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(54) Titre : COMPOSITIONS ET METHODES POUR INHIBER LA BETA-LACTAMASE
(54) Title: COMPOSITIONS AND METHODS FOR INHIBITING BETA-LACTAMASE

(57) **Abrégé/Abstract:**

Provided herein are substituted dihydroisoxazole derivatives useful as inhibitors of β -lactamases. The invention further provides medical uses of substituted dihydroisoxazole derivatives, for example, as antibacterial agents.

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(57) Abstract: Provided herein are substituted dihydroisoxazole derivatives useful as inhibitors of β -lactamases. The invention further provides medical uses of substituted dihydroisoxazole derivatives, for example, as antibacterial agents.



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Compositions and Methods for Inhibiting beta-Lactamase

Related Applications

This application claims the benefit of priority to U.S. Provisional Patent Application serial number 62/236,228, filed October 2, 2015. This application is hereby incorporated
5 by reference in its entirety.

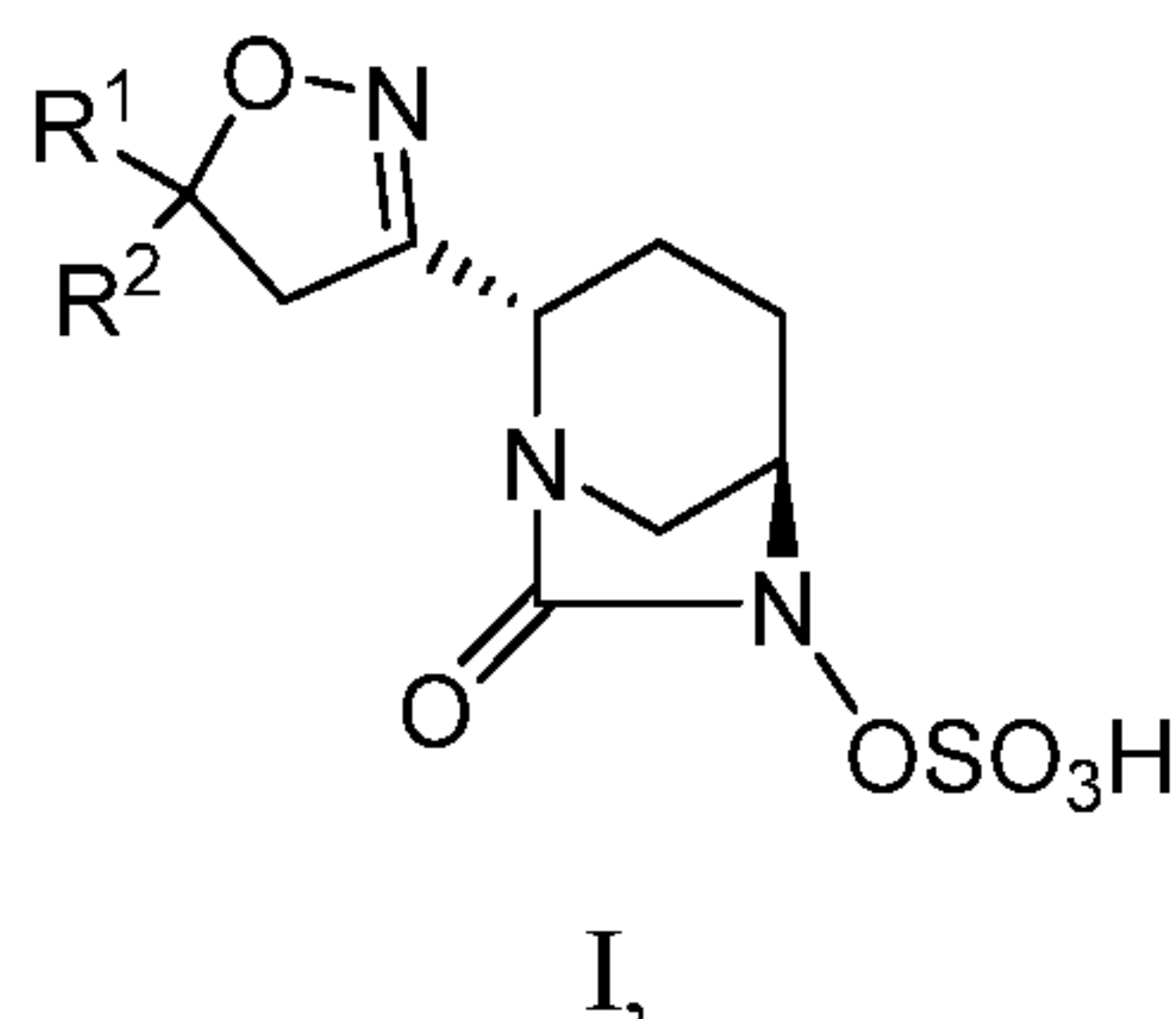
Background

Bacterial resistance to antibiotics is a serious threat to modern medical care. Bacteria have a remarkable ability to develop resistance to new antibiotics rendering them quickly ineffective. For example, the widespread use of penicillins and cephalosporins has
10 resulted in the emergence of β -lactamases, a family of bacterial enzymes that catalyze the hydrolysis of the β -lactam ring common to numerous antibiotics. Hydrolysis of the β -lactam ring leads to the inactivation of the antibiotic and allows the bacteria to resist the antibiotic. β -lactamase inhibitors deactivate or slow down the β -lactamase enzyme activity
15 thus reducing the degradation of β -lactam antibiotics by the bacteria. The continuing evolution of antibacterial resistance could result in bacterial strains against which currently available antibacterial agents will be ineffective.

Therefore, there is a continuing need to discover and develop compounds that inhibit β -lactamases and that may be useful to treat bacterial infections.

Summary of Invention

20 In certain embodiments, the invention relates to compounds having the structure of Formula I:



and pharmaceutically acceptable salts thereof, wherein R^1 and R^2 are as defined herein.

25 The invention also relates to methods for inhibiting β -lactamase, comprising contacting the β -lactamase with a compound of the invention.

The invention further relates to methods for inhibiting growth of a bacterium comprising contacting the bacterium with a β -lactam antibiotic and a compound of the invention.

The invention also relates to methods for treating a bacterial infection, comprising administering a compound of the invention to a subject in need of treatment. In certain embodiments, the method for treating a bacterial infection further comprises administering a β -lactam antibiotic.

Brief Description of the Figures

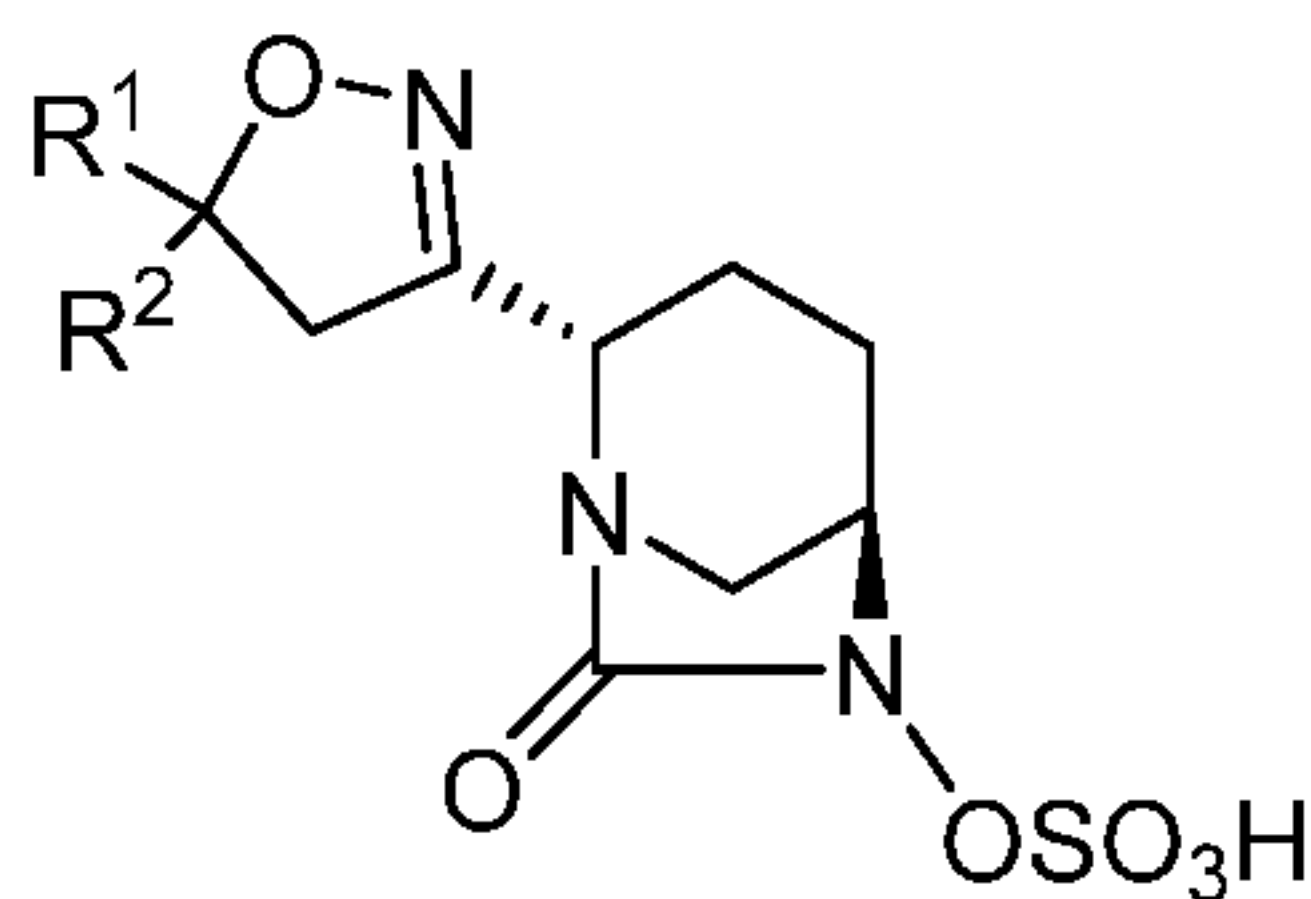
Figure 1 includes 3 panels (Panels A-C) showing the pharmacokinetics of representative compounds of the invention. Panel A is a plot of the pharmacokinetics of the compound of Example 2. Panel B is a plot of the pharmacokinetics of the compound of Example 5. Panel C is a plot of the pharmacokinetics of the compound of Example 17.

Detailed Description of the Invention

In certain aspects, the invention provides substituted dihydroisoxazole derivatives and pharmaceutical compositions thereof. In certain aspects, the compounds, and their pharmaceutically acceptable salts are inhibitors of β -lactamase. In other aspects, the compounds, and their pharmaceutically acceptable salts, are useful in combination with beta-lactam antibiotics, or alone, for inhibiting growth of a bacterium and for the treatment of a bacterial infection, including infections caused by drug resistant organisms, including multi-drug resistant organisms

I. COMPOUNDS

In certain embodiments, the invention relates to compounds having the structure of Formula I, or a pharmaceutically acceptable salt thereof:



I

wherein

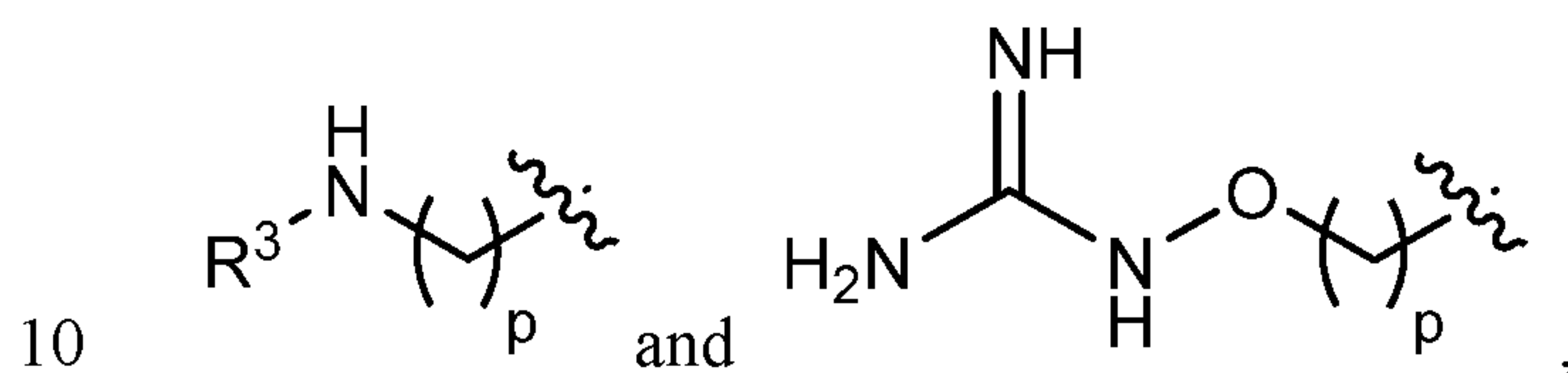
R^1 and R^2 are independently selected from H, hydroxyalkyl, $-C(O)-NH_2$, amido-, amino- or guanidino substituted alkyl, amido-, amino-, or guanidino-substituted alkoxyalkyl, and $-(CH_2)_p-O-NHR^3$, or

- 5 R^1 and R^2 combine to form an amino- or guanidino-substituted cycloalkyl ring, or an optionally substituted nitrogen-containing heterocyclyl ring;

p is an integer from 1 to 6; and

R^3 is, independently for each occurrence, selected from H, lower alkyl, and $-C(=NH)NH_2$.

In certain embodiments, at least one of R^1 and R^2 is independently selected from

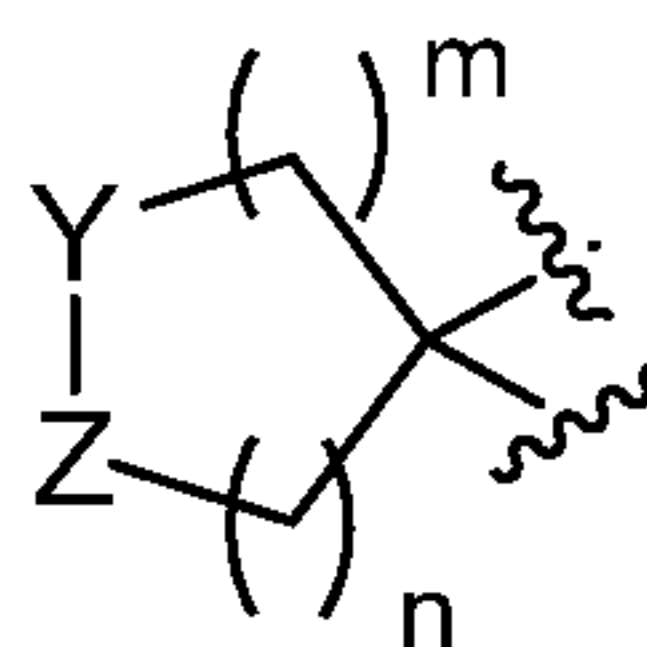


wherein

p is an integer from 1 to 5. In certain such embodiments, both R_1 and R_2 are independently selected from this list, while in other such embodiments, the other of R_1 and R_2 is H.

- 15 In certain embodiments, p is independently an integer from 1 to 5, from 1 to 4, from 2 to 5, from 1 to 3, from 2 to 4, or from 2 to 3.

In certain embodiments, R^1 and R^2 combine to form a structure of Formula A



A

- 20 wherein

Y and Z are each independently CHR^4 , NR^5 , or absent;

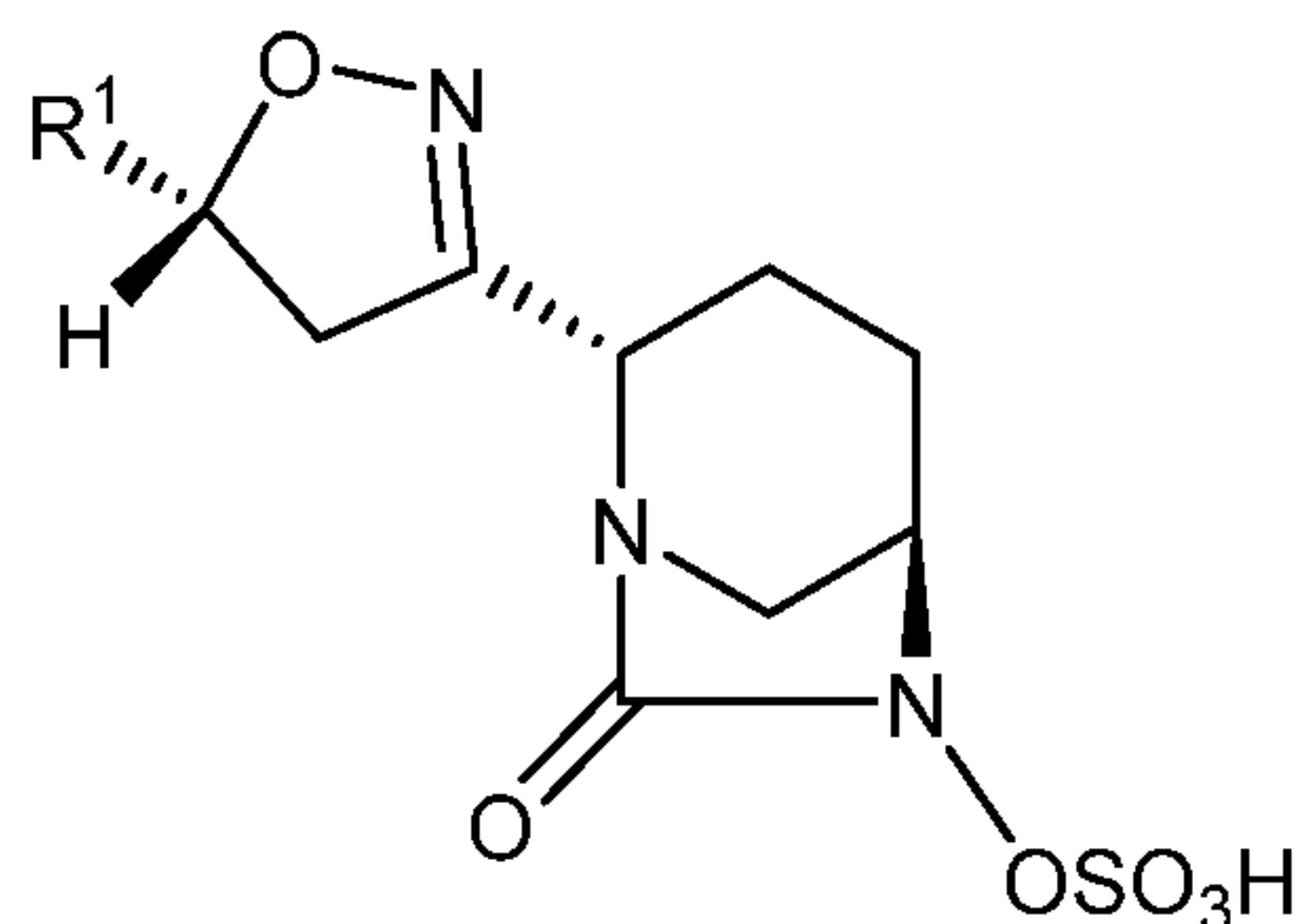
R^4 is, independently for each occurrence, selected from H, amido-, amino-, or guanidino-substituted lower alkyl, and NHR^3 ;

- 25 R^5 is, independently for each occurrence, selected from H, amido-, amino-, or guanidino-substituted lower alkyl, and $-C(=NH)NH_2$; and

m and n are each independently an integer from 1 to 3;

provided that both Y and Z are not absent.

In certain embodiments, the compound has the structure of formula II or a pharmaceutically acceptable salt thereof:

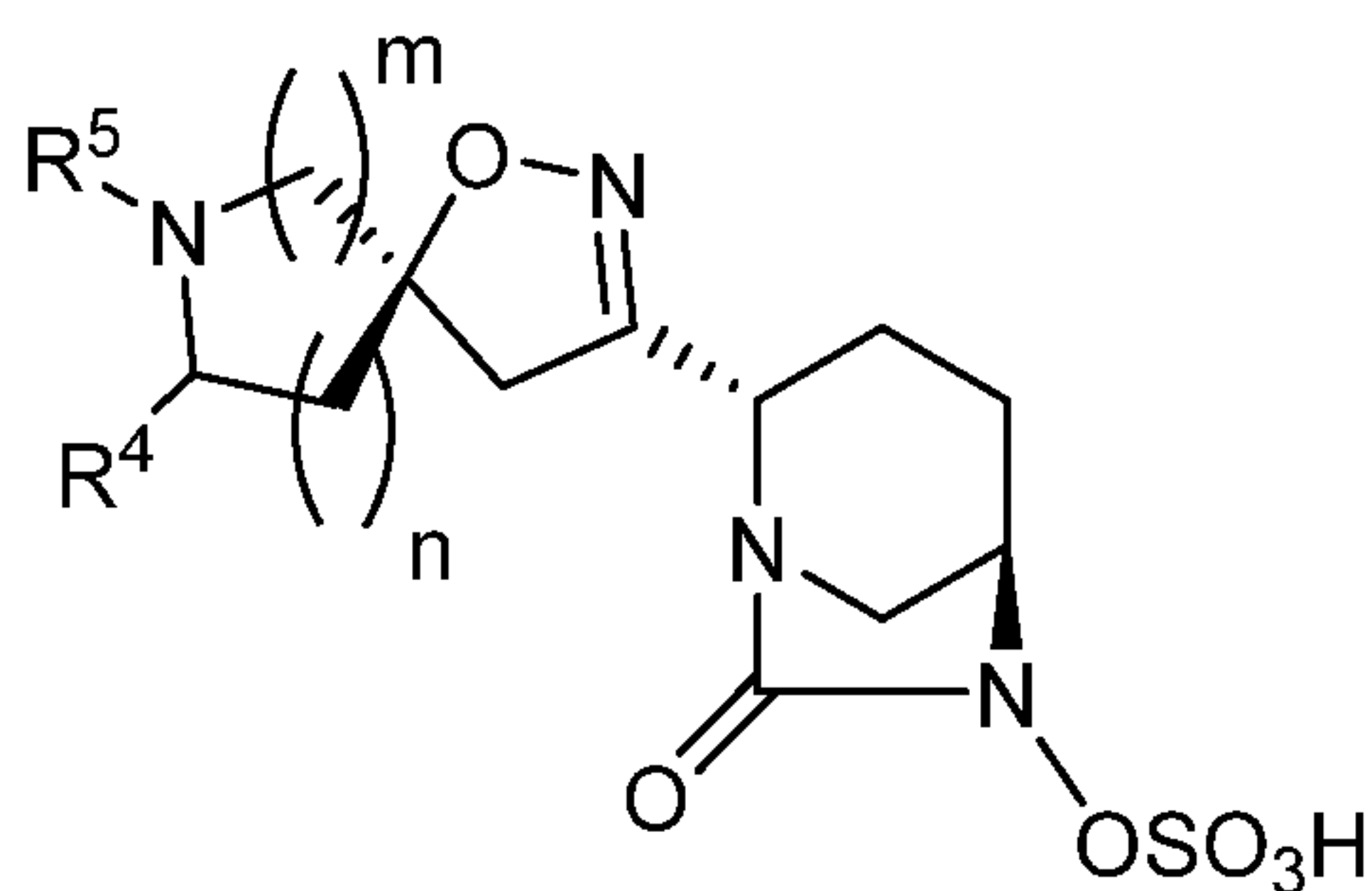


II

wherein R^1 is an amido-, amino- or guanidino substituted alkyl, an amido-, amino-,
guanidino-substituted alkoxyalkyl, or $-(CH_2)_p-O-NHR^3$, and

5 R^3 is as defined for Formula I.

In certain embodiments, the compound has the structure of formula III or a
pharmaceutically acceptable salt thereof:



III

10 wherein R^4 and R^5 are as defined for Formula I and
m is less than or equal to n.

