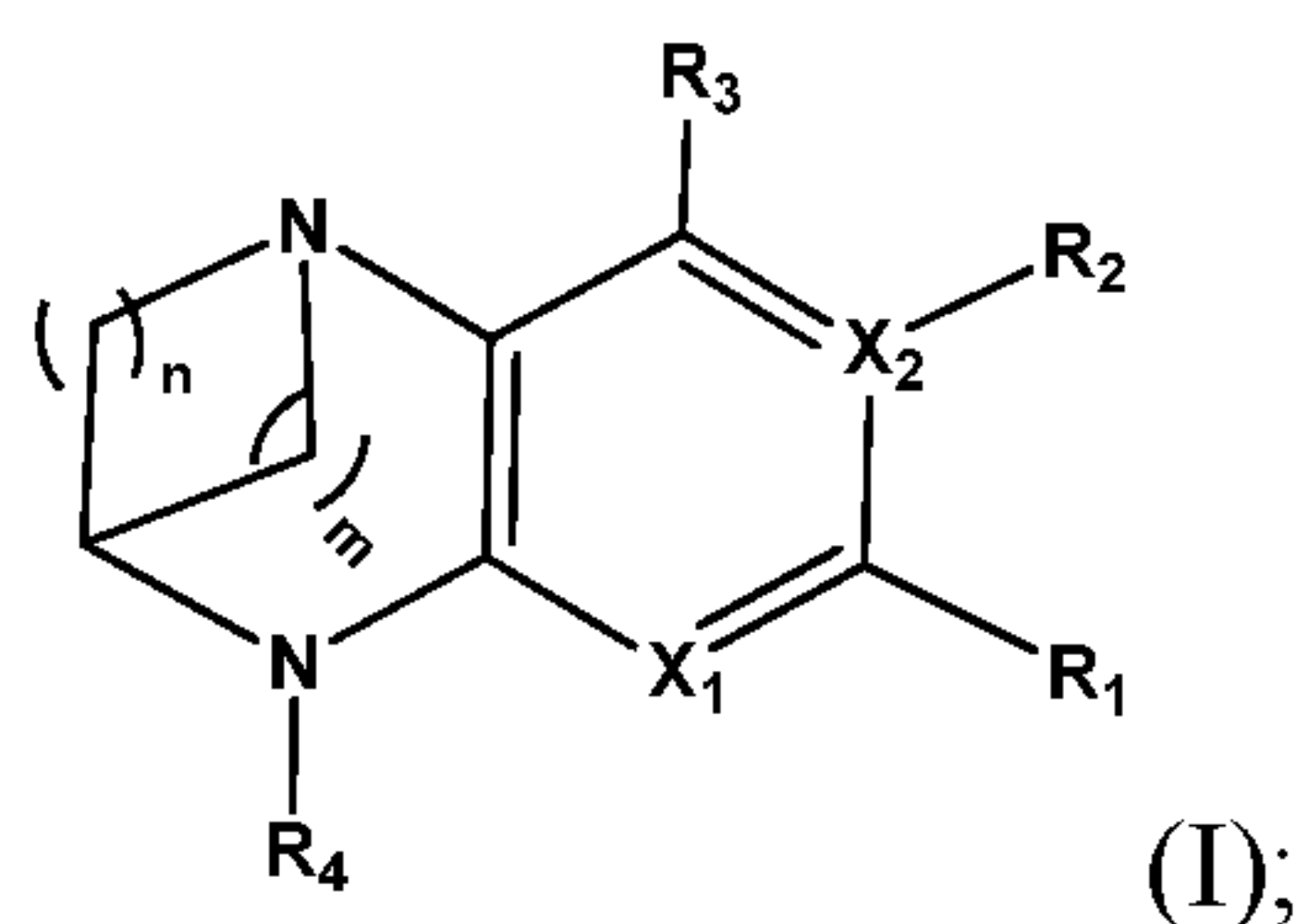
specification. The full scope of the invention should be determined by reference to the

claims, along with their full scope of equivalents, and the specification, along with such variations.

20

CLAIMS

1. A compound of Formula (I):



5 wherein:

X_1 or X_2 independently is selected from -N or -C;

R^1 is hydrogen, halogen, -CN, carbocyclyl, heterocyclyl, -N-substituted heterocyclyl, aryl, heteroaryl, -C(O) R_a or -C(O)-NR $_b$ R $_c$;

10 R^2 is halogen, -straight or branched C $_1$ -C $_6$ alkyl, -straight or branched-C $_1$ -C $_6$ haloalkyl, or -C(O)-NR $_b$ R $_c$;

R^3 is hydrogen, halogen, -hydroxy, -straight or branched C $_1$ -C $_6$ alkyl, or -straight or branched-C $_1$ -C $_6$ haloalkyl;

R^4 is hydrogen or -C(O)NR $_b$ R $_c$;

wherein:

15 when X_2 is -N, R_2 is non-existent; or

when X_2 is -C, R_2 is as defined above;

each R^1 , R^2 , R^3 and R^4 as defined above optionally is further substituted with one or more substituents selected from hydrogen, halogen, -OH, -(CH $_2$) $_x$ OH, -C $\equiv$ N, -NR $_d$ R $_e$, -straight or branched C $_1$ -C $_6$ alkyl, -straight or branched-C $_1$ -C $_6$ haloalkyl, -straight or branched C $_1$ -C $_6$ alkoxy, -straight or branched C $_1$ -C $_6$ haloalkoxy, -O-straight or branched-C $_1$ -C $_6$ haloalkyl, -C $_1$ -C $_6$ cycloalkyl, -(CH $_2$) $_x$ -cycloalkyl, heterocyclyl, aryl, -heteroaryl, -(CH $_2$) $_x$ -heteroaryl, -O-(CH $_2$) $_x$ CH(OH)CH $_2$ (OH), or -C(O)OR $_f$;

25 each R_a , R_b , R_c , R_d , R_e , or R_f as defined above independently is selected from hydrogen, -straight or branched C $_1$ -C $_6$ alkyl, -straight or branched-C $_1$ -C $_6$ haloalkyl, -C $_1$ -C $_6$ -cycloalkyl, -(CH $_2$) $_x$ C $_1$ -C $_6$ -cycloalkyl, heterocyclyl, -N-heterocyclyl, aryl, heteroaryl, or -(CH $_2$) $_x$ heteroaryl, -(CHR $_g$) $_x$ heteroaryl;

wherein:

R_g is -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl;

5 each R_a , R_b , R_c , R_d , R_e , or R_f as defined above optionally is further substituted with one or more substituents selected from hydrogen, halogen, -OH, $-C\equiv N$, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl, -straight or branched C_1 - C_6 alkoxy, -O-straight or branched- C_1 - C_6 haloalkyl, - C_1 - C_6 cycloalkyl, carbocyclyl, $-(CH_2)_x$ -carbocyclyl, -heterocyclyl, -O-heterocyclyl aryl, -heteroaryl, $-(CH_2)_x$ -heteroaryl, -O-
10 $(CH_2)_xCH(OH)CH_2(OH)$, $-(CH_2)_x-OH$, or $-C(O)-OH$;

m is an integer from 1 to 3;

n is an integer selected from 1 to 3;

x is 0 or an integer from 1 to 6; or

a pharmaceutically salt thereof.

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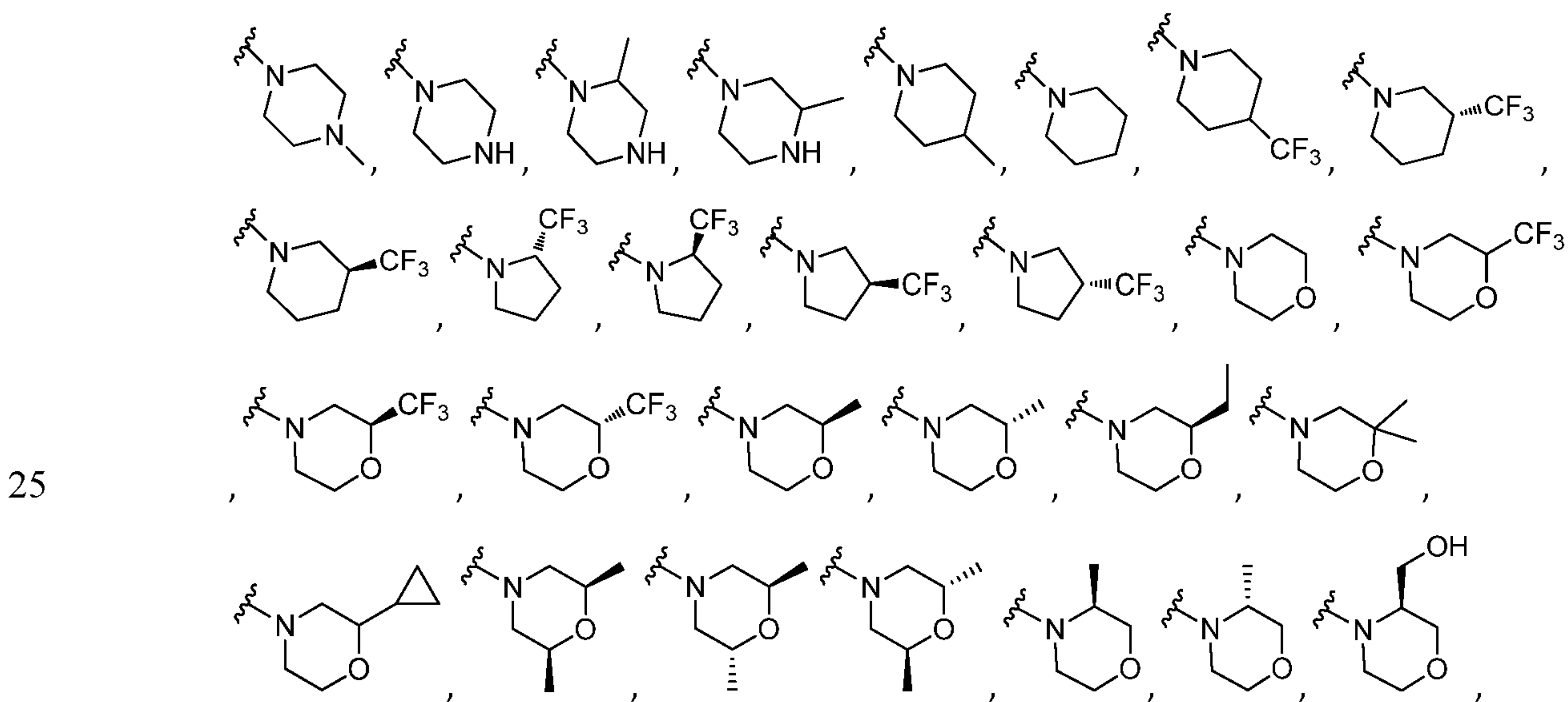
2. The compound of Formula (I) according to claim 1, wherein:

m is 1;