In certain embodiments, the compound is selected from [(2S,5R)-2-[5-(2-aminoethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen
15 sulfate, [(2S, 5R)-2-[5-(aminomethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate, [(2S, 5R)-2-[5-(3-aminopropyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate, [(2S,5R)-2-[5-(guanidinooxymethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate, [(2S, 5R)-2-[5,5-bis(aminomethyl)-4H-isoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate, [(2S, 5R)-2-(1-oxa-2,7,8-triazaspiro[4.4]non-2-en-3-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate,
20 [(2S, 5R)-2-(5-oxa-2,6-diazaspiro[3.4]oct-6-en-7-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate, [(2S, 5R)-2-(2-amino-5-oxa-6-azaspiro[3.4]oct-6-en-7-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate, [(2S, 5R)-2-(1-oxa-2,7-diazaspiro[4.4]non-

2-en-3-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate, [(2S, 5R)-2-(1-oxa-2,8-diazaspiro[4.5]dec-2-en-3-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate, [(2S,5R)-2-(8-carbamimidoyl-1-oxa-2,8-diazaspiro[4.5]dec-2-en-3-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate, [(2S,5R)-2-[7-(2-amino-2-oxo-ethyl)-1-oxa-2,7-diazaspiro[4.4]non-2-en-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate, [(2S,5R)-2-[5-(aminomethyl)-5-(hydroxymethyl)-4H-isoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate, [(2S,5R)-2-[5-(aminomethyl)-5-carbamoyl-4H-isoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate, [(2S)-2-[(5R)-5-(aminomethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate, [(2S)-2-[(5S)-5-(aminomethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate, [(2S,5R)-2-[(5R)-1-oxa-2,7-diazaspiro[4.4]non-2-en-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate, [(2S,5R)-2-[(5S)-1-oxa-2,7-diazaspiro[4.4]non-2-en-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate or a pharmaceutically acceptable salt thereof.

In certain embodiments, compounds of the invention may be racemic. In certain embodiments, compounds of the invention may be enriched in one enantiomer. For example, a compound of the invention may have greater than 30% ee, 40% ee, 50% ee, 60% ee, 70% ee, 80% ee, 90% ee, or even 95% or greater ee. The compounds of the invention have more than one stereocenter. Consequently, compounds of the invention may be enriched in one or more diastereomer. For example, a compound of the invention may have greater than 30% de, 40% de, 50% de, 60% de, 70% de, 80% de, 90% de, or even 95% or greater de.

This invention includes the use of pharmaceutically acceptable salts of compounds of the invention in the compositions and methods of the present invention. The term “pharmaceutically acceptable salt” as used herein includes salts derived from inorganic or organic acids including, for example, hydrochloric, hydrobromic, sulfuric, nitric, perchloric, phosphoric, formic, acetic, lactic, maleic, fumaric, succinic, tartaric, glycolic, salicylic, citric, methanesulfonic, benzenesulfonic, benzoic, malonic, trifluoroacetic, trichloroacetic, naphthalene-2-sulfonic, and other acids. Pharmaceutically acceptable salt forms can include forms wherein the ratio of molecules comprising the salt is not 1:1. For example, the salt may comprise more than one inorganic or organic acid molecule per molecule of base, such as two hydrochloric acid molecules per molecule of compound of Formula I. As

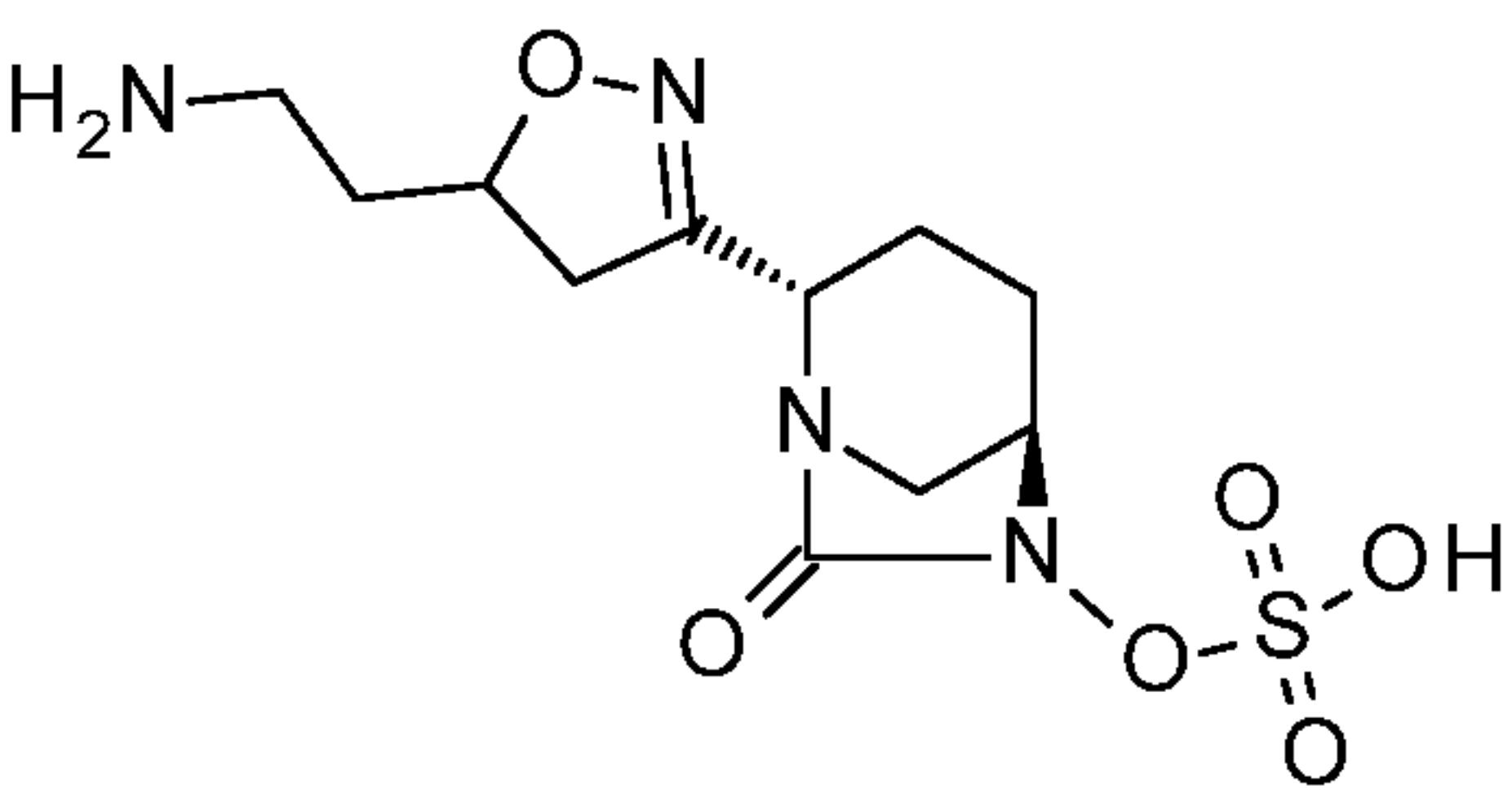
another example, the salt may comprise less than one inorganic or organic acid molecule per molecule of base, such as two molecules of compound of Formula I per molecule of tartaric acid.

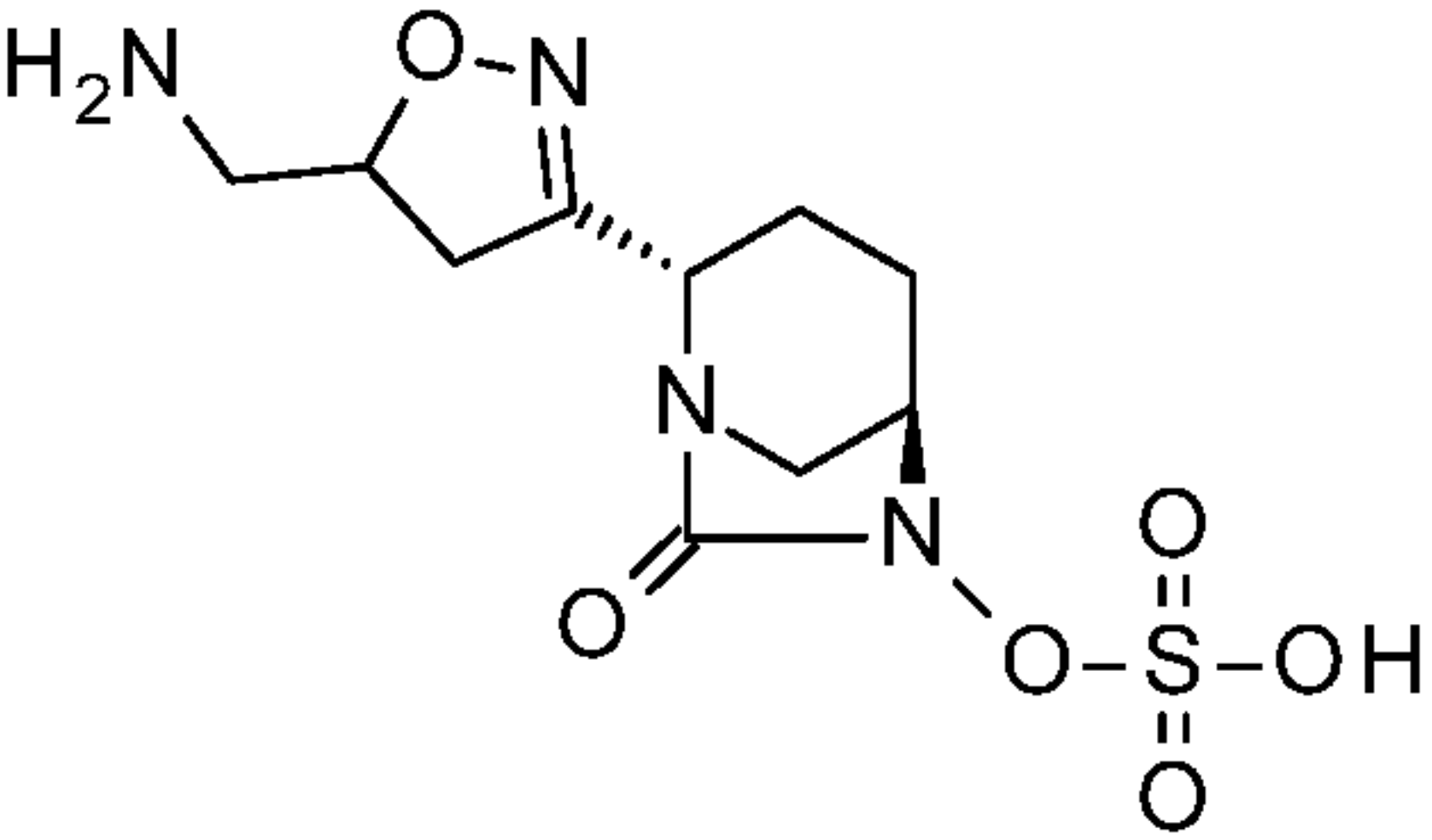
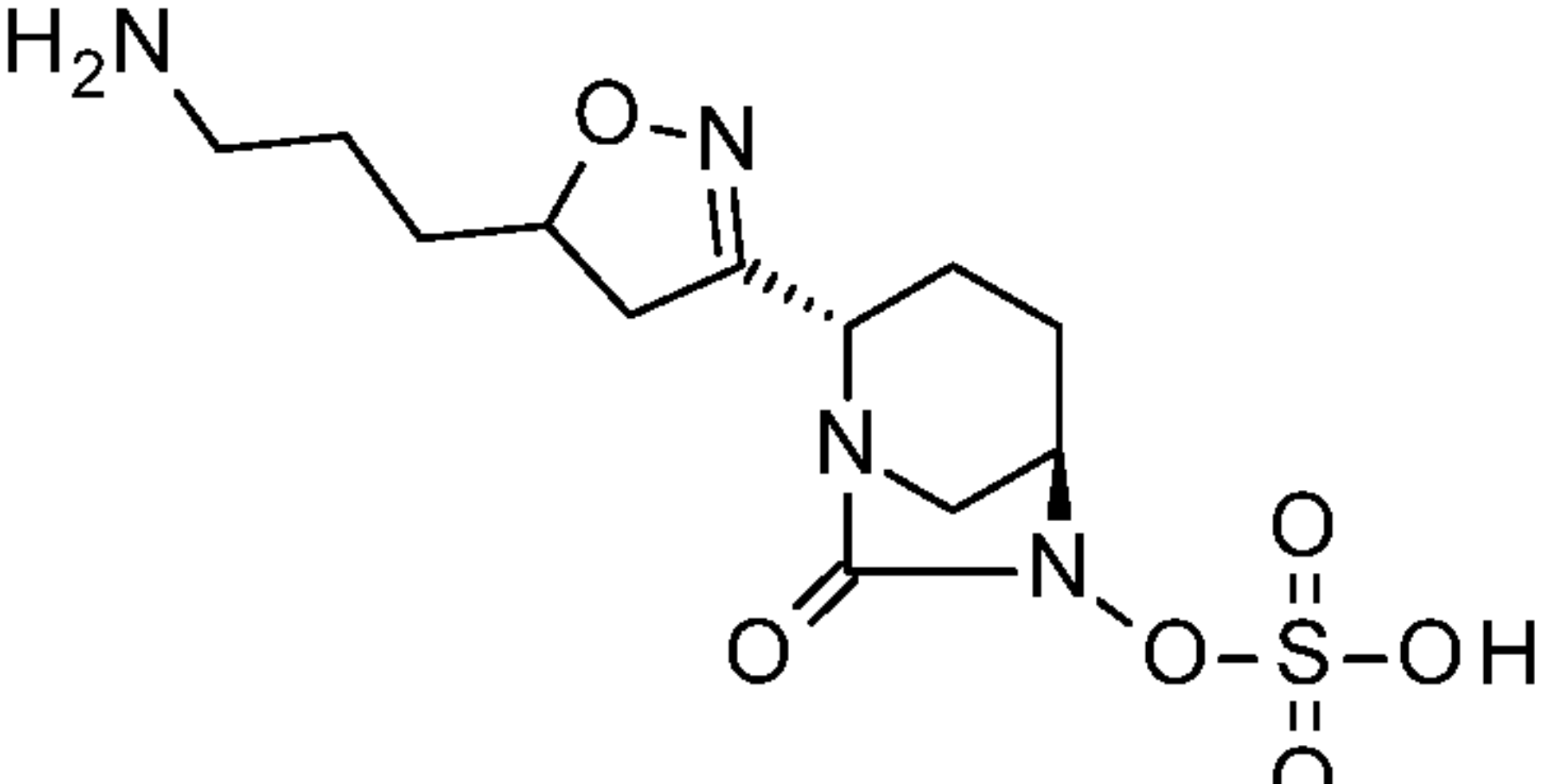
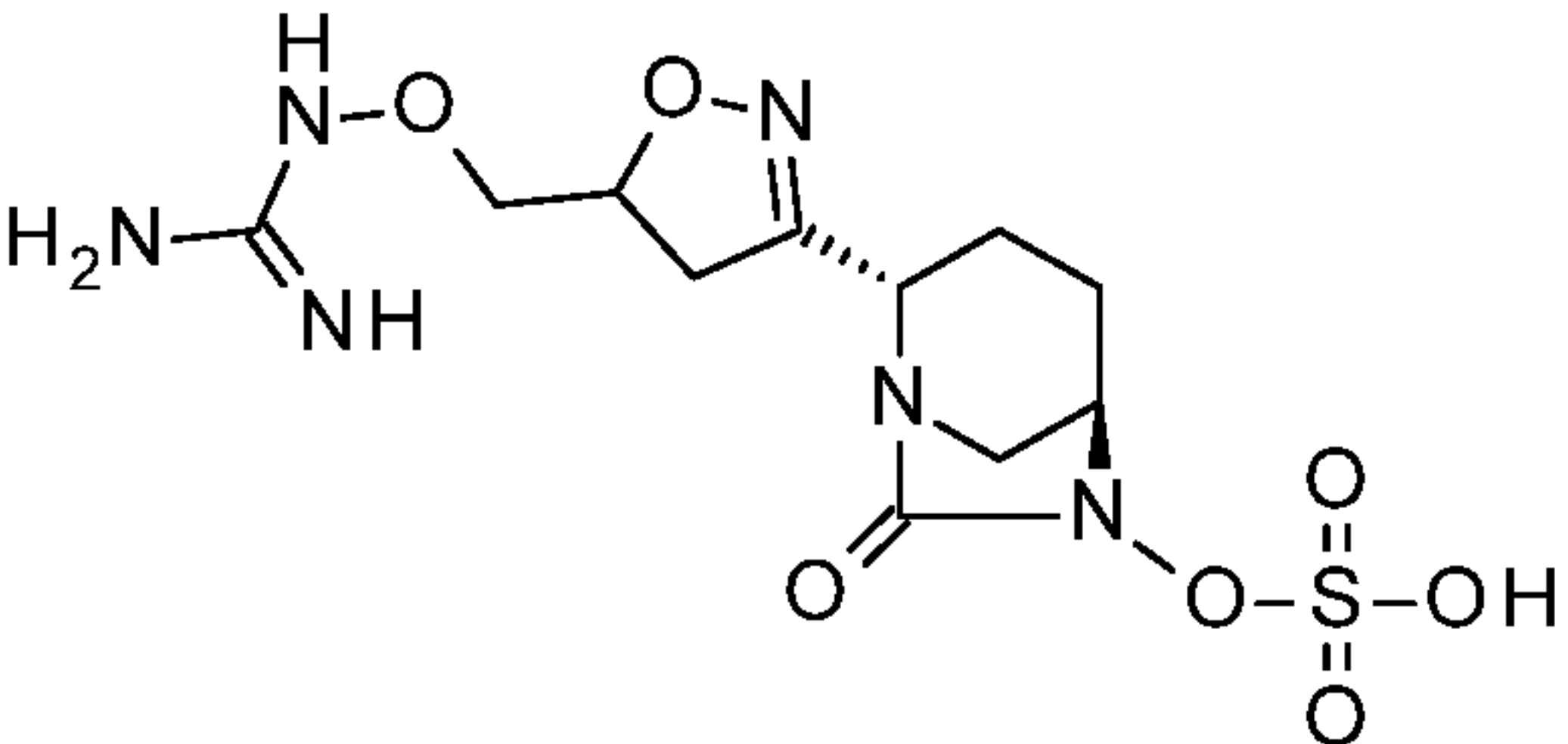
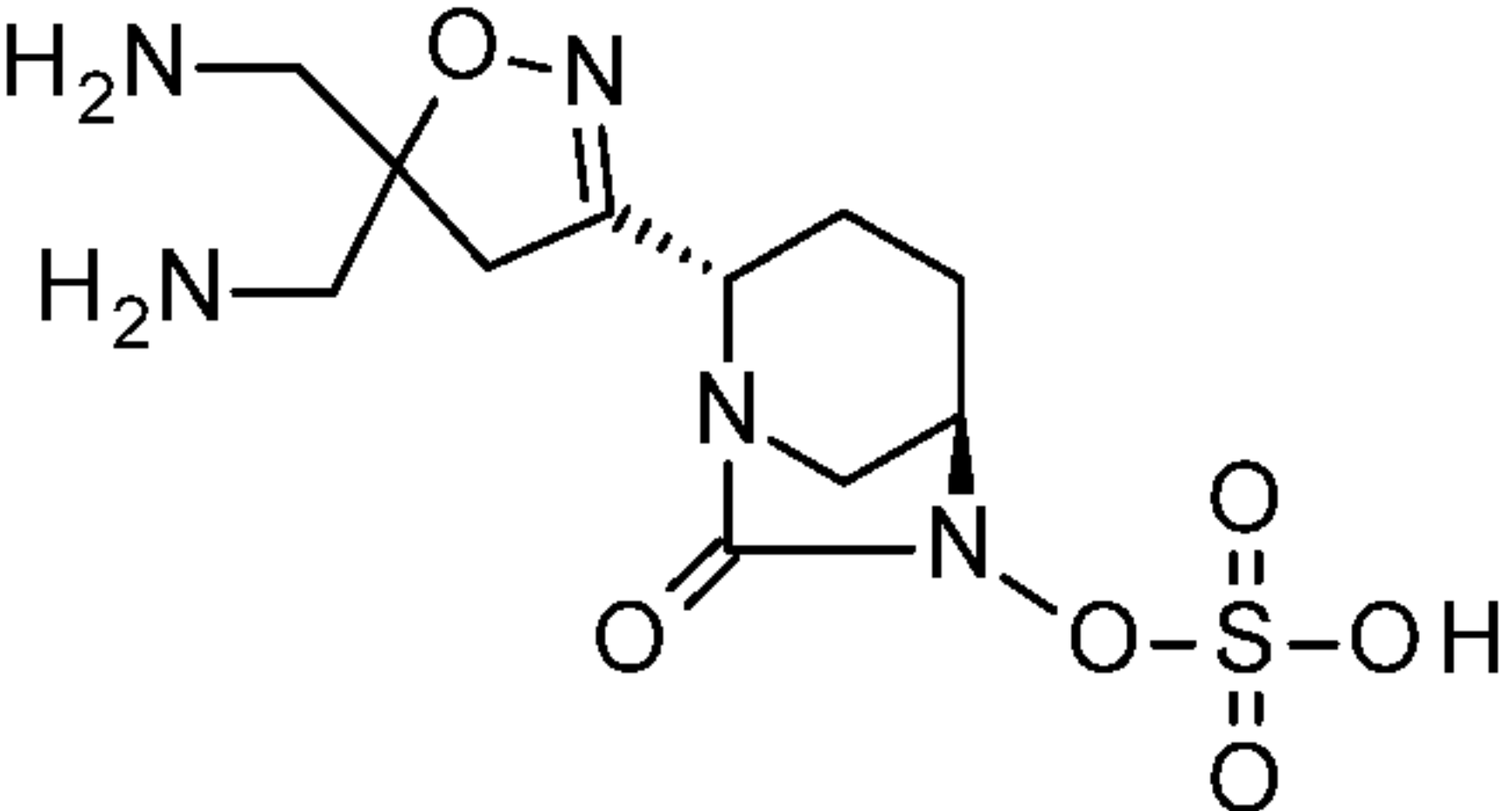
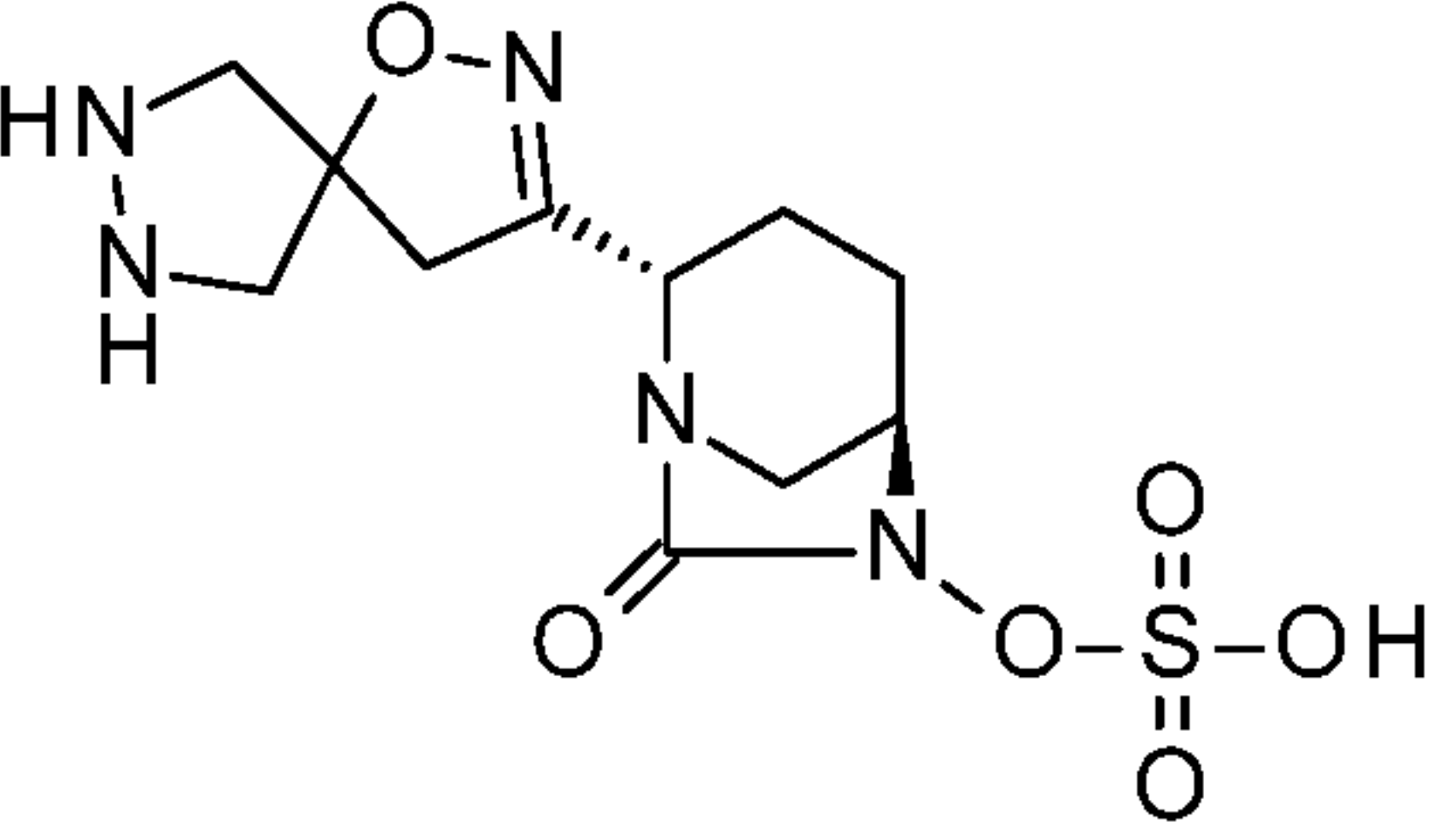
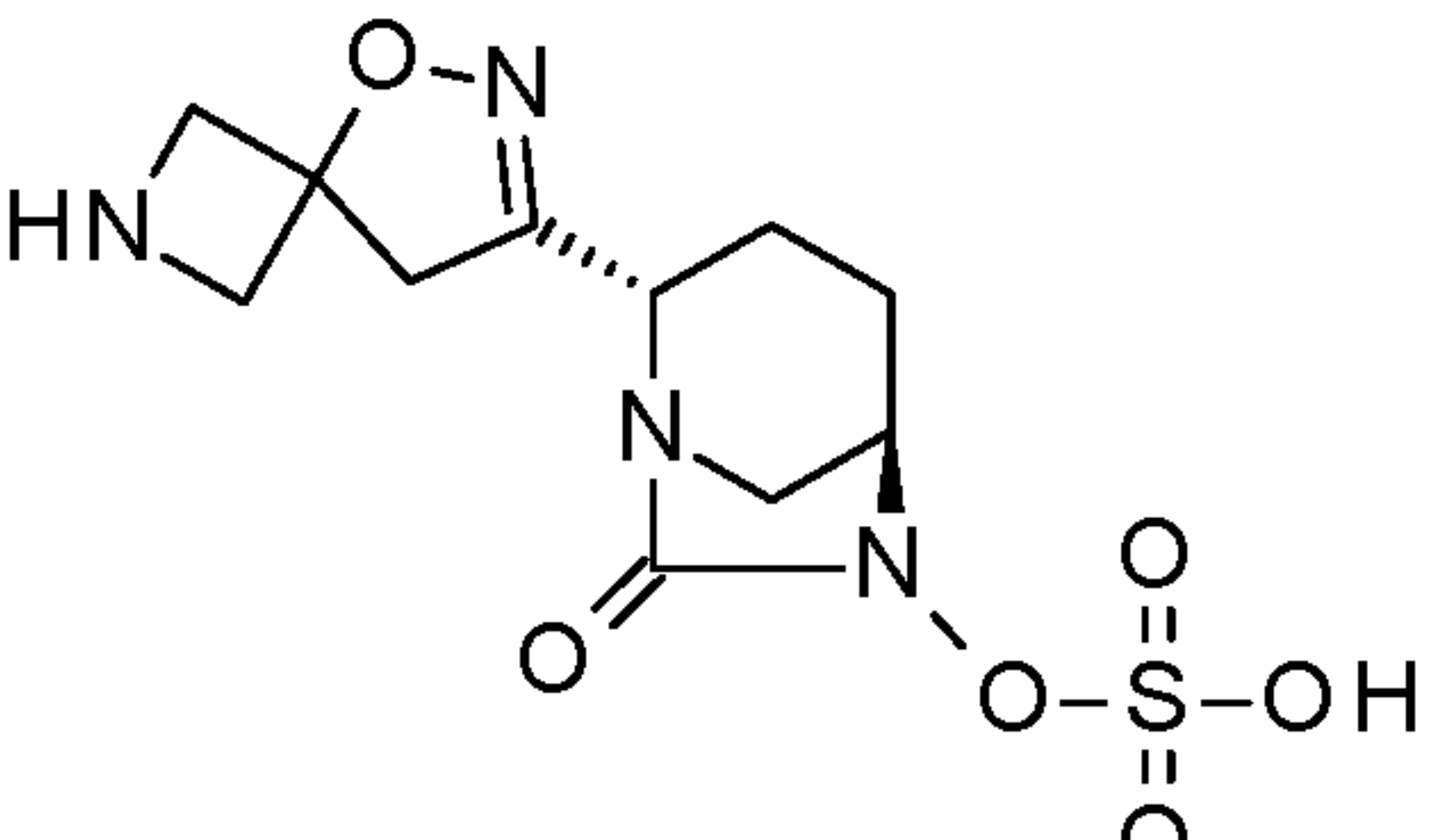
In further embodiments, contemplated salts of the invention include, but are not limited to, alkyl, dialkyl, trialkyl or tetra-alkyl ammonium salts. In certain embodiments, contemplated salts of the invention include, but are not limited to, L-arginine, benenthamine, benzathine, betaine, calcium hydroxide, choline, deanol, diethanolamine, diethylamine, 2-(diethylamino)ethanol, ethanolamine, ethylenediamine, N-methylglucamine, hydrabamine, 1H-imidazole, lithium, L-lysine, magnesium, 4-(2-hydroxyethyl)morpholine, piperazine, potassium, 1-(2-hydroxyethyl)pyrrolidine, sodium, triethanolamine, tromethamine, and zinc salts. In certain embodiments, contemplated salts of the invention include, but are not limited to Na, Ca, K, Mg, Zn or other metal salts.

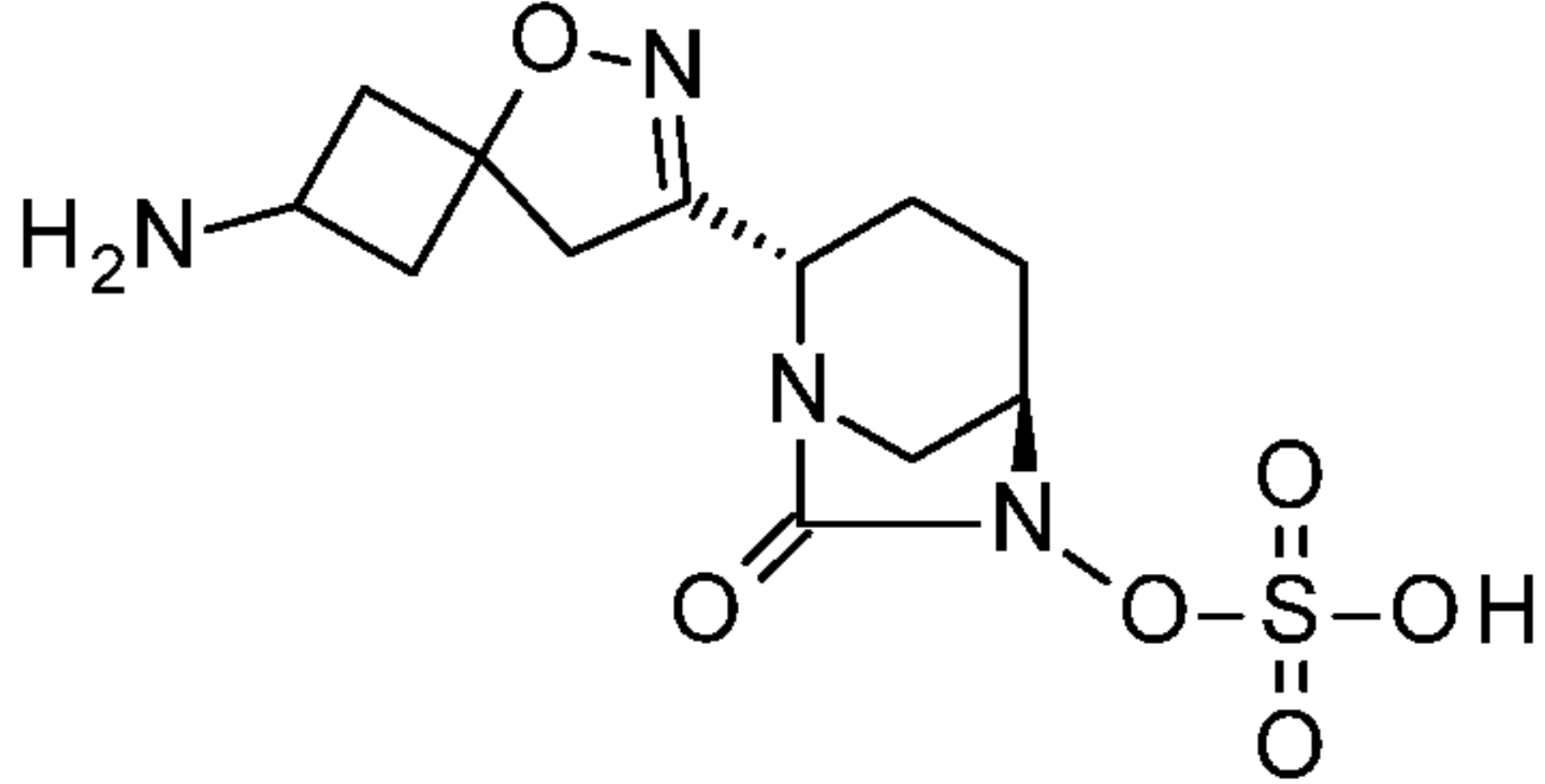
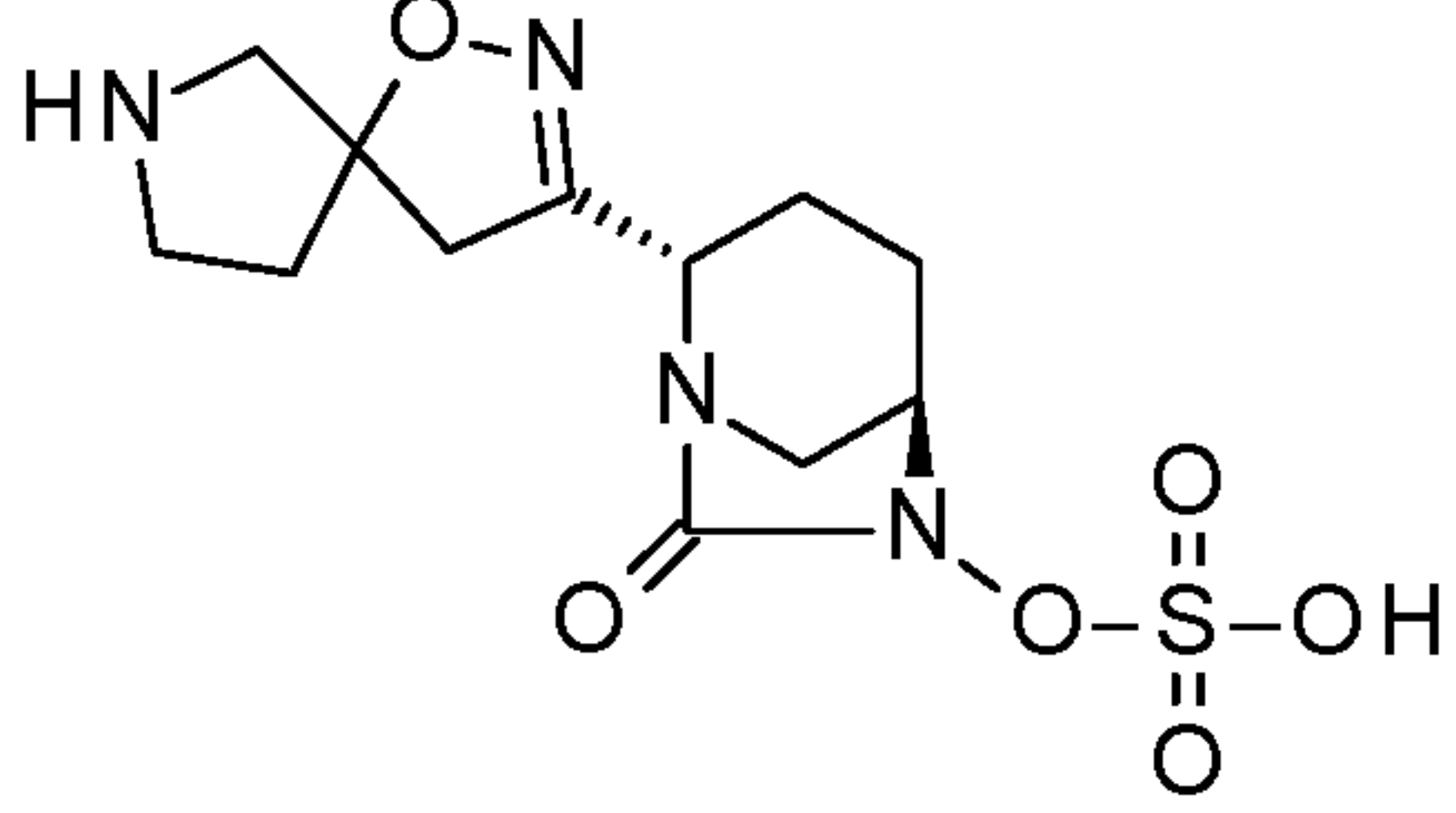
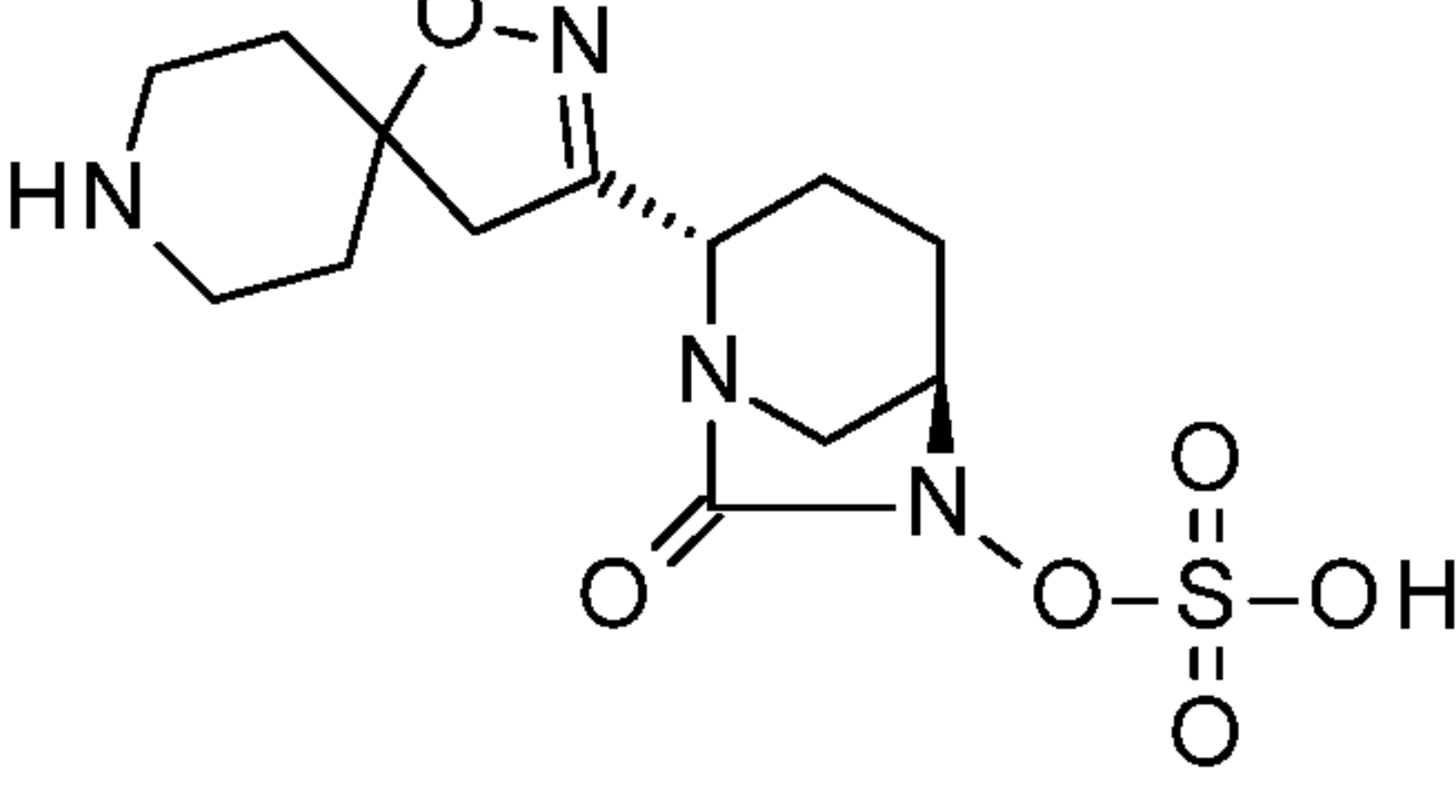
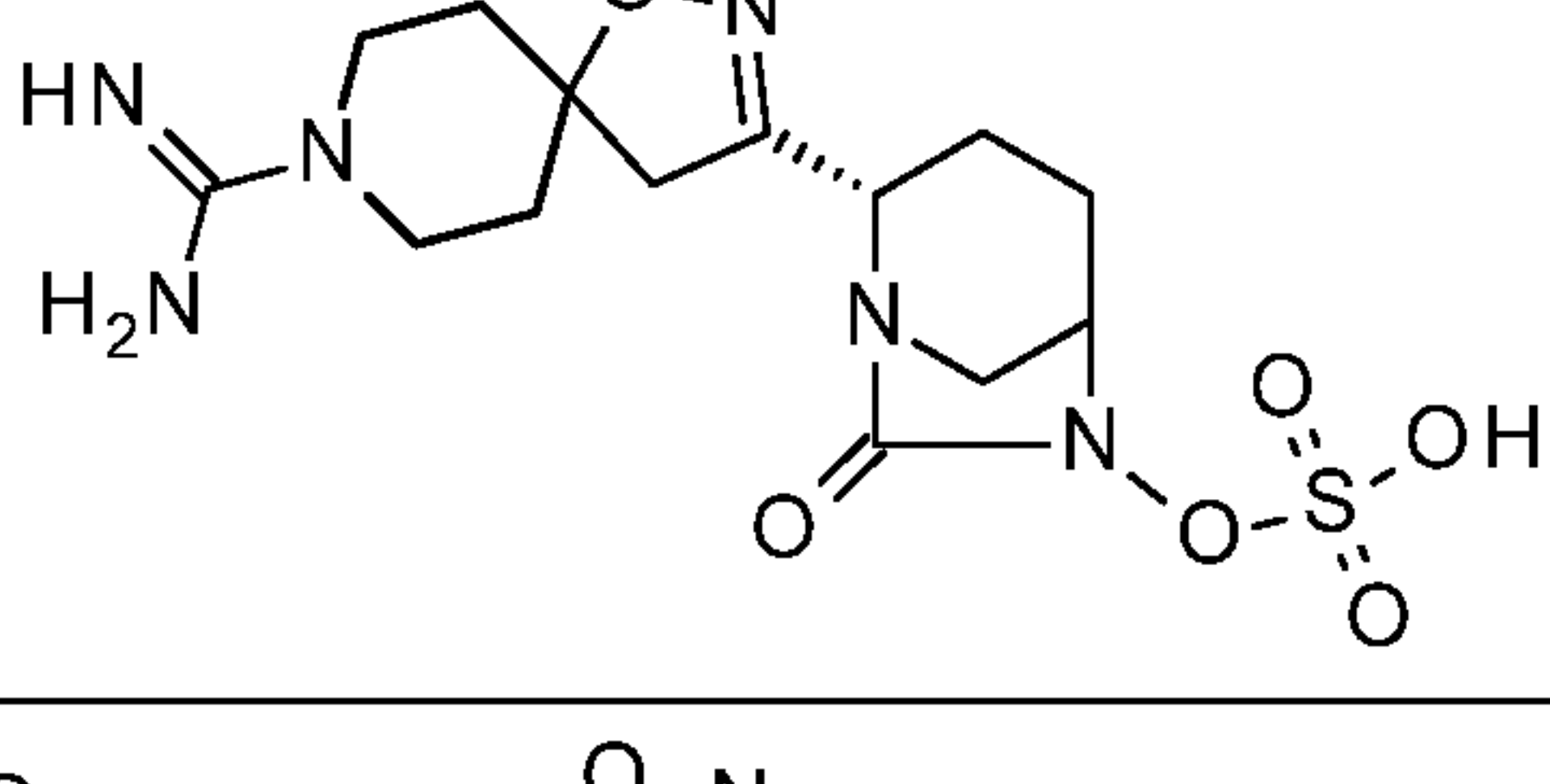
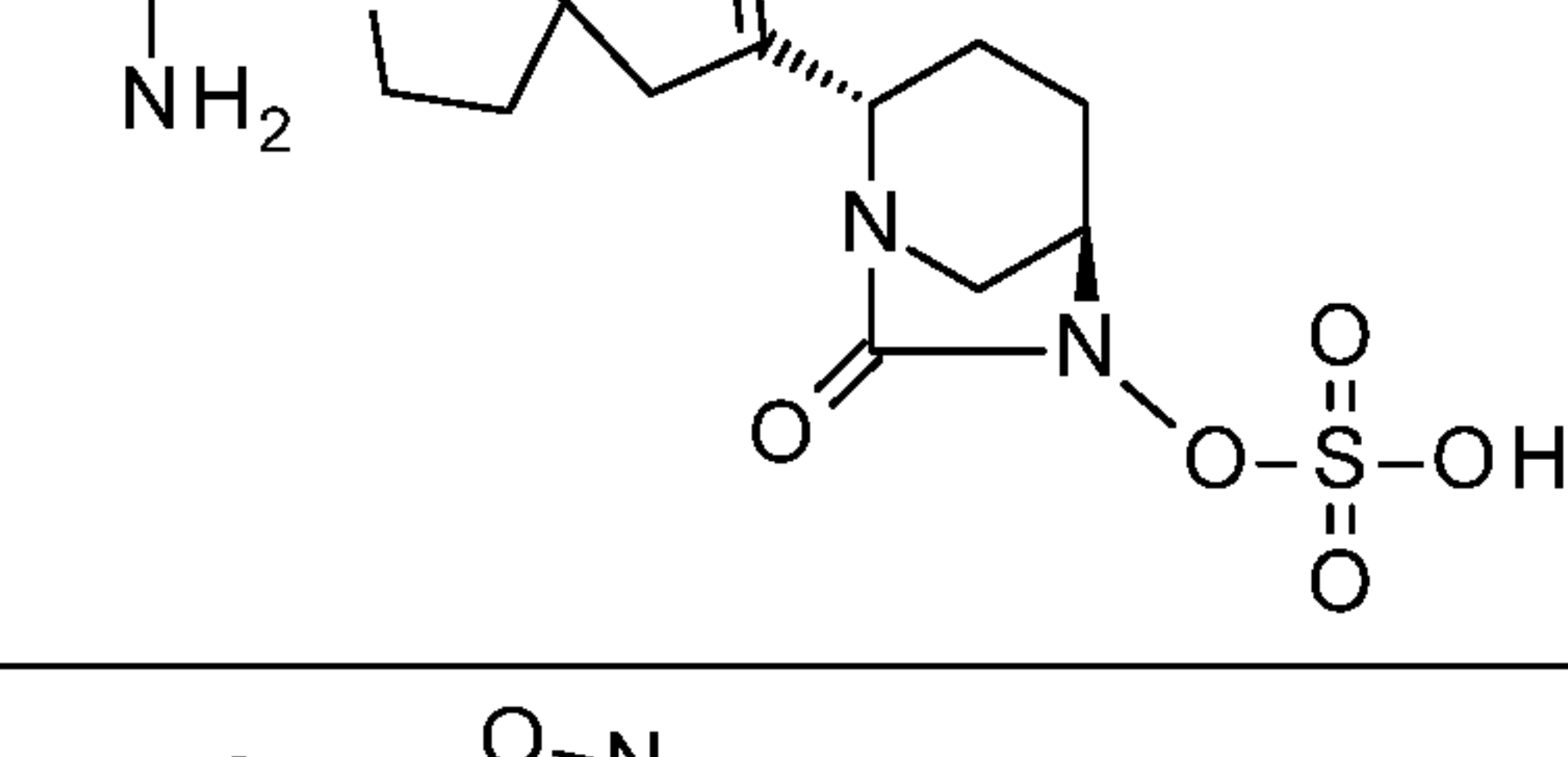
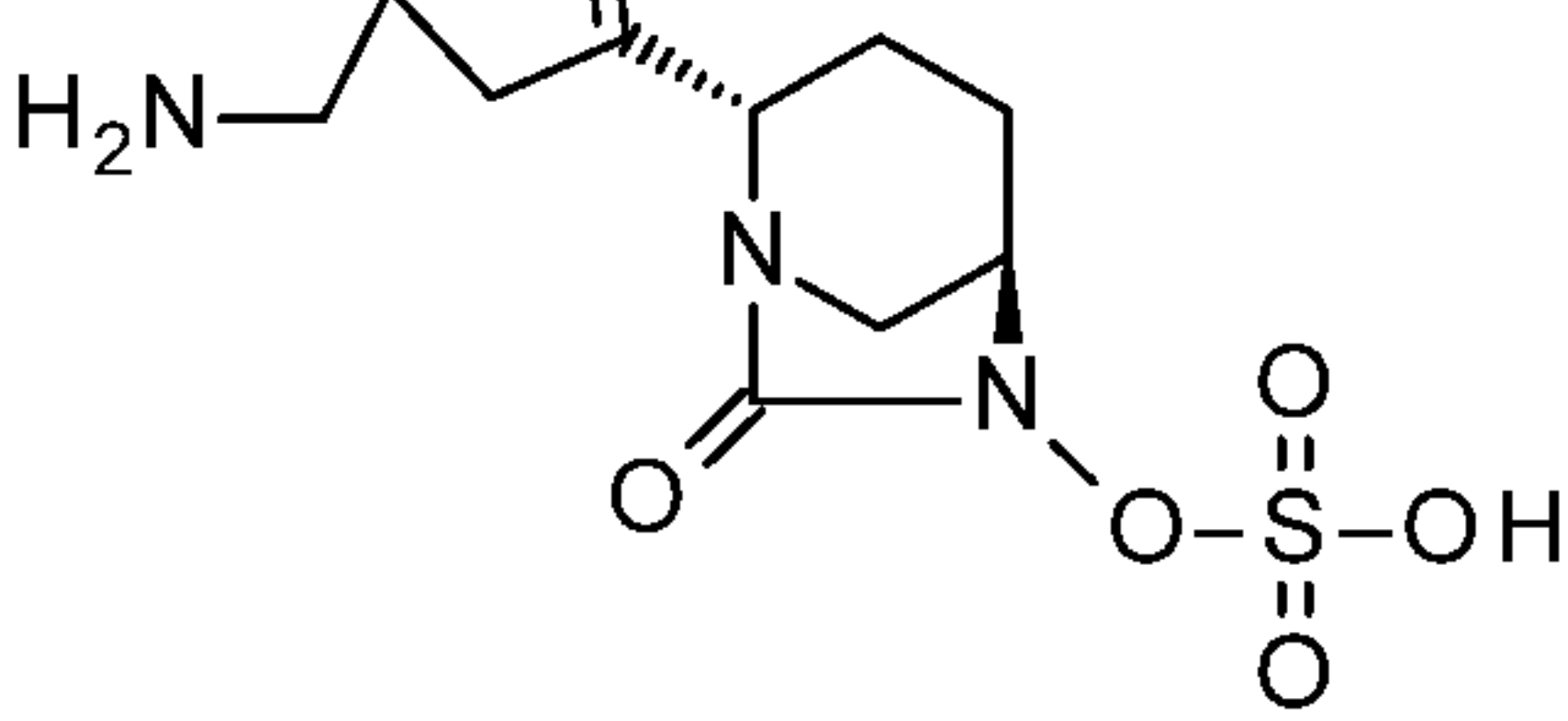
The pharmaceutically acceptable acid addition salts can also exist as various solvates, such as with water, methanol, ethanol, dimethylformamide, and the like. Mixtures of such solvates can also be prepared. The source of such solvate can be from the solvent of crystallization, inherent in the solvent of preparation or crystallization, or adventitious to such solvent.