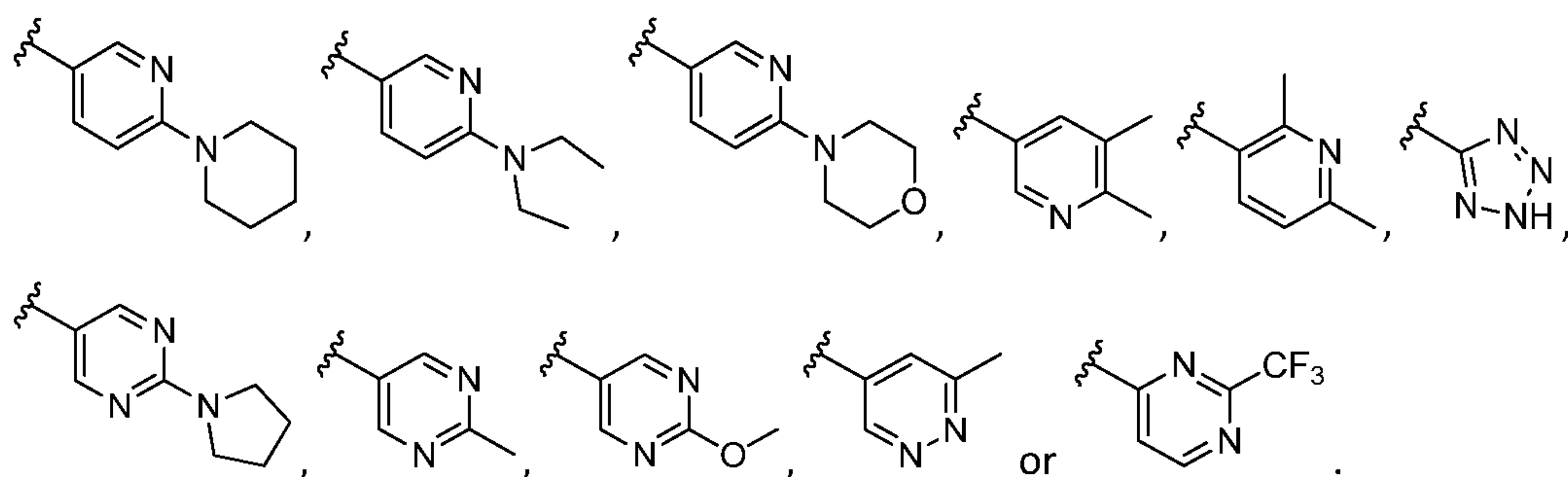
n is 3; and

20 R^4 is R^4 is hydrogen or $-C(O)NR_bR_c$, wherein R_b and R_c is as defined above in claim 1.

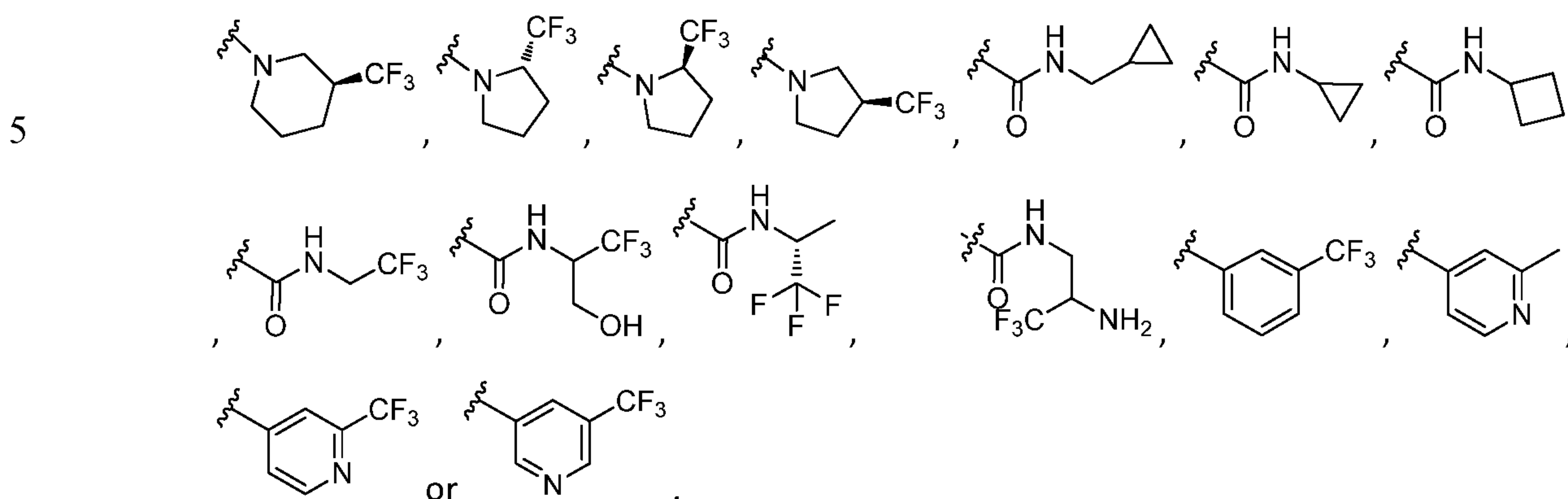
3. The compound of Formula (I) according to claim 1, wherein R^1 is selected from:



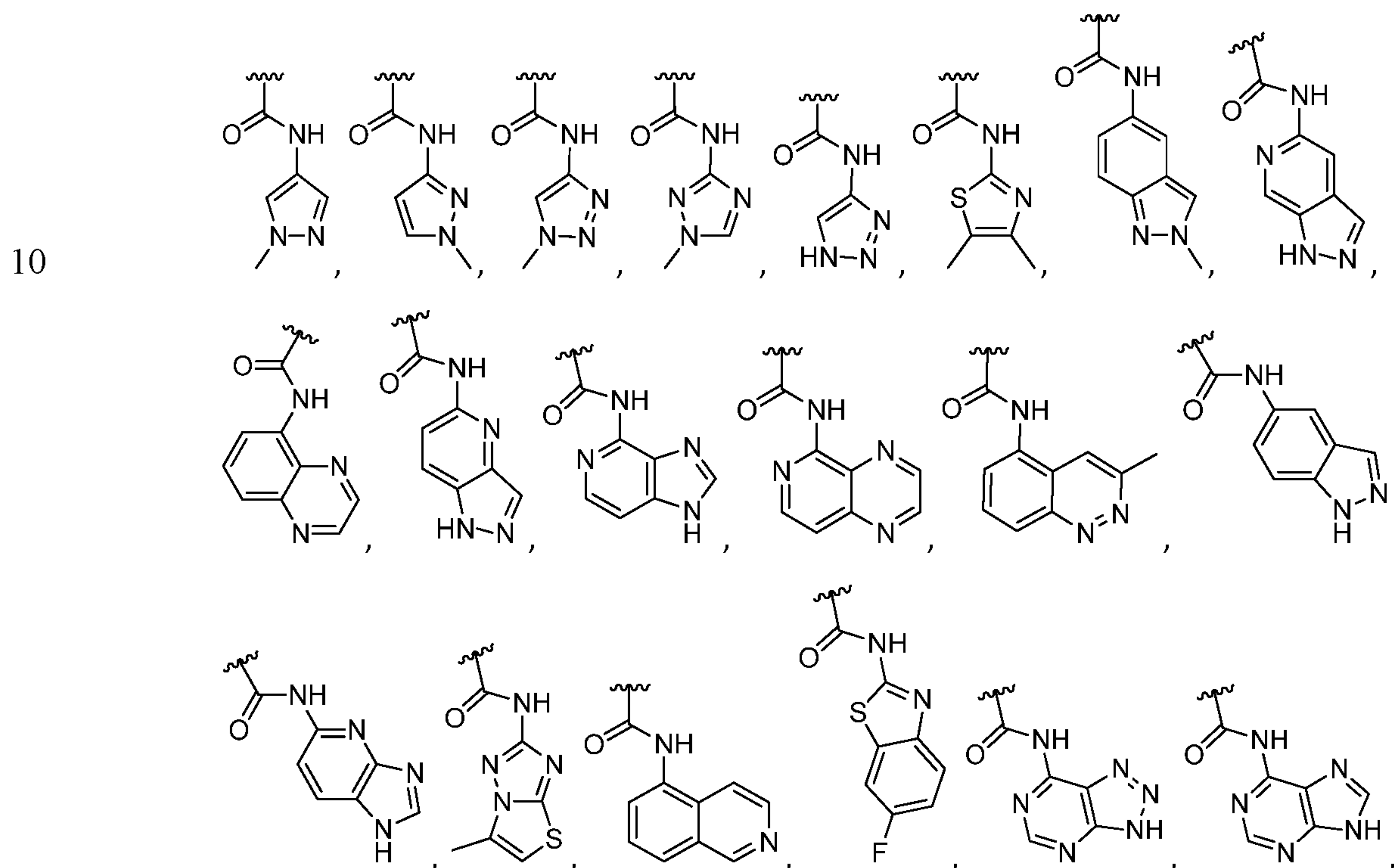




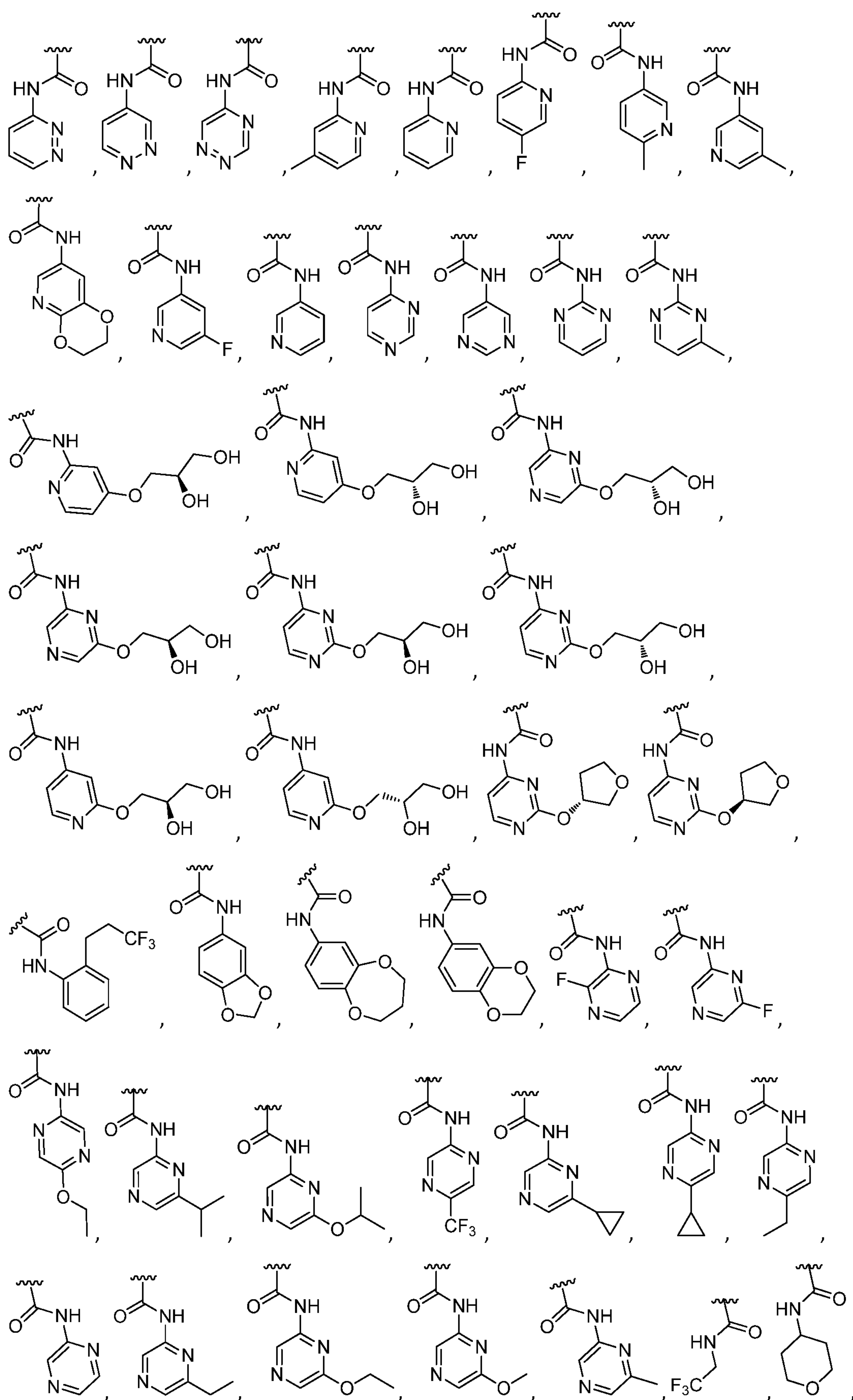
4. The compound of Formula (I) according to claim 1, wherein R^1 is selected from:

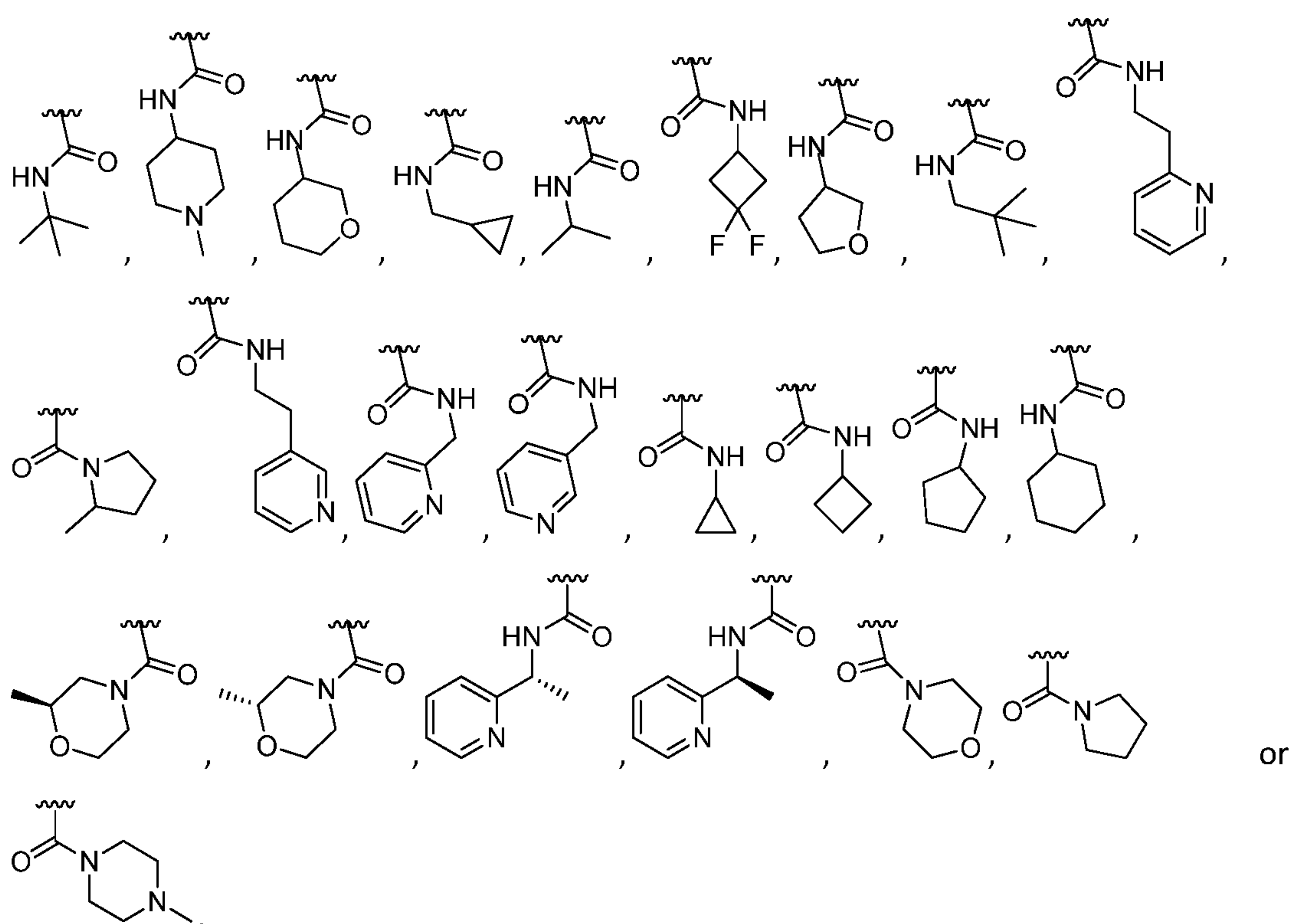


5. The compound of Formula (I) according to claim 1, wherein R^4 is selected from:



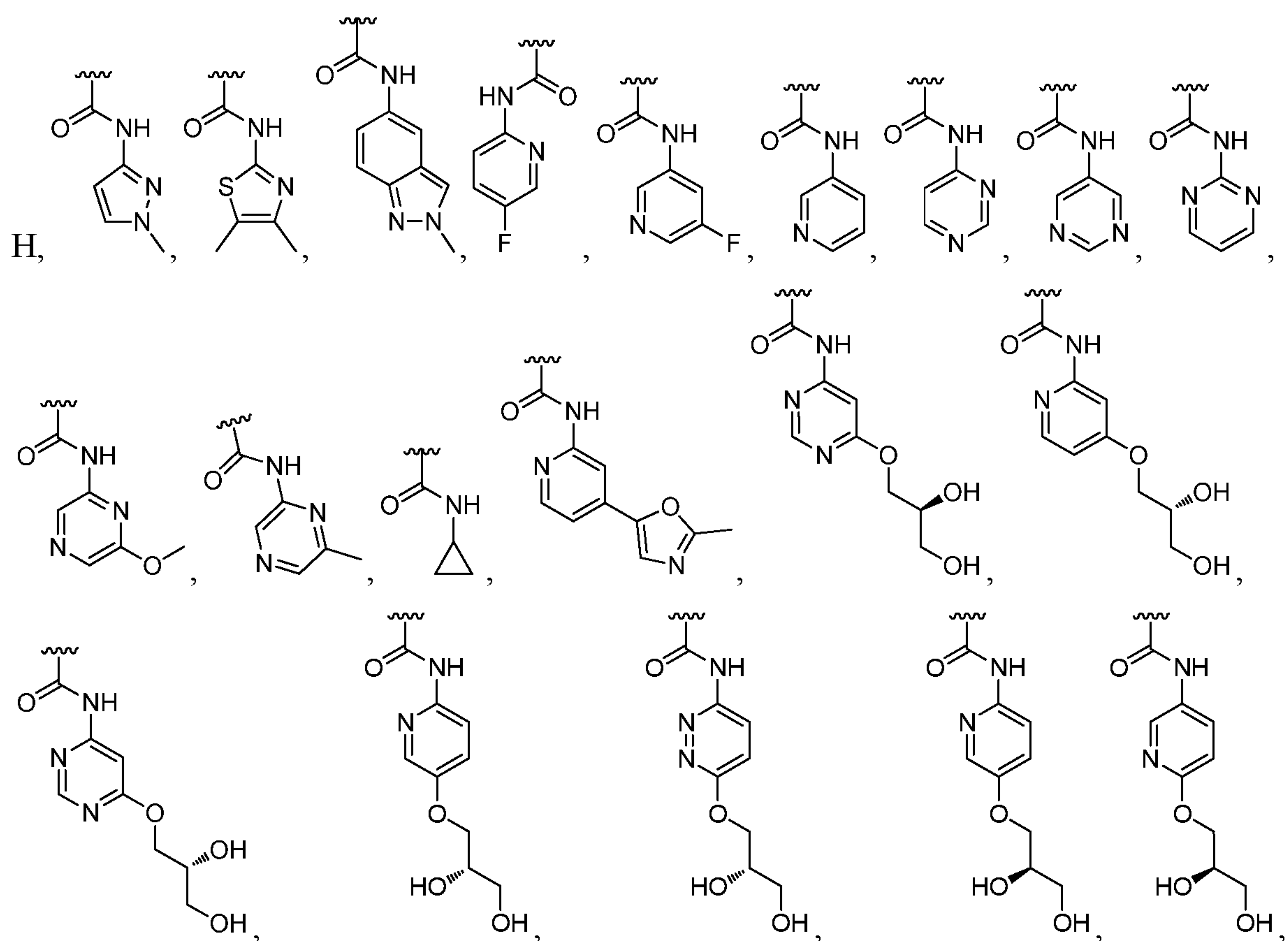
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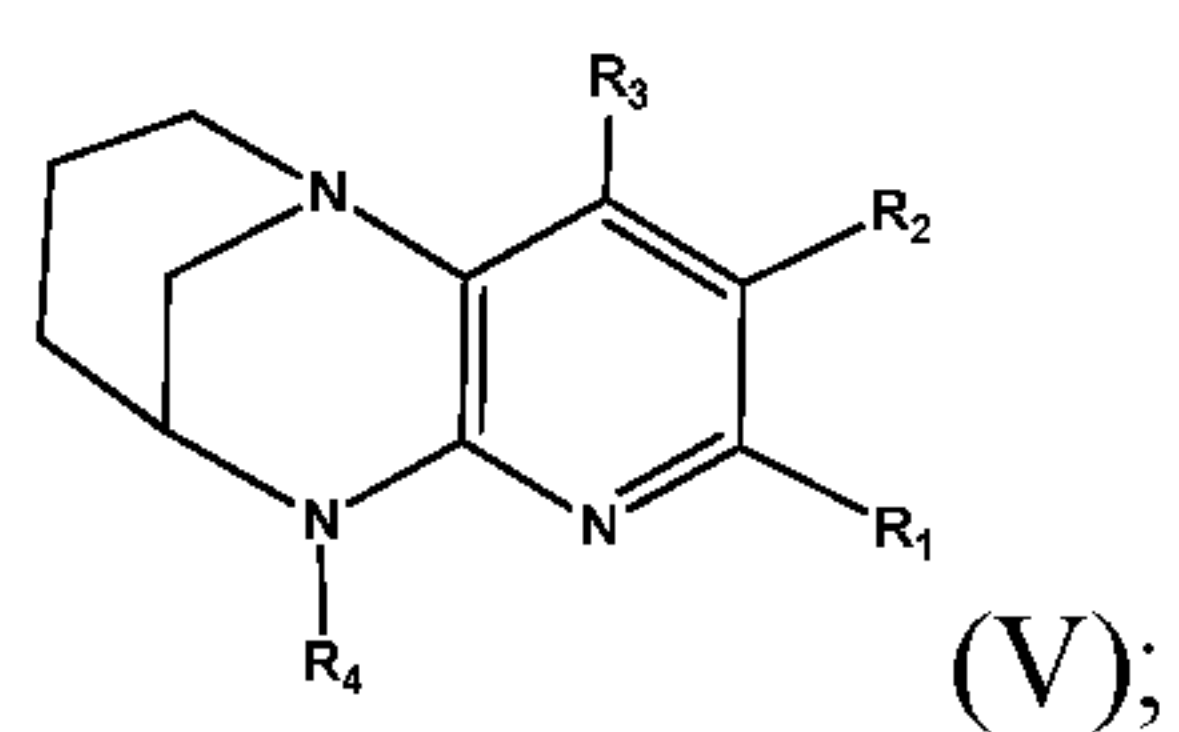
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6. A compound of Formula (I) according to claim 1, wherein R₄ is:





wherein:



R^1 is hydrogen, halogen, -CN, carbocyclyl, heterocyclyl, -N-substituted heterocyclyl, aryl, heteroaryl, -C(O) R_a or -C(O)- NR_bR_c ;

R^2 halogen, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl, or -C(O)- NR_bR_c ;

5 R^3 is hydrogen, halogen, -hydroxy, -straight or branched C_1 - C_6 alkyl, or -straight or branched- C_1 - C_6 haloalkyl;