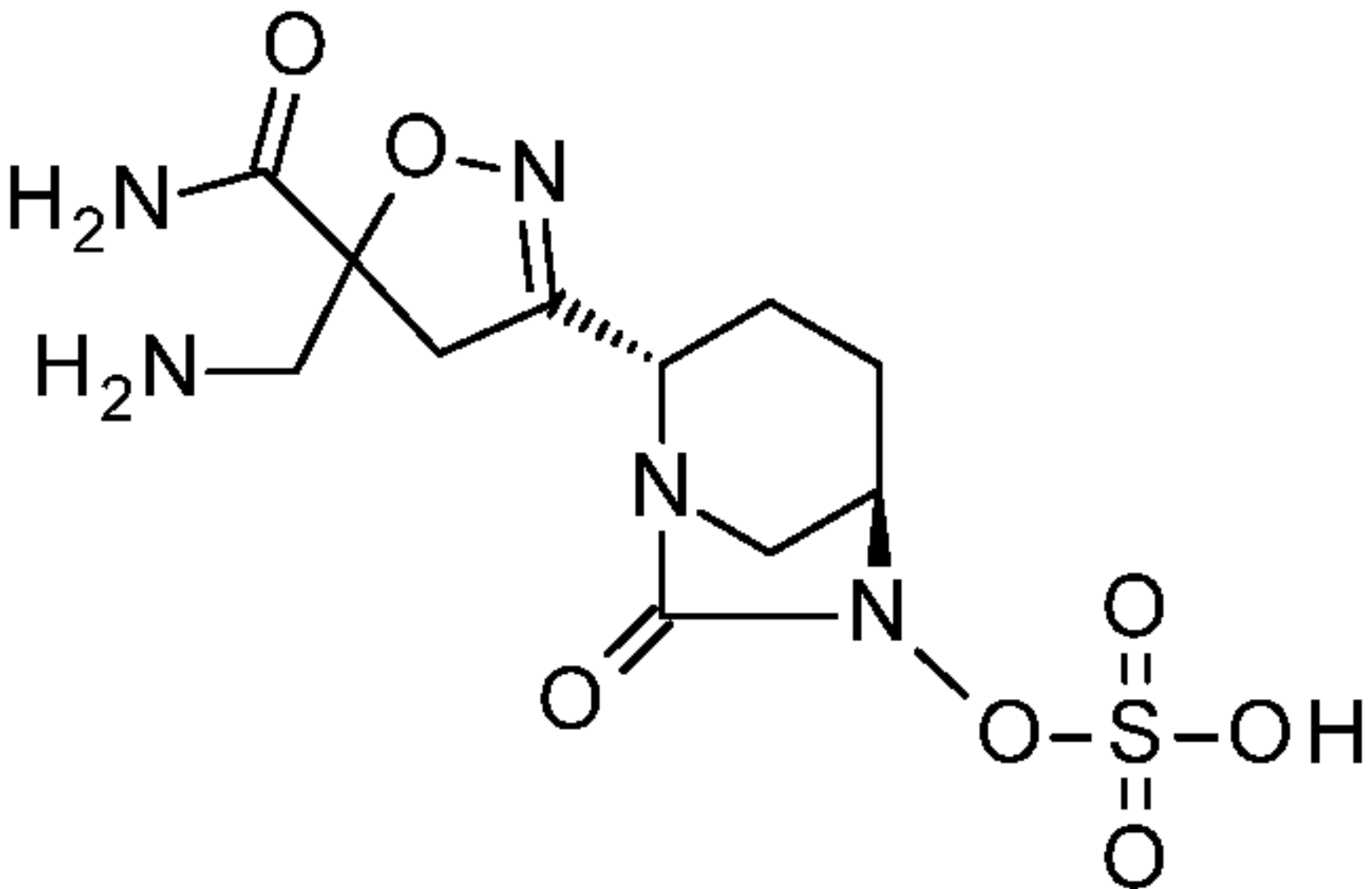
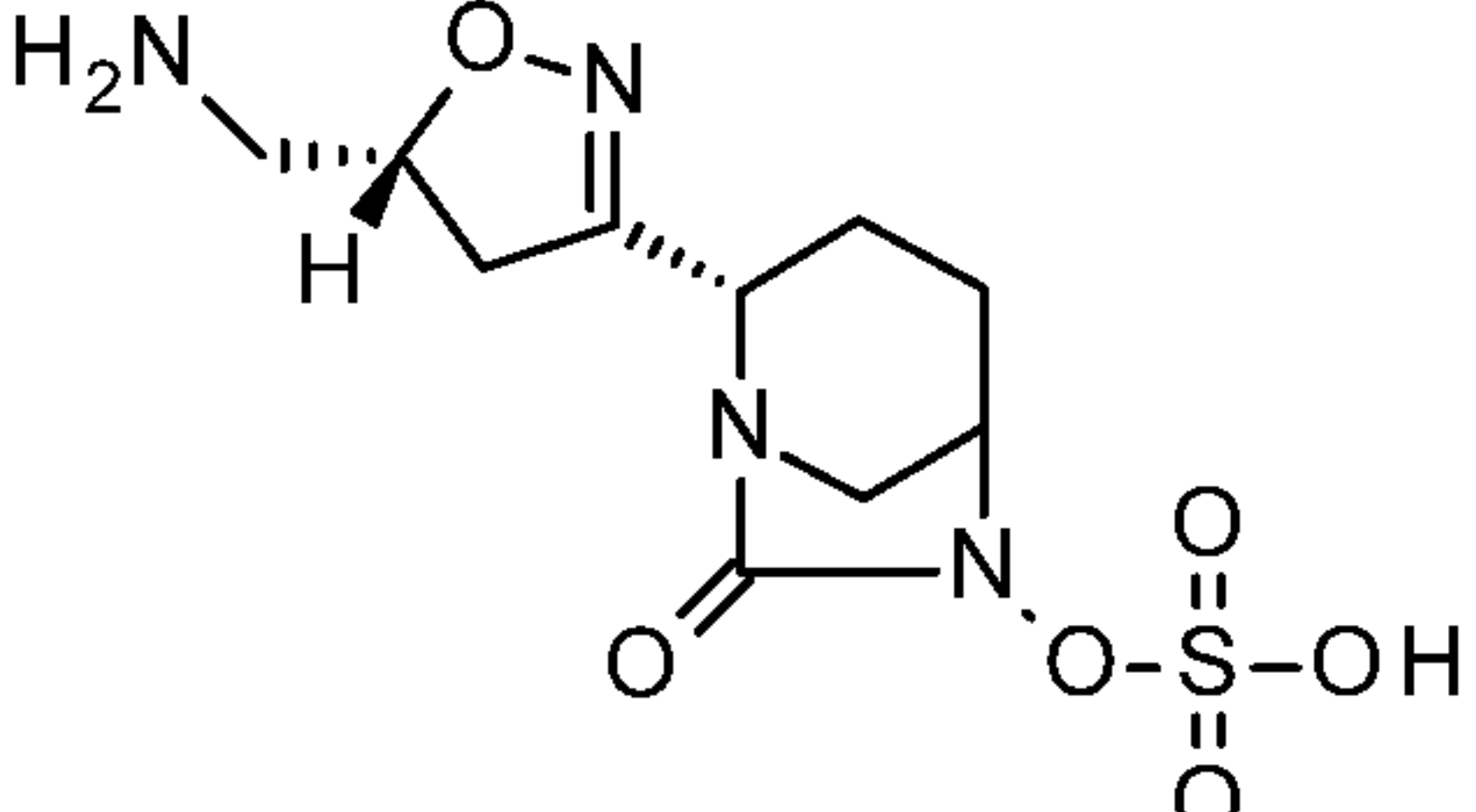
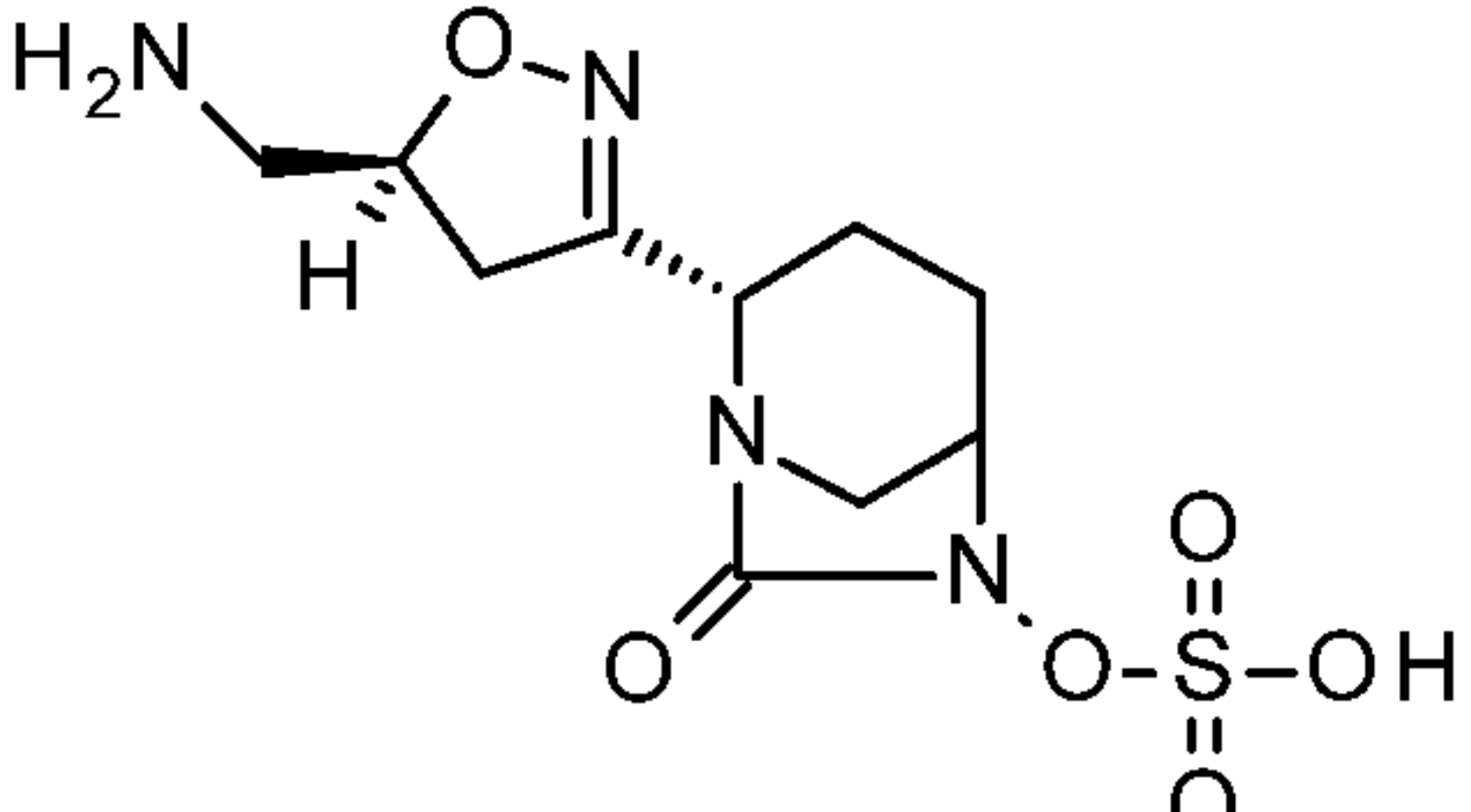
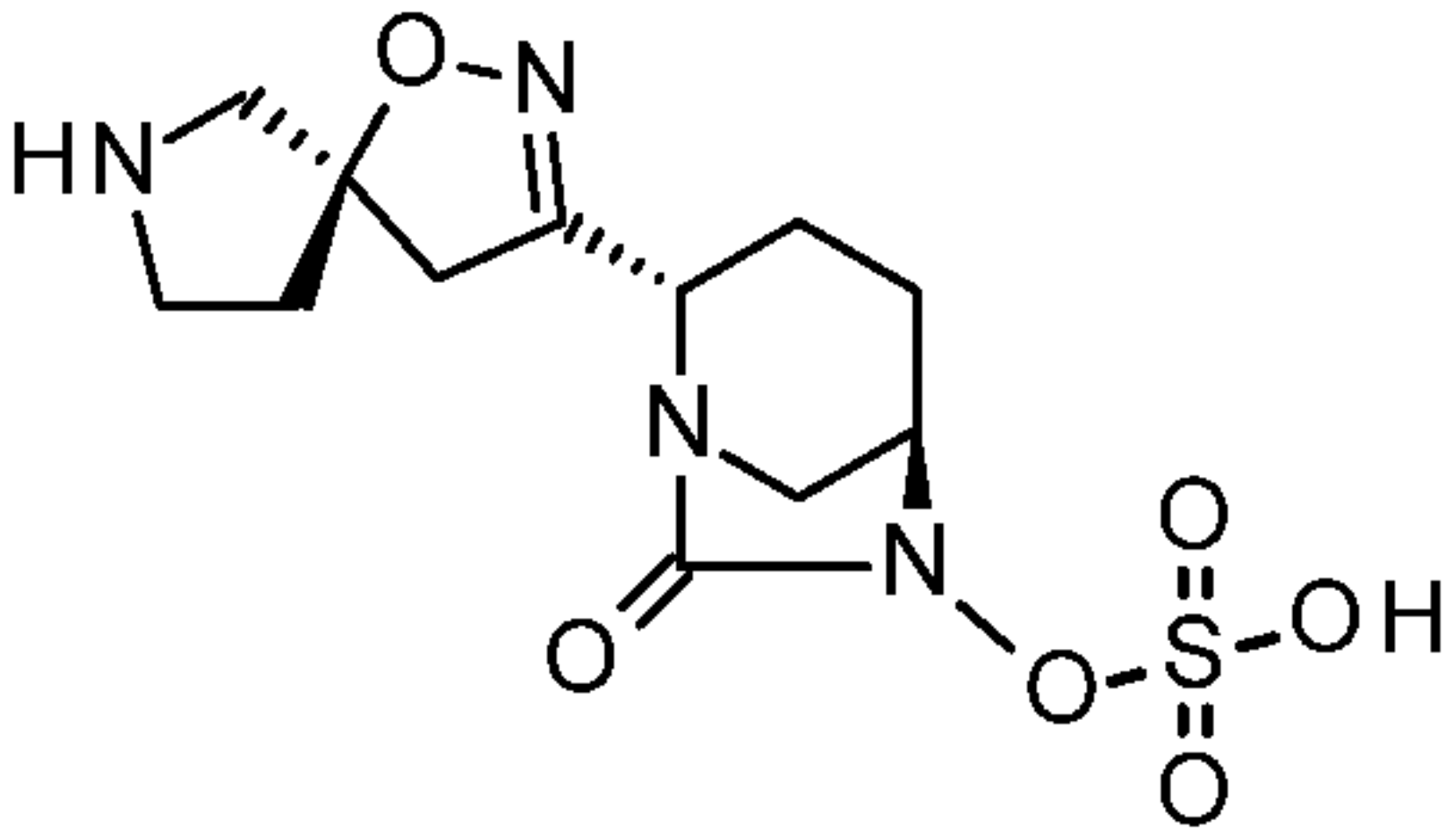
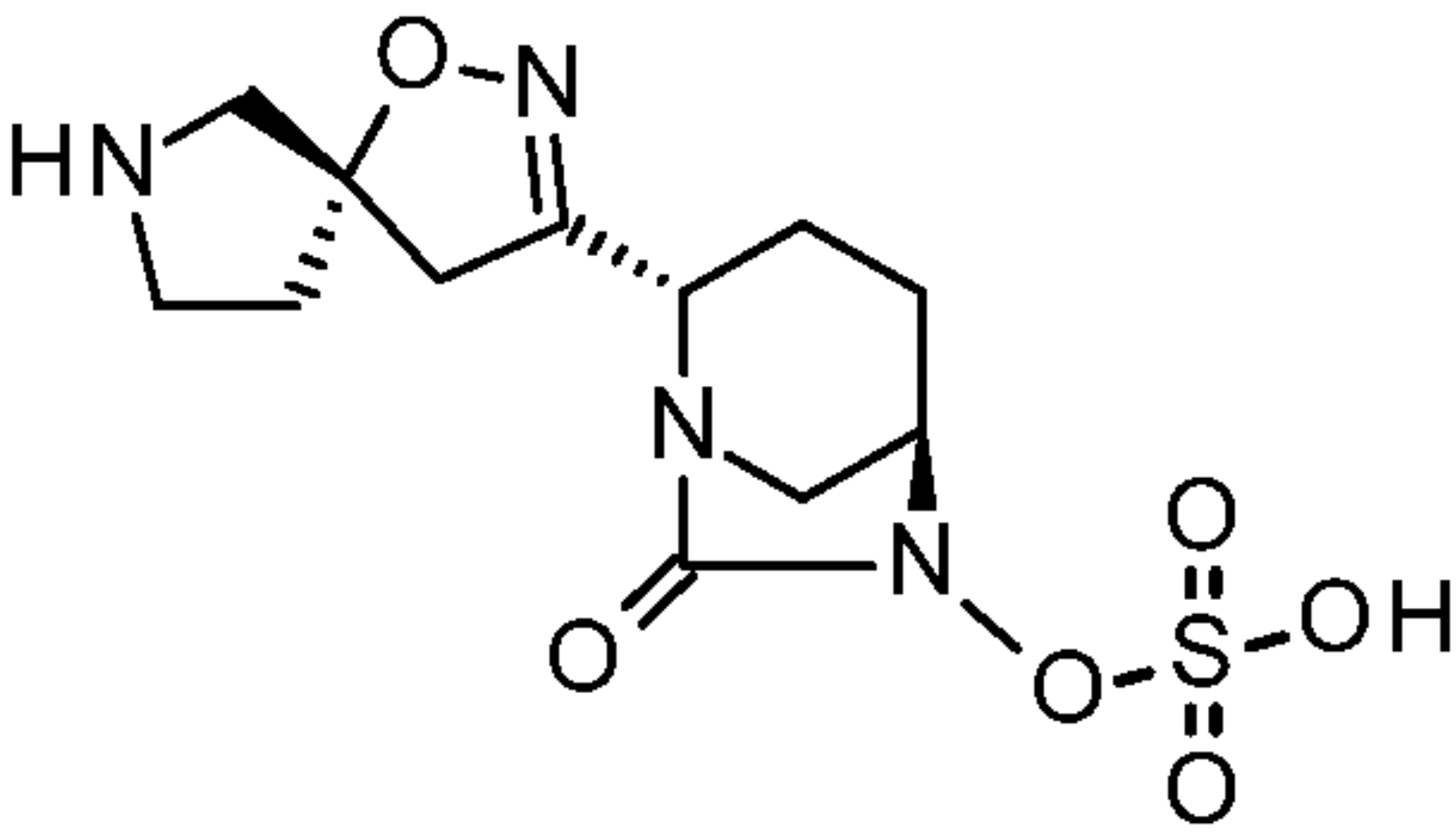
Exemplary compounds of Formula I are depicted in Table 1. The compounds of Table 1 may be depicted as the free base or the conjugate acid. Compounds may be isolated in either the free base form, as a salt (e.g., a sodium salt) or in both forms. In the chemical structures shown below, standard chemical abbreviations are sometimes used.