R^4 is hydrogen or -C(O)- NR_bR_c ;

wherein:

each R^1 , R^2 , R^3 or R^4 as defined above optionally is further substituted with
 10 one or more substituents selected from hydrogen, halogen, -OH, $-(CH_2)_xOH$,
 -C $\equiv$ N, - NR_dR_e , -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6
 haloalkyl, -straight or branched C_1 - C_6 alkoxy, -straight or branched C_1 - C_6
 haloalkoxy, -O-straight or branched- C_1 - C_6 haloalkyl, - C_1 - C_6 cycloalkyl, $-(CH_2)_x$ -
 cycloalkyl, heterocyclyl, aryl, -heteroaryl, $-(CH_2)_x$ -heteroaryl, -O-
 15 $(CH_2)_xCH(OH)CH_2(OH)$, or -C(O) OR_f ;

each R_a , R_b , R_c , R_d , R_e , or R_f as defined above independently is selected
 from hydrogen, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6
 haloalkyl, - C_1 - C_6 -cycloalkyl, $-(CH_2)_xC_1$ - C_6 -cycloalkyl, heterocyclyl, -N-
 heterocyclyl, aryl, heteroaryl, or $-(CH_2)_x$ -heteroaryl;

20 wherein:

each R_a , R_b , R_c , R_d , R_e , or R_f as defined above optionally is further
 substituted with one or more substituents selected from hydrogen, halogen,
 -OH, $-(CH_2)_xOH$, -C $\equiv$ N, - NR_gR_h , -straight or branched C_1 - C_6 alkyl, -
 straight or branched- C_1 - C_6 haloalkyl, -straight or branched C_1 - C_6 alkoxy, -
 25 straight or branched- C_1 - C_6 haloalkoxy, - C_1 - C_6 cycloalkyl, $-(CH_2)_x$ -
 cycloalkyl, heterocyclyl, -heterocyclyl, -O-heterocyclyl, aryl, -heteroaryl, -
 $(CH_2)_x$ -heteroaryl, -O- $(CH_2)_xCH(OH)CH_2(OH)$, $-(CH_2)_x$ -OH, or -C(O) OR_i ;

wherein:

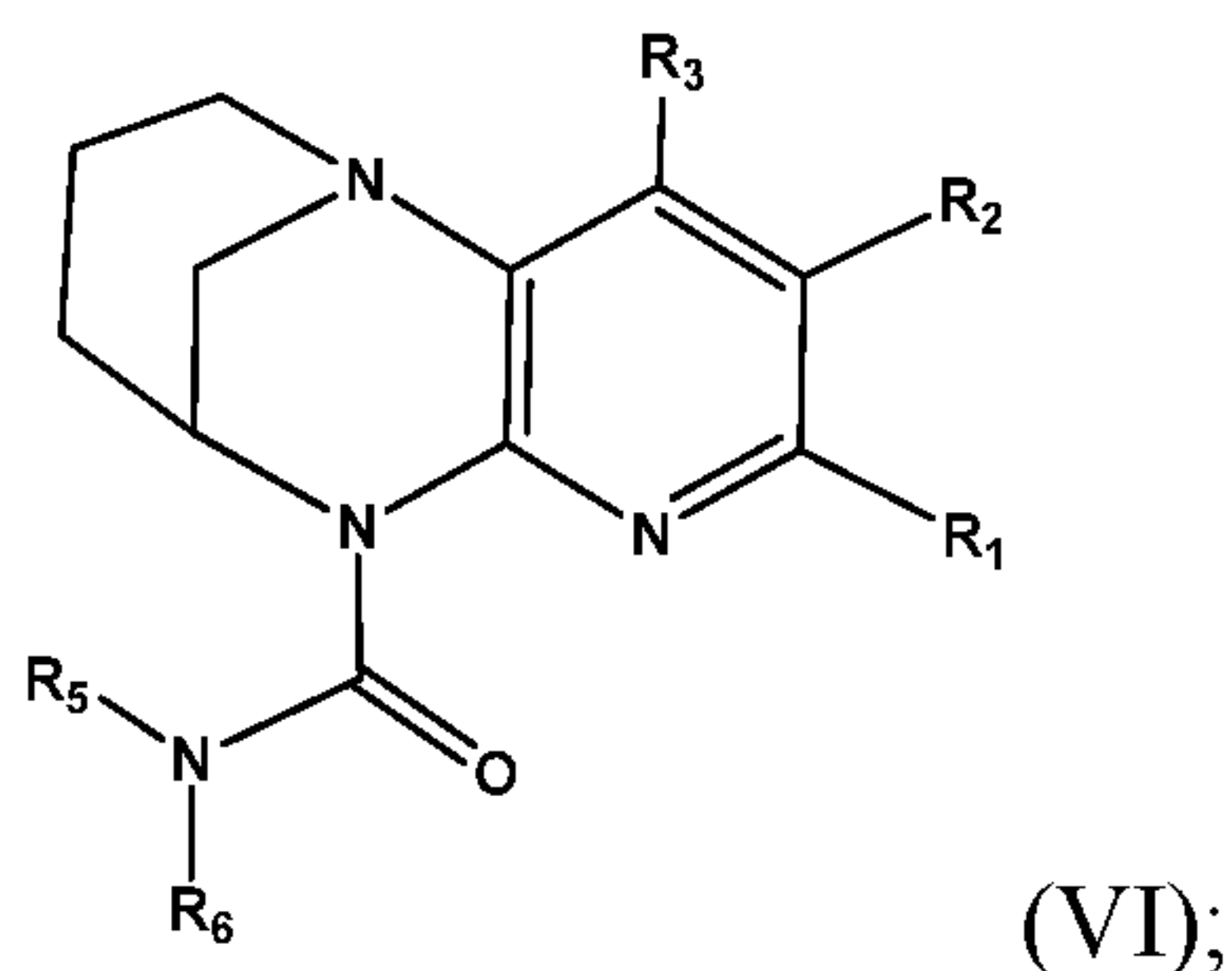
each R_g , R_h and R_i as defined above independently is selected from
 30 hydrogen, -straight or branched C_1 - C_6 alkyl or -straight or branched- C_1 - C_6
 haloalkyl;

m is an integer from 1 to 3;

n is an integer selected from 2 to 3;

x is 0 or an integer from 1 to 6; or
a pharmaceutically salt thereof;