Table 1. Exemplary Compounds of Formula I

<u>Compound of</u>	<u>Structure</u>
Example 1	

<u>Compound of</u>	<u>Structure</u>
Example 2	
Example 3	
Example 4	
Example 5	
Example 6	
Example 7	

<u>Compound of</u>	<u>Structure</u>
Example 8	
Example 9	
Example 10	
Example 11	
Example 12	
Example 13	

<u>Compound of</u>	<u>Structure</u>
Example 14	
Example 15	
Example 16	
Example 17	
Example 18	

II. USES OF COMPOUNDS

In certain aspects, the invention provides methods for inhibiting β -lactamase, the
 5 method comprising contacting the β -lactamase with a compound of Formula I.

In certain aspects, the invention provides methods for inhibiting growth of a bacterium, the method comprising contacting the bacterium with a β -lactam antibiotic and a compound of Formula I.

In certain embodiments, the β -lactam antibiotic includes but is not limited to any
 5 antibiotic belonging, but not limited to, the classes of clavams, carbapenems, monobactams, penicillins, and or cephalosporins, or with any other compound susceptible to serine β -lactamases.

In certain embodiments, the β -lactam antibiotic is a cephalosporin. Examples of cephalosporins include, but are not limited to, Cefacetrile (cephacetrile), Cefadroxil
 10 (cefadroxyl), Cefalexin (cephalexin), Cefaloglycin (cephaloglycin), Cefalonium (cephalonium), Cefaloridine (cephaloradine), Cefalotin (cephalothin), Cefapirin (cephapirin), Cefatrizine, Cefazaflur, Cefazedone, Cefazolin (cephazolin), Cefradine (cephradine), Cefroxadine, Ceftezole, Cefaclor, Cefamandole, Cefmetazole, Cefonicid, Cefotetan, Cefoxitin, Cefprozil (cefproxil), Cefuroxime, Cefuzonam, Cefcapene,
 15 Cefdaloxime, Cefdinir, Cefditoren, Cefetamet, Cefixime, Cefmenoxime, Cefodizime, Cefotaxime, Cefpimizole, Cefpodoxime, Cefteram, Ceftibuten, Ceftiofur, Ceftiolene, Ceftizoxime, Ceftriaxone, Cefoperazone, Ceftazidime, Cefclidine, Cefepime, Cefluprenam, Cefoselis, Cefozopran, Cefpirome, Cefquinome, Cefaclomezine, Cefaloram, Cefaparole, Cefcanel, Cefedrolor, Cefempidone, Cefetrizole, Cefivitril, Cefmatilen, Cefmepidium,
 20 Cefovecin, Cefoxazole, Cefrotil, Cefsumide, Ceftaroline, Ceftioxide, Cefuracetime, cefbuperazone, cefminox, ceforanide, cefotiam, cefpiramide, cefsulodin, ceftobiprole latamoxef, loracarbef and Ceftolozane. In one embodiment the cephalosporin is Ceftolozane or Ceftazidime.

In certain embodiment, the β -lactam antibiotic is a carbapenem. Examples of
 25 carbapenem antibiotics include, but are not limited to, Imipenem, Imipenem/Cilastatin, Biapenem, Doripenem, Meropenem, Ertapenem and Panipenem. In one embodiment the Carbapenem is Imipenem/Cilastatin or Meropenem.

In certain embodiment of the invention, the β -lactam antibiotic is a monobactam. Examples of monobactam antibiotics include, but are not limited to Aztreonam,
 30 Tigemonam, Carumonam, BAL30072 and Nocardicin A.

In certain embodiment of the invention, the β -lactam antibiotic is a penem. In one embodiment of the invention, the β -lactam antibiotic is a penicillin. Examples of penicillin antibiotics include, but are not limited to Amoxicillin, Ampicillin, Azlocillin, Mezlocillin,

Apalcillin, Hetacillin, Becampicillin, Carbenicillin, Sulbenicillin, Ticarcillin, Piperacillin, Azlocillin, Mecillinam, Pivmecillinam, Methicillin, Ciclacillin, Talampicillin, Aspoxicillin, Oxacillin, Cloxacillin, Dicloxacillin, Flucloxacillin, Nafcillin and Pivampicillin.

In certain aspects, the invention provides methods for treating a bacterial infection, comprising administering a compound of Formula I to a subject in need of treatment.

In one aspect, bacterial infection may refer to an infection of any organ or tissue in the body caused by β -lactam resistant bacteria, preferably, Gram-negative β -lactam resistant bacteria. These organs or tissue include, without limitation, skeletal muscle, skin, bloodstream, kidneys, heart, lung and bone. For example, a compound of the invention in conjunction with a β -lactam antibiotic, can be administered to a subject to treat, without limitation, skin and soft tissue infections (e.g., complex skin infections), bacteremia, intra-abdominal infections and urinary tract infections (e.g., UTI). In addition, a compound of the invention may be used to treat community acquired respiratory infections, including, without limitation, otitis media, sinusitis, chronic bronchitis and pneumonia (including community-acquired pneumonia, hospital-acquired pneumonia and ventilator associated pneumonia), including pneumonia caused by drug-resistant *Pseudomonas aeruginosa*. In certain embodiments, a compound of the invention in conjunction with a β -lactam antibiotic, can be administered to a subject to treat mixed infections that comprise different types of Gram-negative bacteria, or which comprise both Gram-positive and Gram-negative bacteria. These types of infections include intra-abdominal infections and obstetrical/gynecological infections. In certain embodiments, a compound of the invention in conjunction with a β -lactam antibiotic, may also be administered to a subject to treat an infection including, without limitation, endocarditis, nephritis, septic arthritis, intra-abdominal sepsis, bone and joint infections and osteomyelitis. In certain embodiments, a compound of the invention in conjunction with a β -lactam antibiotic may also be directly injected or administered into an abscess, ventricle or joint.

In some embodiments, the infection may be a gynecological infection, a respiratory tract infection (RTI), a sexually transmitted disease, syphilis, a urinary tract infection, an acute exacerbation of chronic bronchitis (ACEB), acute otitis media, acute sinusitis, an infection caused by drug resistant bacteria, sepsis, catheter-related sepsis, chancroid, chlamydia, community-acquired pneumonia (CAP), a complicated skin and skin structure infection, a uncomplicated skin and skin structure infection, endocarditis, febrile

neutropenia, gonococcal cervicitis, gonococcal urethritis, hospital-acquired pneumonia (HAP), osteomyelitis, or an intra-abdominal infection (IAI).

In certain embodiments, bacterial infection may refer to an infection caused by Gram-negative bacteria, also referred to as a "Gram-negative infection." In one aspect, the Gram-negative infection is an infection resistant to one or more antibiotics. In one aspect, the Gram-negative infection is a multi-drug resistant infection. Representative Gram-negative pathogens known to express β -lactamases include, but are not limited to *Acinetobacter* spp. (including *Acinetobacter baumannii*), *Citrobacter* spp., *Escherichia* spp. (including *Escherichia coli*), *Haemophilus influenzae*, *Morganella morganii*, *Pseudomonas aeruginosa*, *Klebsiella* spp. (including *Klebsiella pneumoniae*), *Enterobacter* spp. (including *Enterobacter cloacae* and *Enterobacter aerogenes*), *Pasteurella* spp., *Proteus* spp. (including *Proteus mirabilis*), *Serratia* spp. (including *Serratia marcescens*), and *Providencia* spp. Bacterial infections can be caused or exacerbated by Gram-negative bacteria including strains which express β -lactamases that may confer resistance to penicillins, cephalosporins, monobactams and/or carbapenems.

In one embodiment of the invention is a method of treating one or more of the infections listed above comprising administering to a subject suffering from a bacterial infection an effective amount of a compound of Formula I or a pharmaceutically acceptable salt thereof, in combination with an additional antibiotic agent. In certain embodiments, the additional antibiotic agent is a β -lactam antibiotic. In one aspect, the additional antibiotic agent is a penicillin-binding protein inhibitor.

In another aspect of the invention, the compound of Formula I is administered in combination with a β -lactam antibiotic and an additional antibiotic and/or an additional β -lactamase inhibitor. In one aspect of the invention, the additional antibiotic agent is selected from one of the classes of aminoglycosides, spectinomycins, macrolides, ketolides, streptogramins, oxazolidinones, tetracyclines, fluoroquinolones, coumarin antibiotics, glycopeptides, lipoglycopeptides, nitroimidazoles, ansamycins, phenicols, mupirocin, fosfomicin, tobramycin, linezolid, daptomycin, and vancomycin.

In one aspect of the invention, the compound of Formula I is administered in combination with a β -lactam antibiotic and a second agent which is designed to address β -lactam resistance. In one aspect of the invention, the compound of Formula I is administered in combination with a β -lactam antibiotic and a second serine beta-lactamase inhibitor. In one aspect of the invention, the second beta-lactamase inhibitor is selected

from sulbactam, tazobactam, avibactam, clavulanic acid, LK-157, LK-176, SA-1-204, SA-2-13, BLI-489 (Pfizer/Wyeth), BAL0029880 and MK7655. In another aspect of the invention, the second agent designed to address β -lactam resistance may be a metallo- β -lactamase (MBL) inhibitor, also known as a Class B inhibitor.

5 In certain embodiments, the subject is a mammal, e.g., a human.

In certain embodiments, the β -lactamase inhibitor and β -lactam antibiotic are administered simultaneously. In alternative embodiments, the one or more additional β -lactam antibiotics are administered within about 5 minutes to within about 168 hours prior to or after administration of the β -lactamase inhibitor.

10

III. DEFINITIONS

The term “acyl” is art-recognized and refers to a group represented by the general formula hydrocarbonylC(O)-, preferably alkylC(O)-.

15 The term “acylamino” is art-recognized and refers to an amino group substituted with an acyl group and may be represented, for example, by the formula hydrocarbonylC(O)NH-.

The term “acyloxy” is art-recognized and refers to a group represented by the general formula hydrocarbonylC(O)O-, preferably alkylC(O)O-.

20 The term “alkoxy” refers to an alkyl group, preferably a lower alkyl group, having an oxygen attached thereto. Representative alkoxy groups include methoxy, ethoxy, propoxy, tert-butoxy and the like.

The term “alkoxyalkyl” refers to an alkyl group substituted with an alkoxy group and may be represented by the general formula alkyl-O-alkyl.

25 The term “alkenyl”, as used herein, refers to an aliphatic group containing at least one double bond and is intended to include both "unsubstituted alkenyls" and "substituted alkenyls", the latter of which refers to alkenyl moieties having substituents replacing a hydrogen on one or more carbons of the alkenyl group. Such substituents may occur on one or more carbons that are included or not included in one or more double bonds. Moreover, such substituents include all those contemplated for alkyl groups, as discussed below, except where stability is prohibitive. For example, substitution of alkenyl groups by 30 one or more alkyl, carbocyclyl, aryl, heterocyclyl, or heteroaryl groups is contemplated.

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, heptyl and octyl. A C₁-C₆ straight chained or branched alkyl group is also referred to as a "lower alkyl" group.

5 Moreover, the term "alkyl" (or "lower alkyl") as used throughout the specification, examples, and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents, if not otherwise specified, can include, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, 10 an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxy, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in 15 the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. For instance, the substituents of a substituted alkyl may include substituted and unsubstituted forms of amino, azido, imino, amido, phosphoryl (including phosphonate and phosphinate), sulfonyl (including sulfate, sulfonamido, sulfamoyl and sulfonate), and silyl groups, as well as ethers, alkylthios, carbonyls (including ketones, aldehydes, 20 carboxylates, and esters), -CF₃, -CN and the like. Exemplary substituted alkyls are described below. Cycloalkyls can be further substituted with alkyls, alkenyls, alkoxy, alkylthios, aminoalkyls, carbonyl-substituted alkyls, -CF₃, -CN, and the like.

The term "C_{x-y}" when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups that contain from x to 25 y carbons in the chain. For example, the term "C_{x-y}alkyl" refers to substituted or unsubstituted saturated hydrocarbon groups, including straight-chain alkyl and branched-chain alkyl groups that contain from x to y carbons in the chain, including haloalkyl groups such as trifluoromethyl and 2,2,2-trifluoroethyl, etc. C₀ alkyl indicates a hydrogen where the group is in a terminal position, a bond if internal. The terms "C_{2-y}alkenyl" and "C₂₋ 30 yalkynyl" refer to substituted or unsubstituted unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

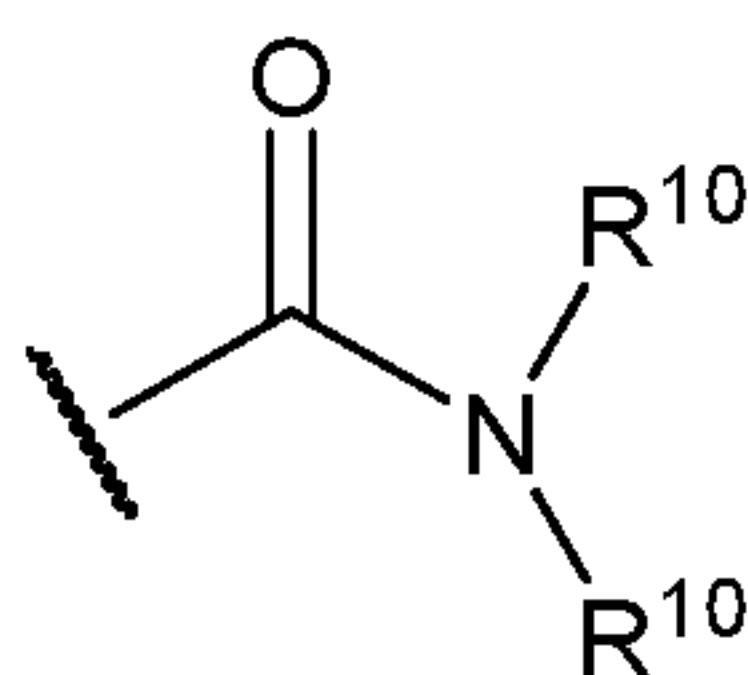
The term “alkylamino”, as used herein, refers to an amino group substituted with at least one alkyl group.

The term “alkylthio”, as used herein, refers to a thiol group substituted with an alkyl group and may be represented by the general formula alkylS-.

5 The term “alkynyl”, as used herein, refers to an aliphatic group containing at least one triple bond and is intended to include both "unsubstituted alkynyls" and "substituted alkynyls", the latter of which refers to alkynyl moieties having substituents replacing a hydrogen on one or more carbons of the alkynyl group. Such substituents may occur on one or more carbons that are included or not included in one or more triple bonds.

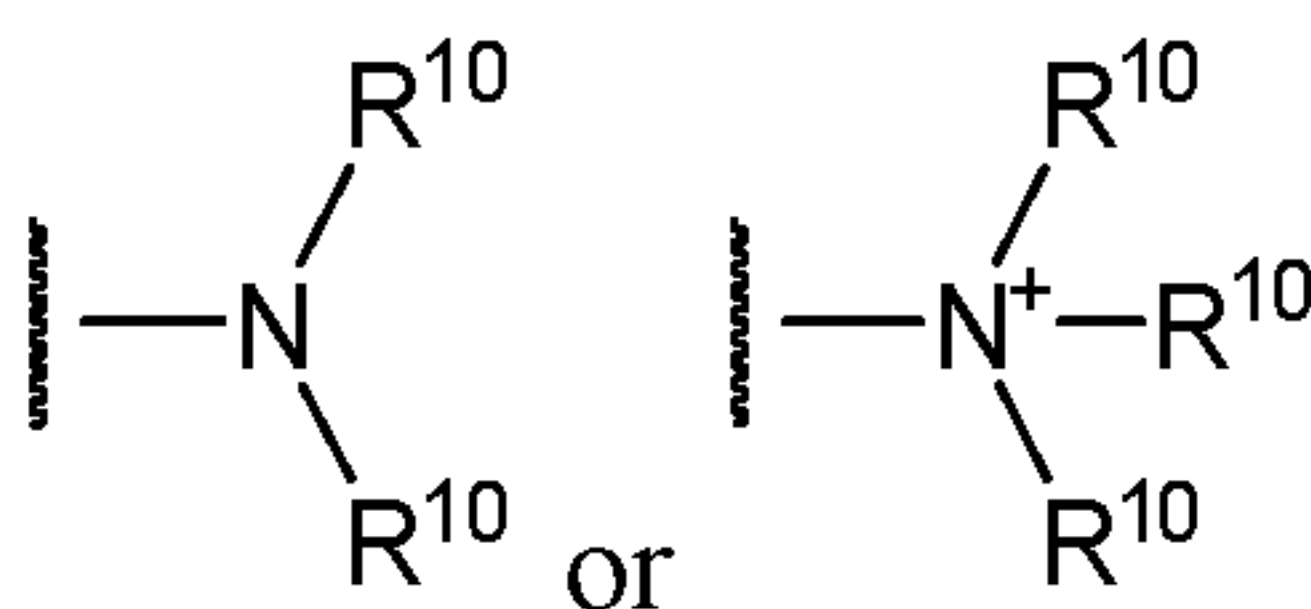
10 Moreover, such substituents include all those contemplated for alkyl groups, as discussed above, except where stability is prohibitive. For example, substitution of alkynyl groups by one or more alkyl, carbocyclyl, aryl, heterocyclyl, or heteroaryl groups is contemplated.

The term “amide”, as used herein, refers to a group



15 wherein each R^{10} independently represent a hydrogen or hydrocarbyl group, or two R^{10} are taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

The terms “amine” and “amino” are art-recognized and refer to both unsubstituted and substituted amines and salts thereof, e.g., a moiety that can be represented by



20 wherein each R^{10} independently represents a hydrogen or a hydrocarbyl group, or two R^{10} are taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

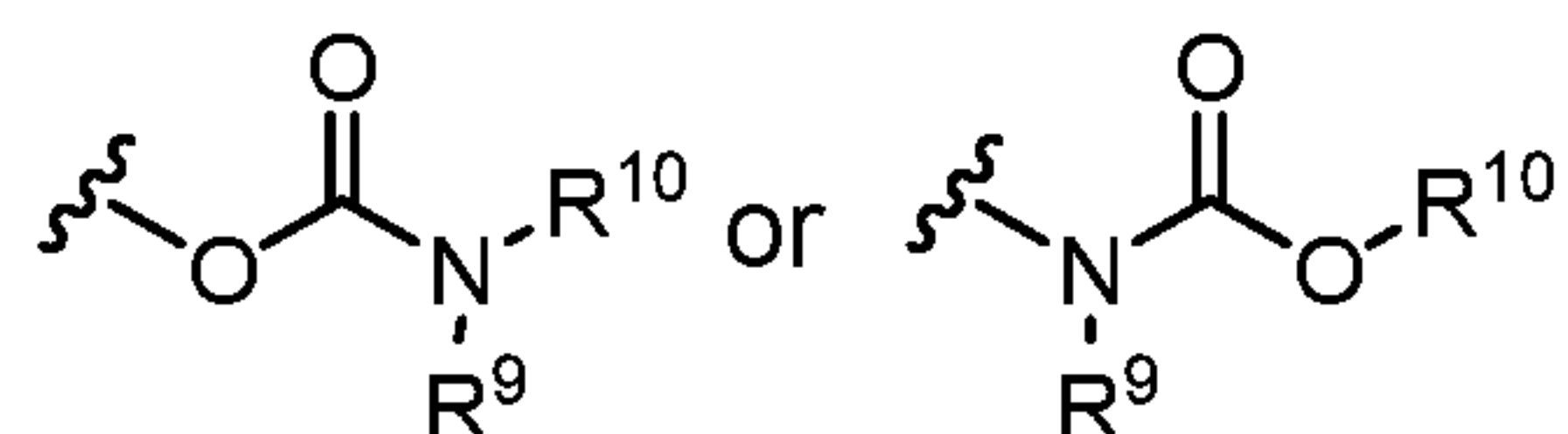
25 The term “aminoalkyl”, as used herein, refers to an alkyl group substituted with an amino group.

The term “aralkyl”, as used herein, refers to an alkyl group substituted with an aryl group.

The term “aryl” as used herein include substituted or unsubstituted single-ring aromatic groups in which each atom of the ring is carbon. Preferably the ring is a 5- to 7-

membered ring, more preferably a 6-membered ring. The term “aryl” also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is aromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Aryl groups include benzene, naphthalene, phenanthrene, phenol, aniline, and the like.

The term “carbamate” is art-recognized and refers to a group



wherein R^9 and R^{10} independently represent hydrogen or a hydrocarbyl group, such as an alkyl group, or R^9 and R^{10} taken together with the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

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 saturated, unsaturated 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 saturated, unsaturated and aromatic rings. In an exemplary embodiment, an aromatic ring, e.g., phenyl, may be fused to a saturated or unsaturated ring, e.g., cyclohexane, cyclopentane, or cyclohexene. Any combination of saturated, unsaturated and aromatic bicyclic rings, as valence permits, is included in the definition of carbocyclic. Exemplary “carbocycles” include 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 hydrogen atom.

A “cycloalkyl” group is a cyclic hydrocarbon which is completely saturated. “Cycloalkyl” includes monocyclic and bicyclic rings. Typically, a monocyclic cycloalkyl group has from 3 to about 10 carbon atoms, more typically 3 to 8 carbon atoms unless otherwise defined. The second ring of a bicyclic cycloalkyl may be selected from saturated, 5 unsaturated and aromatic rings. Cycloalkyl includes bicyclic molecules in which one, two or three or more atoms are shared between the two rings. The term “fused cycloalkyl” refers to a bicyclic cycloalkyl in which each of the rings shares two adjacent atoms with the other ring. The second ring of a fused bicyclic cycloalkyl may be selected from saturated, unsaturated and aromatic rings. A “cycloalkenyl” group is a cyclic hydrocarbon containing 10 one or more double bonds.

The term “carbocyclalkyl”, as used herein, refers to an alkyl group substituted with a carbocycle group.

The term “carbonate” is art-recognized and refers to a group $-\text{OCO}_2\text{-R}^{10}$, wherein R^{10} represents a hydrocarbyl group.

15 The term “carboxy”, as used herein, refers to a group represented by the formula $-\text{CO}_2\text{H}$.

The term “ester”, as used herein, refers to a group $-\text{C}(\text{O})\text{OR}^{10}$ wherein R^{10} represents a hydrocarbyl group.

The term “ether”, as used herein, refers to a hydrocarbyl group linked through an 20 oxygen to another hydrocarbyl group. Accordingly, an ether substituent of a hydrocarbyl group may be hydrocarbyl-O-. Ethers may be either symmetrical or unsymmetrical. Examples of ethers include, but are not limited to, heterocycle-O-heterocycle and aryl-O-heterocycle. Ethers include “alkoxyalkyl” groups, which may be represented by the general formula alkyl-O-alkyl.

25 The terms “halo” and “halogen” as used herein means halogen and includes chloro, fluoro, bromo, and iodo.

The terms “hetaralkyl” and “heteroaralkyl”, as used herein, refers to an alkyl group substituted with a hetaryl group.

The term “heteroalkyl”, as used herein, refers to a saturated or unsaturated chain of 30 carbon atoms and at least one heteroatom, wherein no two heteroatoms are adjacent.

The terms “heteroaryl” and “hetaryl” include 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 terms “heteroaryl” and “hetaryl” also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heteroaromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, and pyrimidine, and the like.

The term “heteroatom” as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, and sulfur.

The terms “heterocyclyl”, “heterocycle”, and “heterocyclic” refer to substituted or unsubstituted non-aromatic ring structures, preferably 3- to 10-membered rings, more preferably 3- to 7-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms “heterocyclyl” and “heterocyclic” also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heterocyclic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heterocyclyl groups include, for example, piperidine, piperazine, pyrrolidine, morpholine, lactones, lactams, and the like. Heterocyclyl groups can also be substituted by oxo groups. For example, “heterocyclyl” encompasses both pyrrolidine and pyrrolidinone.

The term “heterocyclylalkyl”, as used herein, refers to an alkyl group substituted with a heterocycle group.

The term “hydrocarbyl”, as used herein, refers to a group that is bonded through a carbon atom that does not have a =O or =S substituent, and typically has at least one carbon-hydrogen bond and a primarily carbon backbone, but may optionally include heteroatoms. Thus, groups like methyl, ethoxyethyl, 2-pyridyl, and trifluoromethyl are considered to be hydrocarbyl for the purposes of this application, but substituents such as acetyl (which has a =O substituent on the linking carbon) and ethoxy (which is linked through oxygen, not carbon) are not. Hydrocarbyl groups include, but are not limited to aryl, heteroaryl, carbocycle, heterocyclyl, alkyl, alkenyl, alkynyl, and combinations thereof.

The term “hydroxyalkyl”, as used herein, refers to an alkyl group substituted with a hydroxy group.

The term “lower” when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups where there are ten or fewer non-hydrogen atoms in the substituent, preferably six or fewer. A “lower alkyl”, for example, refers to an alkyl group that contains ten or fewer carbon atoms, preferably six or fewer. In certain embodiments, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy substituents defined herein are respectively lower acyl, lower acyloxy, lower alkyl, lower alkenyl, lower alkynyl, or lower alkoxy, whether they appear alone or in combination with other substituents, such as in the recitations hydroxyalkyl and aralkyl (in which case, for example, the atoms within the aryl group are not counted when counting the carbon atoms in the alkyl substituent).

As used herein, the term “oxo” refers to a carbonyl group. When an oxo substituent occurs on an otherwise saturated group, such as with an oxo-substituted cycloalkyl group (e.g., 3-oxo-cyclobutyl), the substituted group is still intended to be a saturated group. When a group is referred to as being substituted by an “oxo” group, this can mean that a carbonyl moiety (i.e., -C(=O)-) replaces a methylene unit (i.e., $\text{-CH}_2\text{-}$).

The terms “polycyclyl”, “polycycle”, and “polycyclic” refer to two or more rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls) in which two or more atoms are common to two adjoining rings, e.g., the rings are “fused rings”. Each of the rings of the polycycle can be substituted or unsubstituted. In certain embodiments, each ring of the polycycle contains from 3 to 10 atoms in the ring, preferably from 5 to 7.

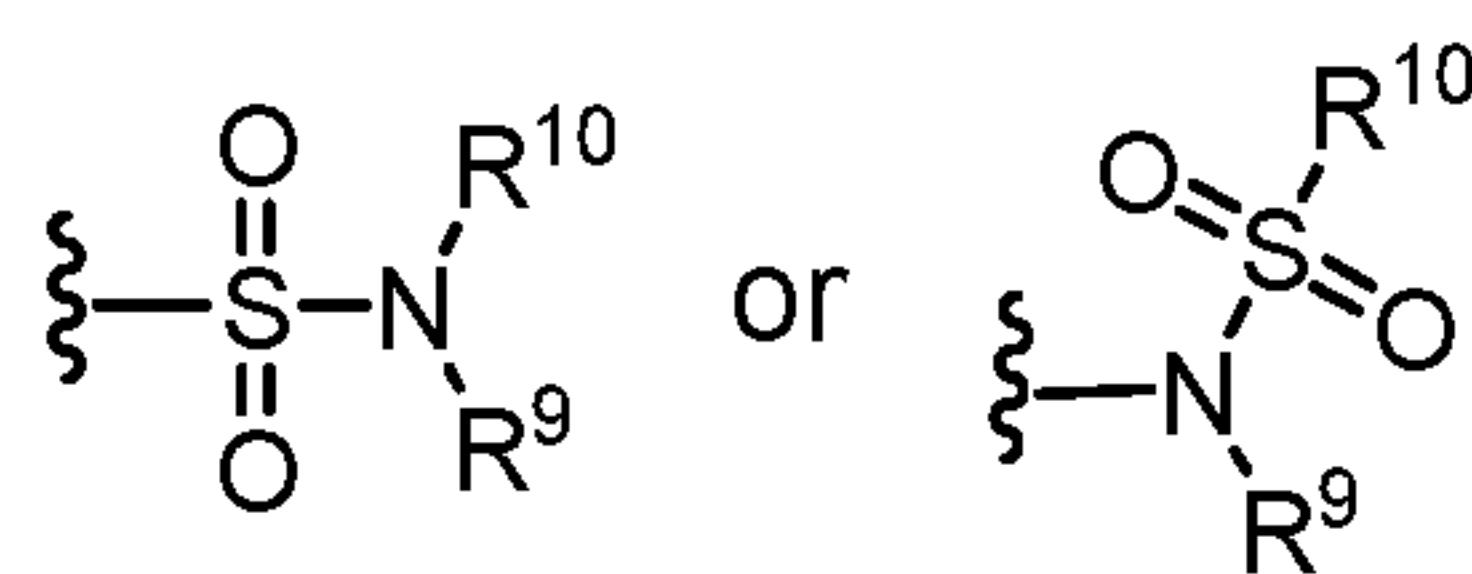
The term “silyl” refers to a silicon moiety with three hydrocarbyl moieties attached thereto.

The term “substituted” refers to moieties having substituents replacing a hydrogen on one or more carbons of the backbone. It will be understood that “substitution” or “substituted with” includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. As used herein, the term “substituted” is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and non-aromatic substituents of organic compounds. The permissible substituents can be one or more and the same or

different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. Substituents can include any substituents described herein, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxy, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a , a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that substituents can themselves be substituted, if appropriate. Unless specifically stated as “unsubstituted,” references to chemical moieties herein are understood to include substituted variants. For example, reference to an “aryl” group or moiety implicitly includes both substituted and unsubstituted variants.

The term “sulfate” is art-recognized and refers to the group $-\text{OSO}_3\text{H}$, or a pharmaceutically acceptable salt thereof.

The term “sulfonamide” is art-recognized and refers to the group represented by the general formulae



wherein R^9 and R^{10} independently represents hydrogen or hydrocarbyl, such as alkyl, or R^9 and R^{10} taken together with the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

The term “sulfoxide” is art-recognized and refers to the group $-\text{S}(\text{O})-\text{R}^{10}$, wherein R^{10} represents a hydrocarbyl.

The term “sulfonate” is art-recognized and refers to the group SO_3H , or a pharmaceutically acceptable salt thereof.

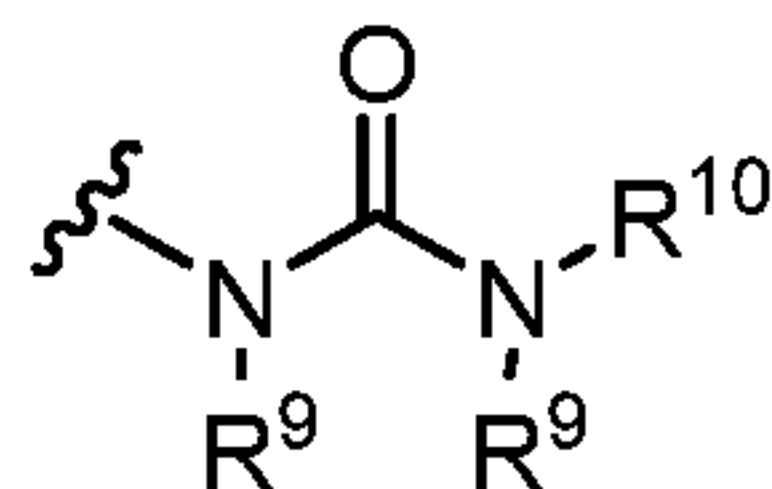
The term “sulfone” is art-recognized and refers to the group $-\text{S}(\text{O})_2-\text{R}^{10}$, wherein R^{10} represents a hydrocarbyl.

The term “thioalkyl”, as used herein, refers to an alkyl group substituted with a thiol group.

The term “thioester”, as used herein, refers to a group $-\text{C}(\text{O})\text{SR}^{10}$ or $-\text{SC}(\text{O})\text{R}^{10}$ wherein R^{10} represents a hydrocarbyl.

The term “thioether”, as used herein, is equivalent to an ether, wherein the oxygen is replaced with a sulfur.

The term “urea” is art-recognized and may be represented by the general formula



5 wherein R⁹ and R¹⁰ independently represent hydrogen or a hydrocarbyl, such as alkyl, or either occurrence of R⁹ taken together with R¹⁰ and the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

“Protecting group” refers to a group of atoms that, when attached to a reactive functional group in a molecule, mask, reduce or prevent the reactivity of the functional group. Typically, a protecting group may be selectively removed as desired during the course of a synthesis. Examples of protecting groups can be found in Greene and Wuts, *Protective Groups in Organic Chemistry*, 3rd Ed., 1999, John Wiley & Sons, NY and Harrison et al., *Compendium of Synthetic Organic Methods*, Vols. 1-8, 1971-1996, John Wiley & Sons, NY. Representative nitrogen protecting groups include, but are not limited to, formyl, acetyl, trifluoroacetyl, benzyl, benzyloxycarbonyl (“CBZ”), tert-butoxycarbonyl (“Boc”), trimethylsilyl (“TMS”), 2-trimethylsilyl-ethanesulfonyl (“TES”), trityl and substituted trityl groups, allyloxycarbonyl, 9-fluorenylmethyloxycarbonyl (“Fmoc”), nitro-veratryloxycarbonyl (“NVOC”) and the like. Representative hydroxylprotecting groups include, but are not limited to, those where the hydroxyl group is either acylated (esterified) or alkylated such as benzyl and trityl ethers, as well as alkyl ethers, tetrahydropyranyl ethers, trialkylsilyl ethers (e.g., TMS or TIPS groups), glycol ethers, such as ethylene glycol and propylene glycol derivatives and allyl ethers.

As used herein, a therapeutic that “prevents” a disorder or condition refers to a compound that, in a statistical sample, reduces the occurrence of the disorder or condition in the treated sample relative to an untreated control sample, or delays the onset or reduces the severity of one or more symptoms of the disorder or condition relative to the untreated control sample.

The term “treating” includes prophylactic and/or therapeutic treatments. The term “prophylactic or therapeutic” treatment is art-recognized and includes administration to the host of one or more of the subject compositions. 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 it is administered after manifestation of the unwanted condition, the treatment is therapeutic, (i.e., it is intended to diminish, ameliorate, or stabilize the existing unwanted condition or side effects thereof).

5 The term “prodrug” is intended to encompass compounds which, under physiologic conditions, are converted into the therapeutically active agents of the present invention (e.g., a compound of formula I). A common method for making a prodrug is to include one or more selected moieties which are hydrolyzed under physiologic conditions to reveal the desired molecule. In other embodiments, the prodrug is converted by an enzymatic activity of the host animal. For example, esters or carbonates (e.g., esters or carbonates of alcohols
10 or carboxylic acids) are preferred prodrugs of the present invention. In certain embodiments, some or all of the compounds of formula I in a formulation represented above can be replaced with the corresponding suitable prodrug, e.g., wherein a hydroxyl in the parent compound is presented as an ester or a carbonate or carboxylic acid present in the parent compound is presented as an ester.

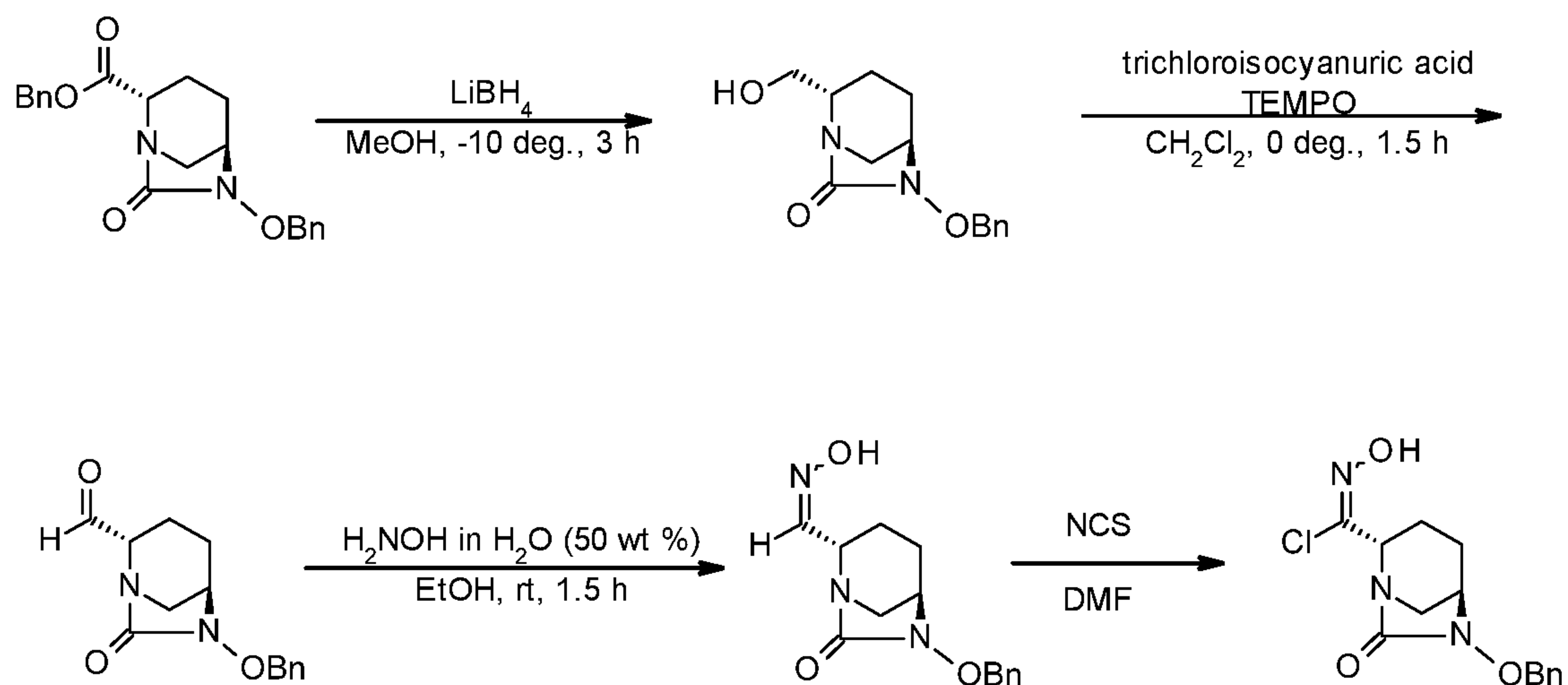
15 **Examples**

Examples of compounds of Formula I or pharmaceutically acceptable salts thereof are listed above in Table 1. The ability of compounds Formula I or pharmaceutically acceptable salts thereof to inhibit growth of a bacterium was established with the representative compounds of Formula I listed in Tables 1 and 2 using the assays described
20 below.

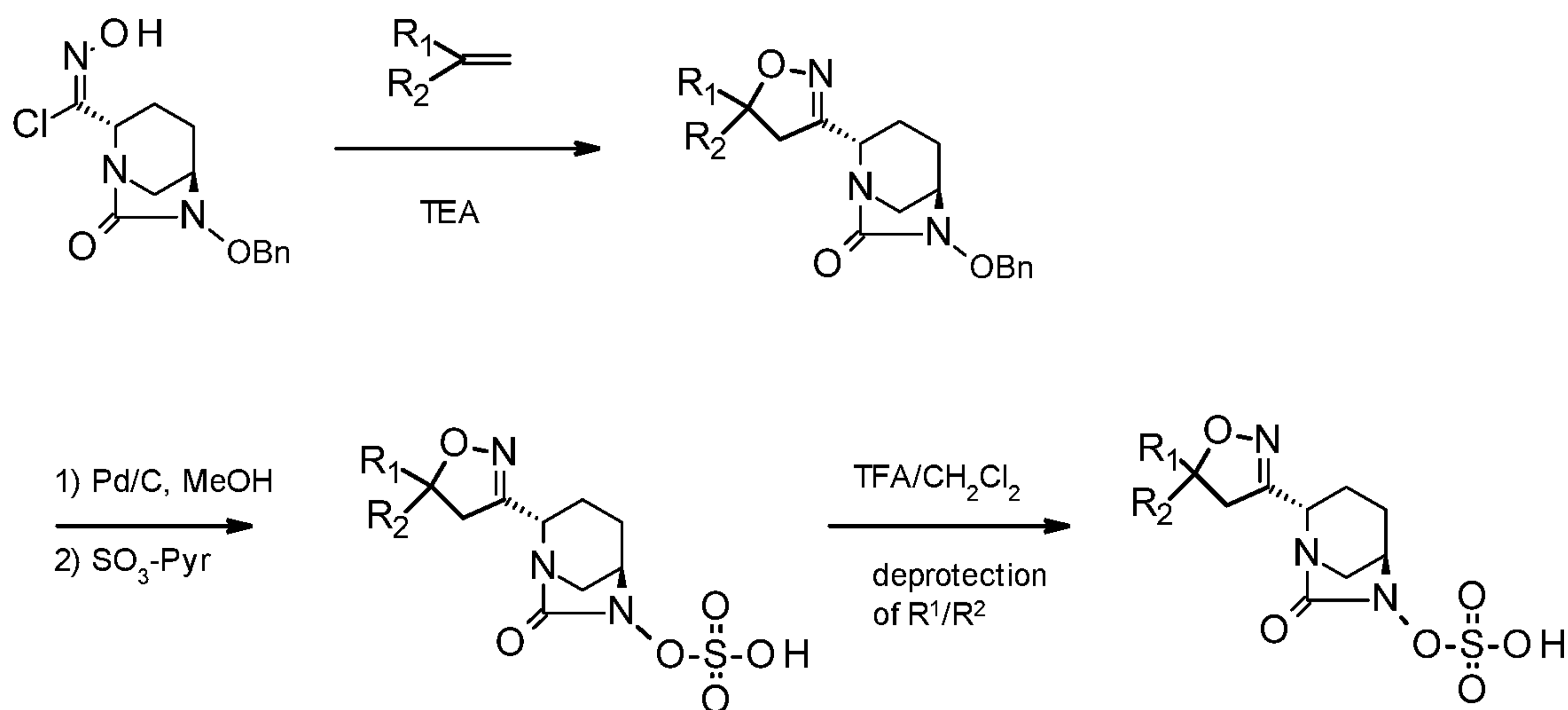
A. Chemical Syntheses

The general procedures used in the methods to prepare the compounds of the present invention are outlined in Schemes 1 and 2 and are described below.