8. A compound of Formula (VI):



wherein:

R^1 is hydrogen, halogen, -CN, carbocyclyl, heterocyclyl, -N-substituted heterocyclyl, aryl, heteroaryl, -C(O) R_a or -C(O)-NR $_b$ R $_c$;

R^2 halogen, -straight or branched C₁-C₆ alkyl, -straight or branched-C₁-C₆ haloalkyl, or -C(O)-NR $_b$ R $_c$;

R^3 is hydrogen, halogen, -hydroxy, -straight or branched C₁-C₆ alkyl, or -straight or branched-C₁-C₆ haloalkyl;

each R^5 and R^6 independently is selected from hydrogen, -straight or branched C₁-C₆ alkyl, -straight or branched-C₁-C₆ haloalkyl, -C₁-C₆cycloalkyl, -(CH₂)_xC₁-C₆cycloalkyl, heterocyclyl, -N-heterocyclyl, aryl, heteroaryl, or -(CH₂)_xheteroaryl, -(CHR_g)_xheteroaryl;

wherein:

each R^1 , R^2 , R^3 , R^5 and R^6 as defined above optionally is further substituted with one or more substituents selected from hydrogen, halogen, -OH, -(CH₂)_xOH, -C≡N, -NR $_d$ R $_e$, -straight or branched C₁-C₆ alkyl, -straight or branched-C₁-C₆ haloalkyl, -straight or branched C₁-C₆ alkoxy, -straight or branched C₁-C₆ haloalkoxy, -O-straight or branched-C₁-C₆ haloalkyl, -C₁-C₆ cycloalkyl, -(CH₂)_x-cycloalkyl, heterocyclyl, aryl, -heteroaryl, -(CH₂)_x-heteroaryl, -O-(CH₂)_xCH(OH)CH₂(OH), or -C(O)OR $_f$;

each R_a , R_b , R_c , R_d , R_e , R_f or R_g as defined above independently is selected from hydrogen, -straight or branched C₁-C₆ alkyl, -straight or branched-C₁-C₆ haloalkyl, -C₁-C₆-cycloalkyl, -(CH₂)_xC₁-C₆-cycloalkyl, heterocyclyl, -N-heterocyclyl, aryl, heteroaryl, or -(CH₂)_xheteroaryl;

wherein:

each R_a , R_b , R_c , R_d , R_e , R_f or R_g as defined above optionally is further substituted with one or more substituents selected from hydrogen, halogen, -OH, $-(CH_2)_xOH$, $-C\equiv N$, $-NR_hR_i$, -straight or branched C_1 - C_6 alkyl, -straight or branched- C_1 - C_6 haloalkyl, -straight or branched C_1 - C_6 alkoxy, -straight or branched- C_1 - C_6 haloalkoxy, $-C_1$ - C_6 cycloalkyl, $-(CH_2)_x$ -cycloalkyl, heterocyclyl, -heterocyclyl, -O-heterocyclyl, aryl, -heteroaryl, $-(CH_2)_x$ -heteroaryl, $-O-(CH_2)_xCH(OH)CH_2(OH)$, $-(CH_2)_x-OH$, or $-C(O)OR_j$;

wherein:

each R_h , R_i and R_j as defined above independently is selected from hydrogen, -straight or branched C_1 - C_6 alkyl or -straight or branched- C_1 - C_6 haloalkyl;

m is an integer from 1 to 3;

n is an integer selected from 2 to 3;

x is 0 or an integer from 1 to 6; or

a pharmaceutically salt thereof;

9. A compound which is as set forth in Table I or a pharmaceutically acceptable salt thereof.

10. A pharmaceutical composition comprising a compound of any one of claims 1 to 9 and a pharmaceutically acceptable carrier.

11. The pharmaceutical composition of claim 13, further comprising an additional active agent.

12. A method for treating insulin resistance, a metabolic syndrome, metabolic dysfunctions, diabetes, or complications thereof, or for increasing insulin sensitivity, comprising administering a compound according to any one of claims 1 to 9 or a pharmaceutical composition according to any one of claims 10 or 11 to a subject in need thereof.

13. A method for treating diseases or disorders resulting from diminished SIRT1 expression or activity, which comprises administering a compound or a

pharmaceutically acceptable salt thereof according to any one of claims 1 to 9 or a pharmaceutical composition according to any one of claims 11 or 12 to a subject in need thereof.

5 14. The method according to claim 13, wherein the diseases or disorders resulting from diminished SIRT1 expression or activity are selected from, but not limited to aging or stress, diabetes, metabolic dysfunctions, neurodegenerative diseases, cardiovascular disease, cancer or inflammatory disease.

10 15. The method according to claim 14, wherein diseases related to aging or stress, diabetes, metabolic dysfunctions, neurodegenerative diseases, cardiovascular disease, cancer or inflammatory disease are selected from psoriasis, atopic dermatitis, acne, rosacea, inflammatory bowel disease, osteoporosis, sepsis, arthritis, COPD, systemic lupus erythematosus and ophthalmic inflammation.

15

 16. A method for treating psoriasis, which comprises administering a compound according to any one of claims 1 to 9 or a pharmaceutical composition according to claims 11 or 12 to a subject in need thereof.

20

 17. A compound as defined in claims 1 to 9 for use in therapy in treating a subject suffering from or susceptible to insulin resistance, a metabolic syndrome, diabetes, or complications thereof, or for increasing insulin sensitivity in a subject.

25

 18. The use of compound as defined in claims 1 to 9 in the manufacture of a medicament for use in the treatment of insulin resistance, a metabolic syndrome, diabetes, or complications thereof, or for increasing insulin sensitivity in a subject.