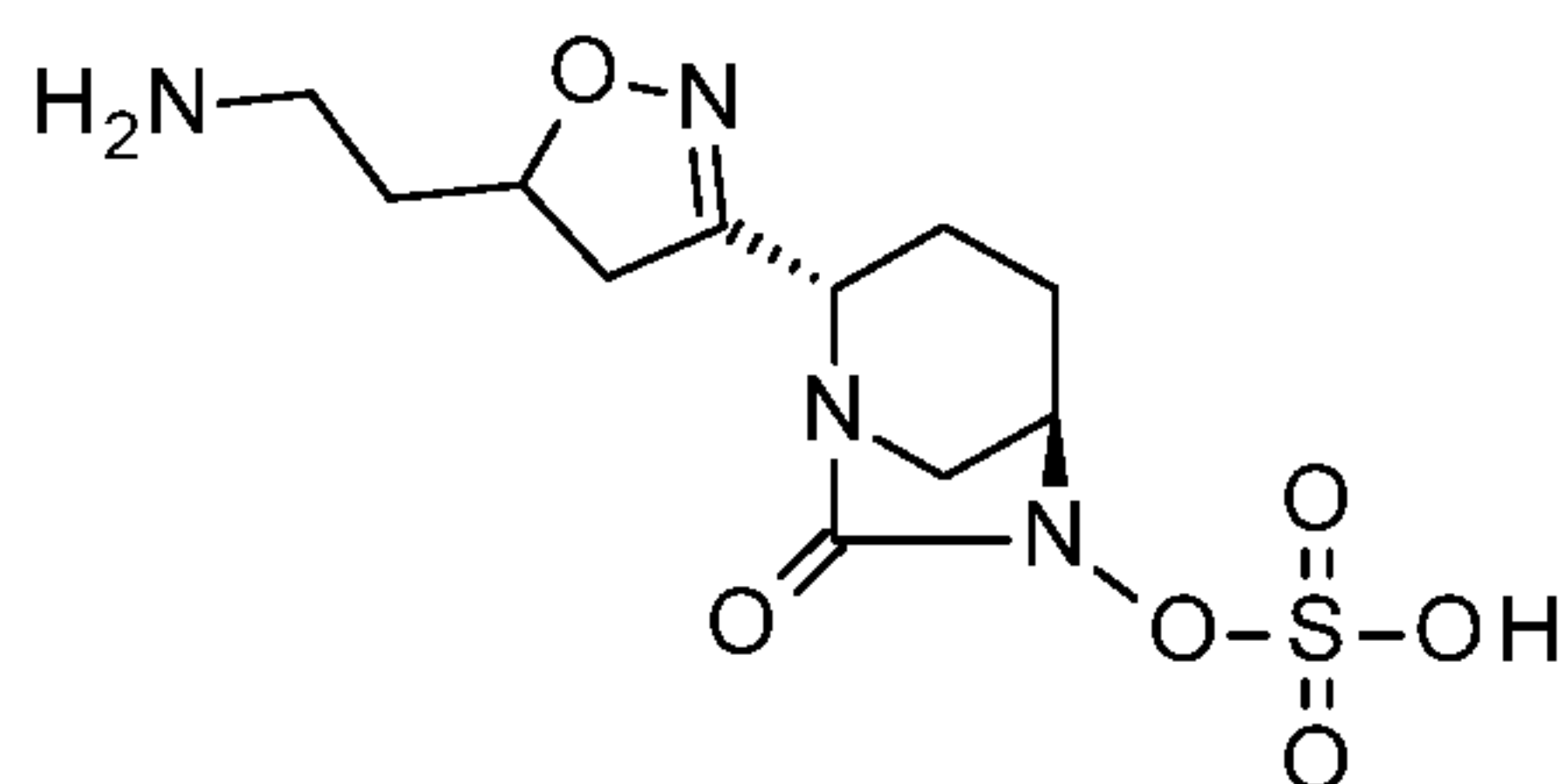
Scheme 1



Scheme 2

**Example 1:**

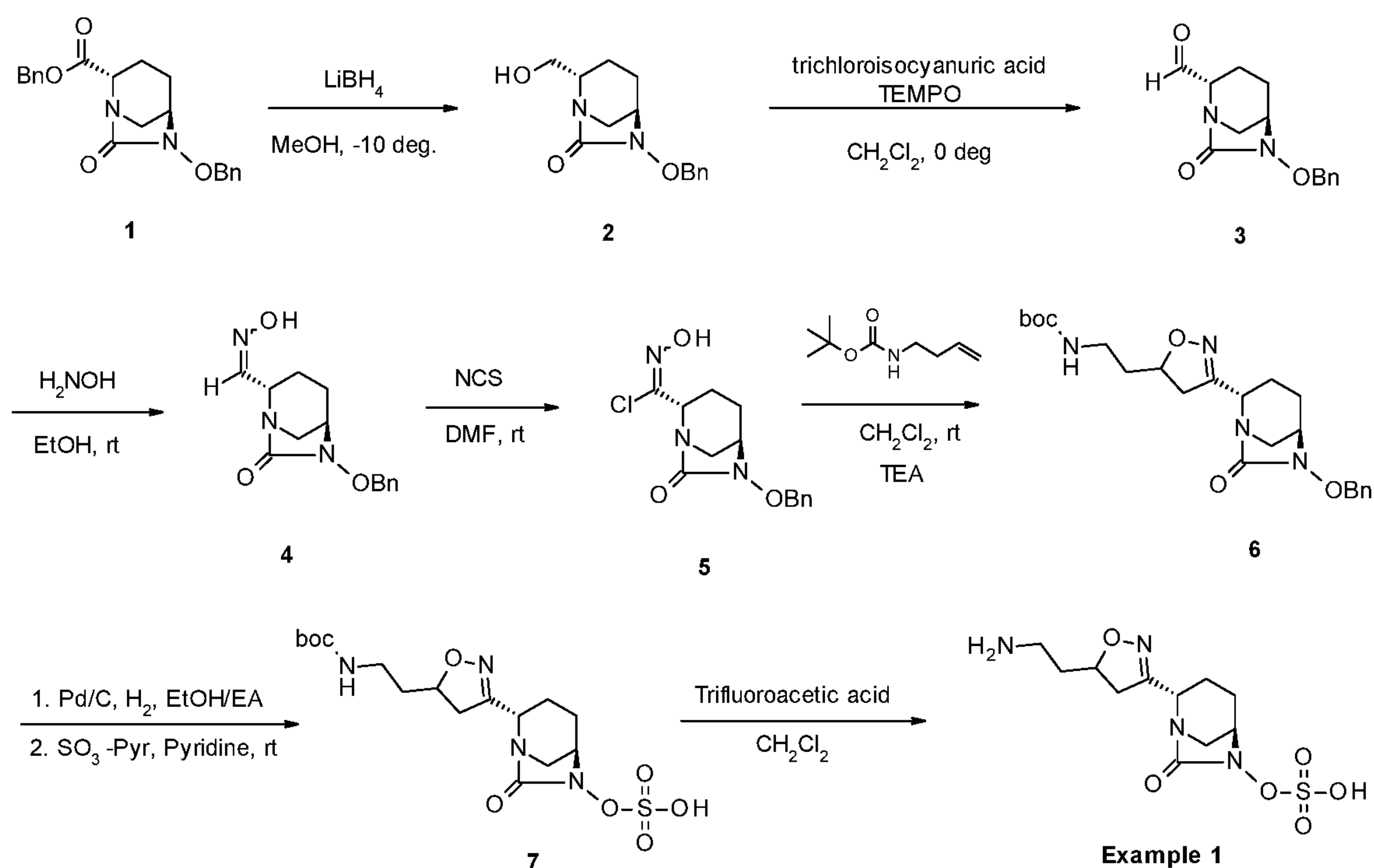
[(2S,5R)-2-[5-(2-aminoethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate



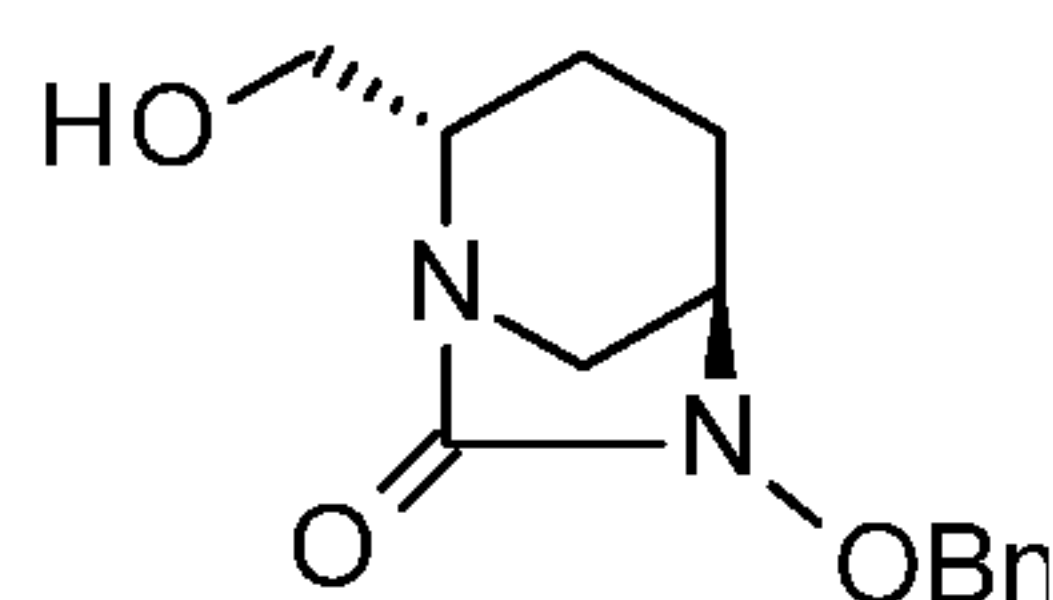
The synthesis of [(2S,5R)-2-[5-(2-aminoethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate is carried out according to Scheme 3 and as outlined in the steps below.

5

Scheme 3



Step 1: (2S, 5R)-6-benzyloxy-2-(hydroxymethyl)-1,6-diazabicyclo[3.2.1]octan-7-one



2

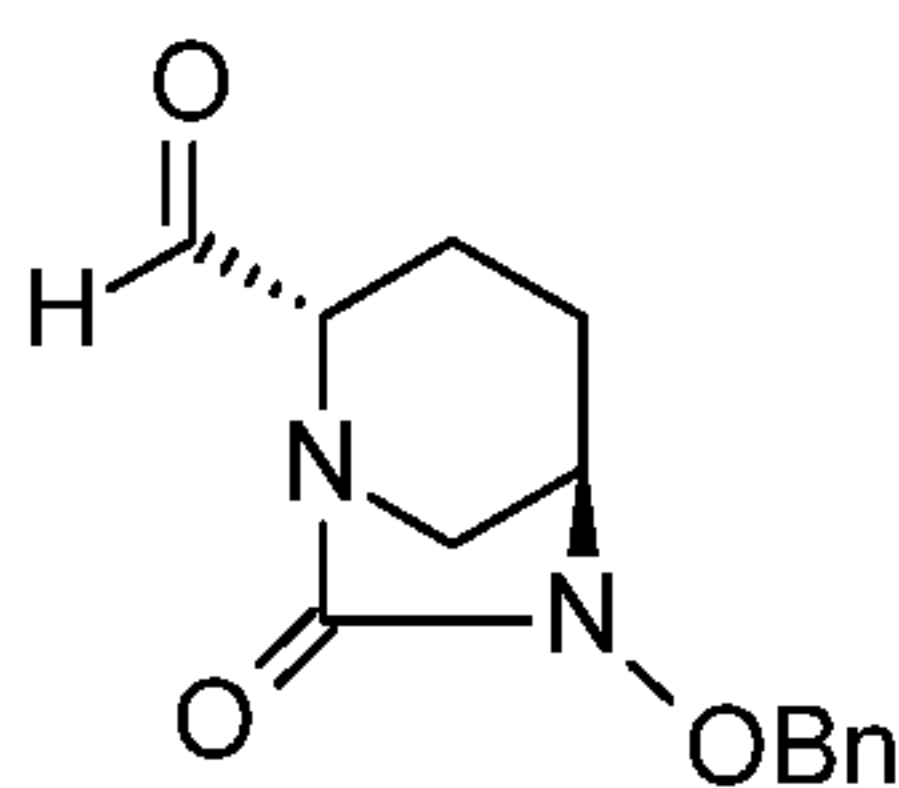
10

To a solution of (2S, 5R)-benzyl 6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylate (10g, 27.3 mmol) in MeOH (70 mL) was added 2.0 M solution of LiBH₄ in THF (20.5 mL, 40.9 mmol) was added at -10 °C. After 10 minutes, another portion of 2.0 M solution of LiBH₄ (20.5 mL, 40.9 mmol) in THF was

added and the mixture was stirred for 2h at -10°C to 0°C. The reaction mixture was carefully quenched by addition of saturated NaH₂PO₄ solution (100 mL) at 0°C. The reaction mixture was diluted with water and extracted with methylene chloride (250mL x 3). The combined organic layer was concentrated and purified by silica gel column chromatography (50-100% EtOAc/n-Hexane) to give **Compound-2** (5.8g, 81%).

¹H NMR (400 MHz, CDCl₃) δ 7.46-7.37 (m, 5H), 5.07 (d, *J* = 11.6Hz, 1H), 4.92 (d, *J* = 11.6Hz, 1H), 3.73 (br, 1H), 3.62-3.57 (m, 2H), 3.35 (m, 1H), 3.01 (d, *J* = 11.6Hz, 1H), 2.93 (br d, *J* = 11.6Hz, 1H) 2.23 (br, 1H), 2.07-1.93(m, 2H), 1.57-1.35 (m, 2H).

Step 2: (2S, 5R)-6-benzyloxy-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carbaldehyde

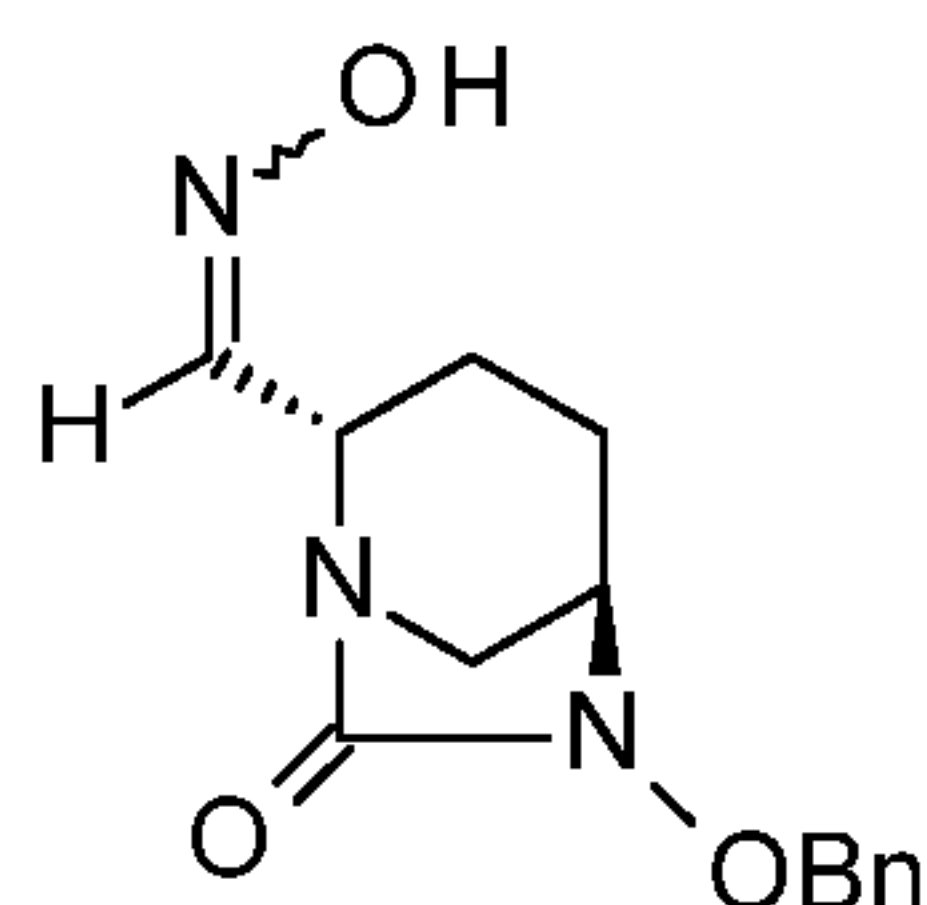


3

To a solution of **Compound-2** (5 g, 19.1mmol) was added TEMPO (0.3g, 1.9 mmol) and trichloroisocyanuric acid (5.3 g, 22.8 mmol) in methylene chloride (100mL) at 0°C. The mixture was stirred for 2h at 0°C, and filtered through celite. The mixture was washed with saturated NaHCO₃ (200 mL) and brine (50 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give **Compound-3** as a yellow oil with quantitative yield.

¹H NMR (400 MHz, CDCl₃) δ 9.74 (s, 1H), 7.44-7.37 (m, 5H), 5.07 (d, *J* = 11.6 Hz, 1H), 4.90 (d, *J* = 11.6 Hz, 1H), 3.85 (d, *J* = 8.0 Hz, 1H), 3.28 (br, 1H), 3.14 (d, *J* = 13.2 Hz, 1H), 2.56 (d, *J* = 11.6 Hz, 1H), 2.20-1.91 (m, 3H), 1.46 (m, 1H).

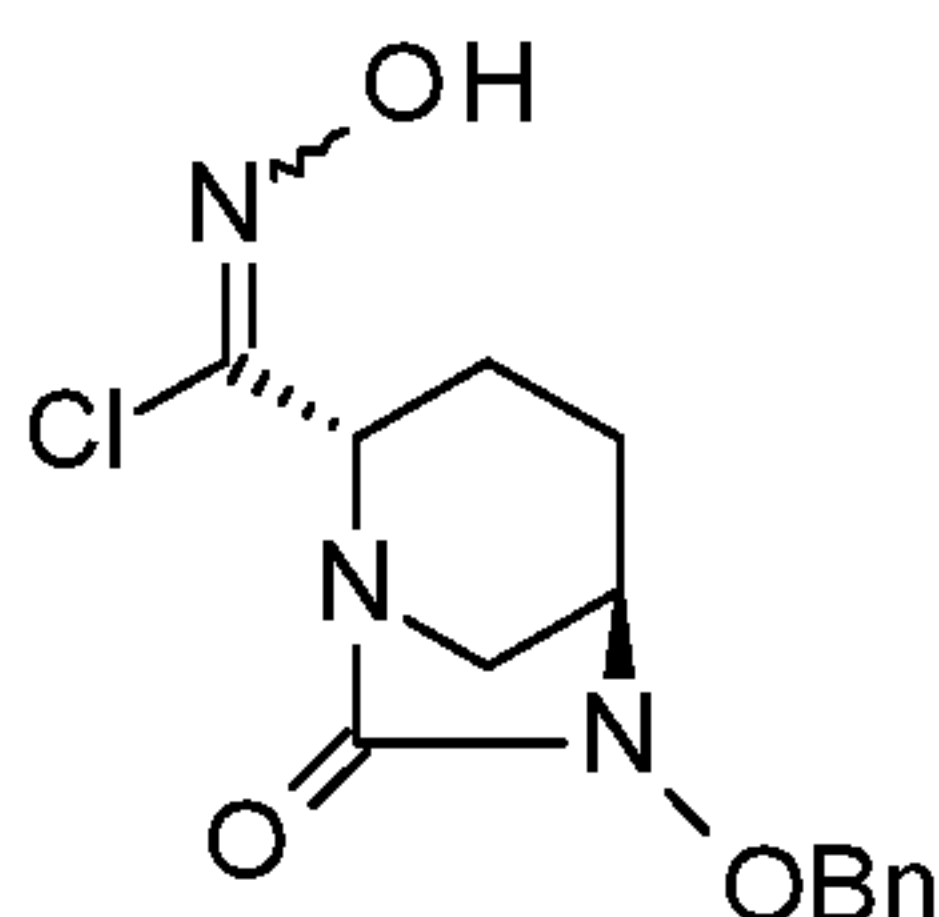
Step 3: (2S, 5R)-6-benzyloxy-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carbaldehyde oxime



4

To a solution of **Compound-3** (19.1 mmol) in EtOH (50 mL) was added Hydroxylamine (1.25 mL, 19.1 mmol, 50% aq. Sol.) at 0 °C. The reaction mixture was stirred for 1h at 0 °C and concentrated under reduced pressure to give **Compound-4** as a light yellow foam with quantitative yield.

Step 4: (2S, 5R)-6-benzyloxy-N-hydroxy-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboximidoyl chloride



5

10

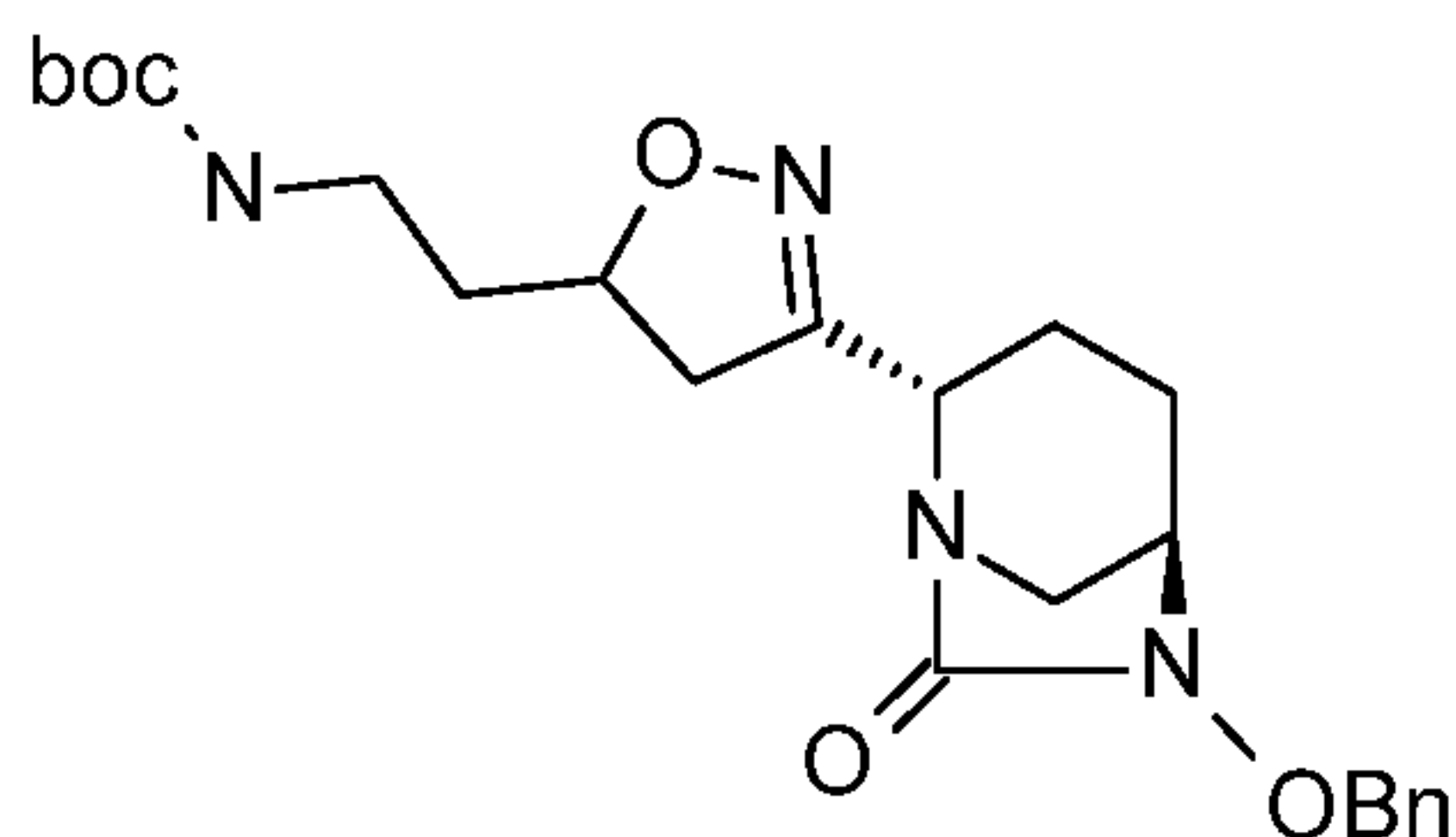
To a solution of **Compound-4** (0.3 g, 1.09 mmol) in DMF (5 mL) was added (*N*-chlorosuccinimide (0.15 g, 1.14 mmol) at room temperature. After stirring for 1h at room temperature, the reaction mixture was diluted with EA (20 mL), washed with water (20 mL) twice, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give a light yellow foam with quantitative yield.

15

¹H NMR (400 MHz, CDCl₃) δ 7.67 (s, 1H), 7.44-7.35 (m, 5H), 5.07 (d, *J* = 12.0 Hz, 1H), 4.92 (d, *J* = 12.0 Hz, 1H), 4.24 (d, *J* = 6.0 Hz, 1H), 3.34 (bs, 1H), 2.97 (d, *J* = 12.0 Hz, 1H), 2.87 (d, *J* = 12.0 Hz, 1H), 2.14-2.01 (m, 3H), 1.75 (m, 1H).

20

Step 5: *tert*-butyl *N*-[2-[3-[(2S, 5R)-6-benzyloxy-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]-4,5-dihydroisoxazol-5-yl]ethyl]carbamate

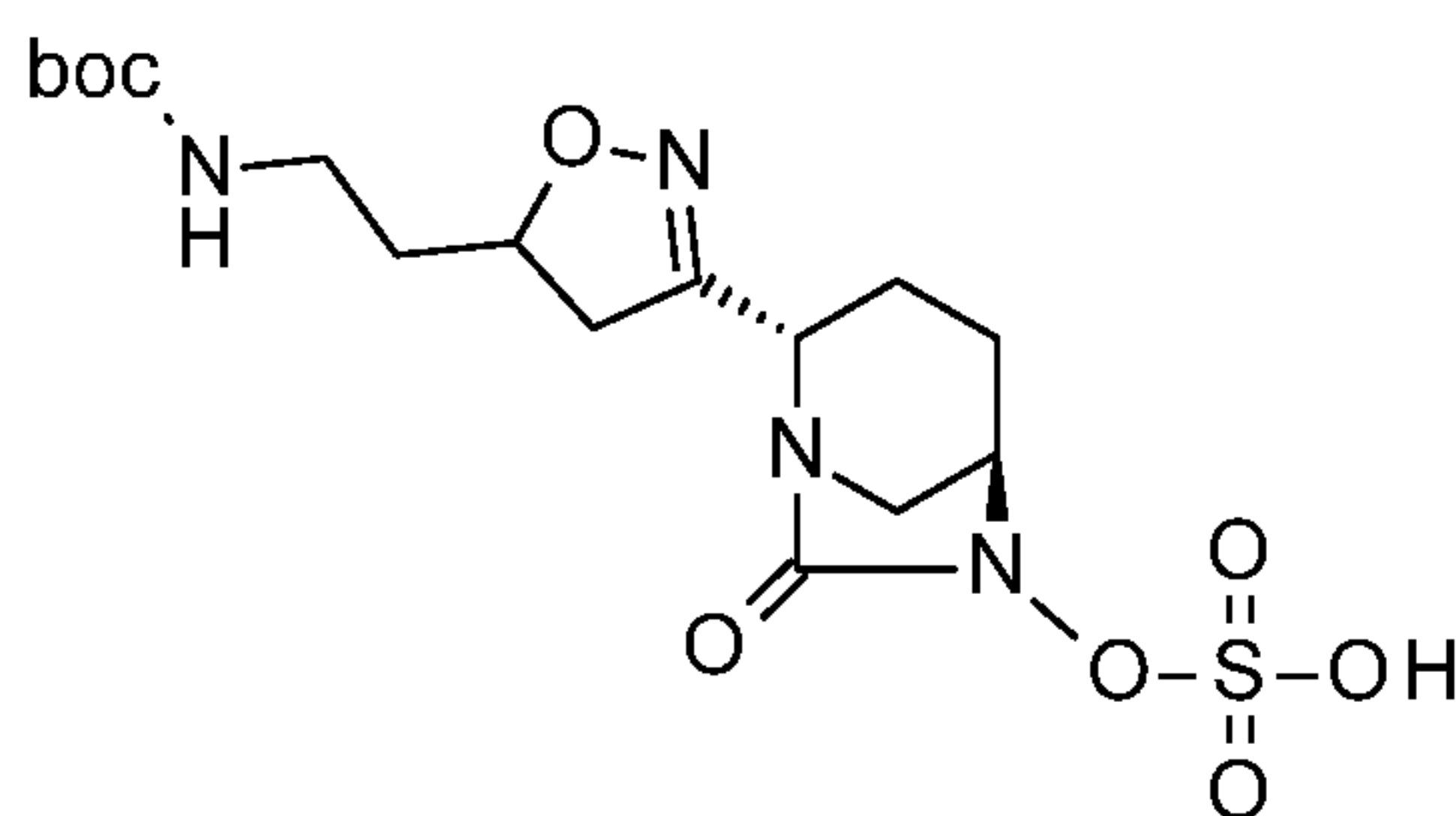


6

To a solution of **Compound-5** (1.09 mmol) and *tert*-Butyl but-3-enylcarbamate (0.72 g, 4.2 mmol) in methylene chloride (10 mL) was added TEA (0.18 mL, 1.3 mmol) drop-wisely at room temperature. The mixture was stirred overnight at room temperature. The mixture was washed with saturated NaHCO₃ (10 mL) and dried over anhydrous sodium sulfate. The combined organic layer was concentrated under reduced pressure and purified by silica gel column chromatography (30% EA/Hexane) to give **Compound-6** (0.23 g, 47%).

¹H NMR (400 MHz, DMSO-*d*₆) δ 7.46-7.36 (m, 5H), 6.87 (m, 1H), 4.97-4.91 (m, 2H), 4.56 (m, 1H), 4.05 (d, *J* = 6.40 Hz, 1H), 3.67 (m, 1H), 3.09-2.82 (m, 4H), 2.75-2.57 (m, 1H), 2.00 (m, 1H), 1.86-1.61 (m, 5H), 1.37 (s, 9H).

Step 6: *tert*-butyl N-[2-[3-[(2*S*, 5*R*)-7-oxo-6-sulfooxy-1,6-diazabicyclo[3.2.1]octan-2-yl]-4,5-dihydroisoxazol-5-yl]ethyl]carbamate



7

To a solution of **Compound-6** (0.23 g, 0.52 mmol) in EtOH/EA (4 mL/4 mL) was added Pd/C (0.1 g). The reaction mixture was stirred for 1h under hydrogen atmosphere. The mixture was filtered through Celite®. The filtrate was washed with Ethyl Acetate (4 mL) and concentrated under reduced pressure. The resulting intermediate was used without purification for next step.

To a solution of crude intermediate in pyridine (2 mL) was added $\text{SO}_3 \cdot \text{Pyr}$ (0.41 g, 2.6 mmol). The reaction mixture was stirred overnight at room temperature. The resulting mixture was filtered, concentrated and purified by silica gel column chromatography (5% MeOH/MC) to give **Compound-7** (0.15 g, 66%).

- 5 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 4.56 (m, 1H), 4.03 (d, $J = 6.8$ Hz, 1H), 4.00 (br, 1H), 3.11-2.91 (m, 4H), 2.78-2.62 (m, 2H), 2.00-1.86 (m, 2H), 1.83-1.61 (m, 4H), 1.37 (s, 9H).

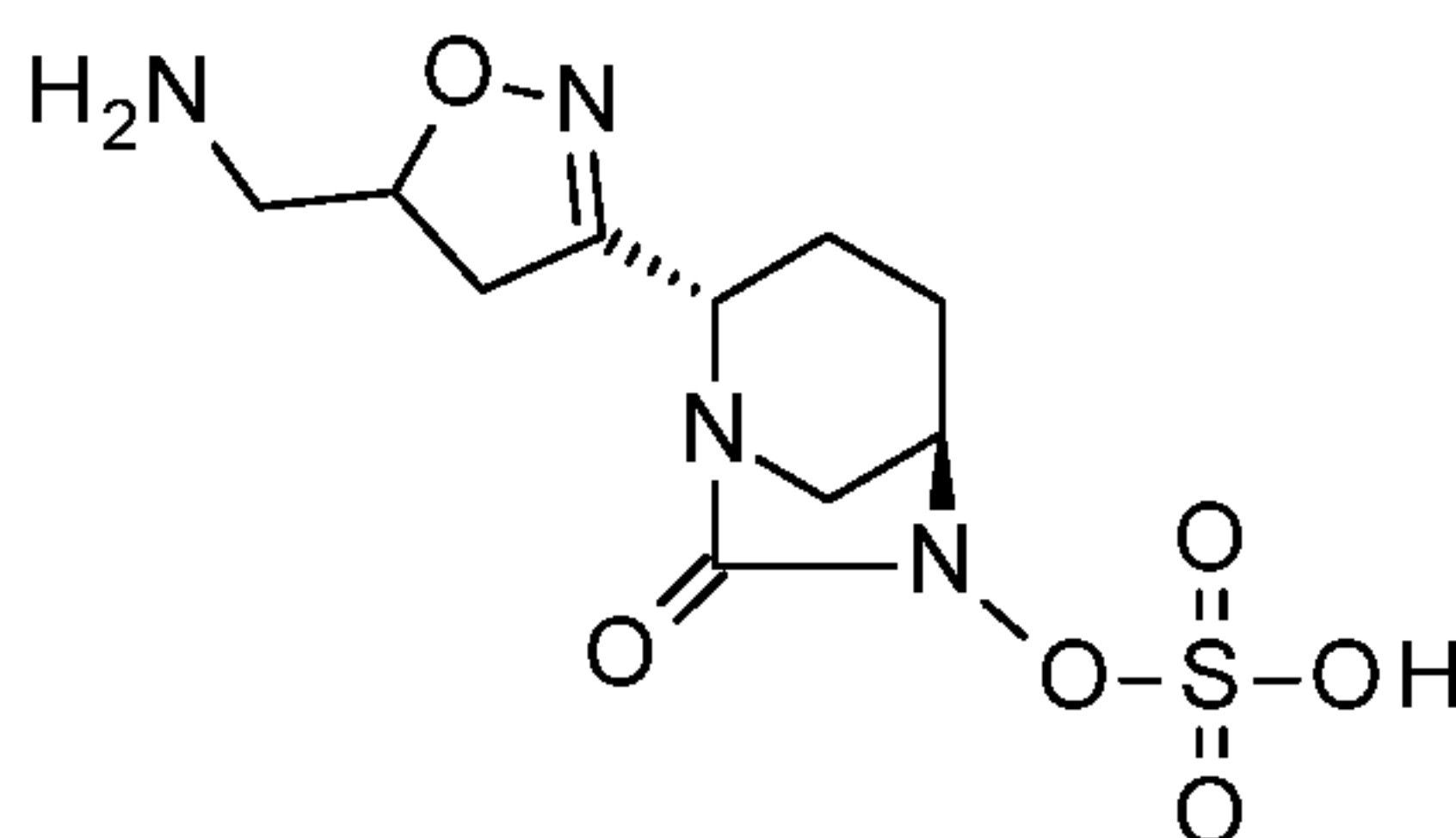
Step 7: [(2S, 5R)-2-[5-(2-aminoethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate

- 10 To a solution of **Compound-7** (0.15 g, 0.35 mmol) in methylene chloride (2 mL) was added trifluoroacetic acid (1 mL) at 0°C . After stirring for 1 h at room temperature, the reaction mixture was diluted with diethyl ether (6 mL) and stirred for an additional 0.5 h at room temperature. The resulting precipitate was collected by filtration to give **Example 1** (0.10 g, 85%) as a white solid.

- 15 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.70 (br, 3H), 4.69 (m, 1H), 4.09 (m, 1H), 4.03 (br, 1H), 3.15 (dd, $J = 17.6, 10.8$ Hz, 1H), 2.97-2.86 (m, 3H), 2.77-2.71 (m, 2H), 2.02-1.68 (m, 6H).

Example 2:

- 20 [(2S, 5R)-2-[5-(aminomethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate

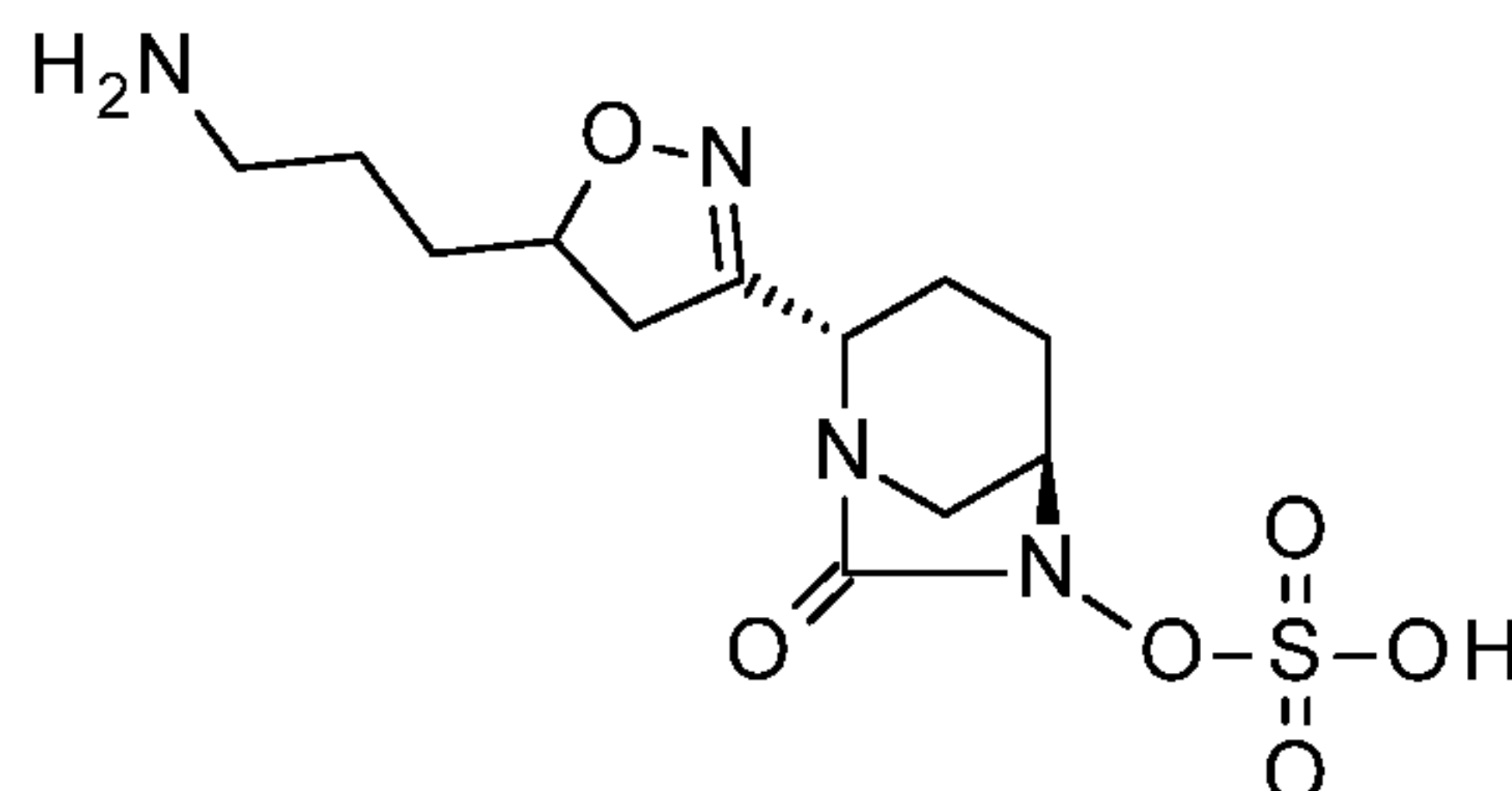


[(2S, 5R)-2-[5-(aminomethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate was prepared by following steps 5-7 in Example 1 using *tert*-butyl N-allylcarbamate for the olefin.

- 25 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.97 (br, 2H), 7.92 (br, 1H), 4.81 (m, 1H), 4.09 (br, 1H), 4.03 (br, 1H), 3.21 (m, 1H), 2.97-2.72 (m, 5H), 2.03-1.65 (m, 4H).

Example 3:

[(2S, 5R)-2-[5-(3-aminopropyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate

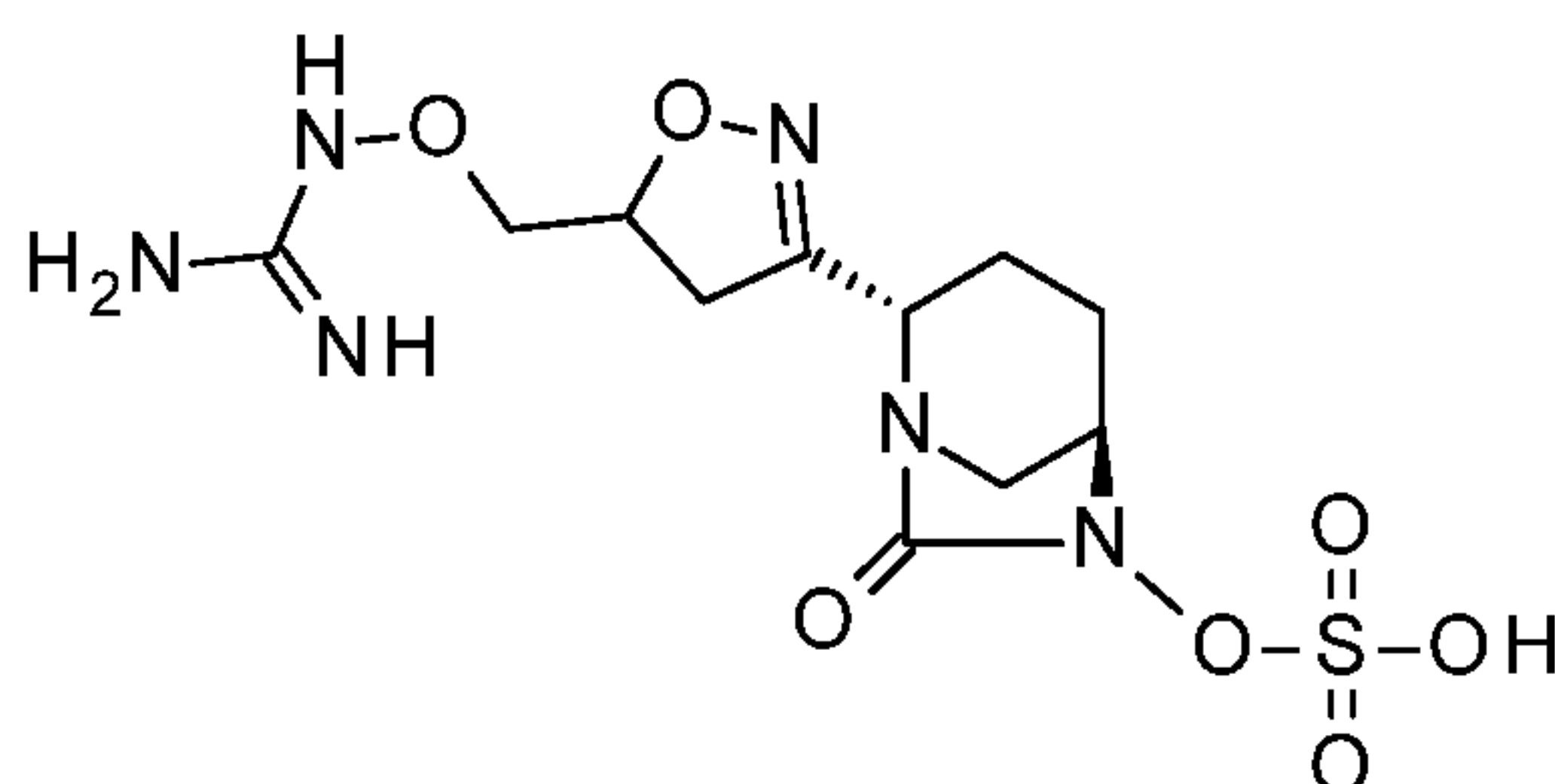


- 5 [(2S, 5R)-2-[5-(3-aminopropyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate was prepared following steps 5-7 in Example 1 using, *tert*-butyl *N*-pent-4-enylcarbamate for the olefin.

¹H NMR (400 MHz, DMSO-*d*₆) δ 7.66 (br, 3H), 4.62 (m, 1H), 4.07-4.12 (m, 2H), 3.12 (m, 1H), 2.95 (m, 1H), 2.82-2.60 (m, 4H), 2.02-1.88 (m, 2H), 1.79-1.58 (m, 6H).

10 **Example 4:**

[(2S,5R)-2-[5-(guanidinooxymethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate

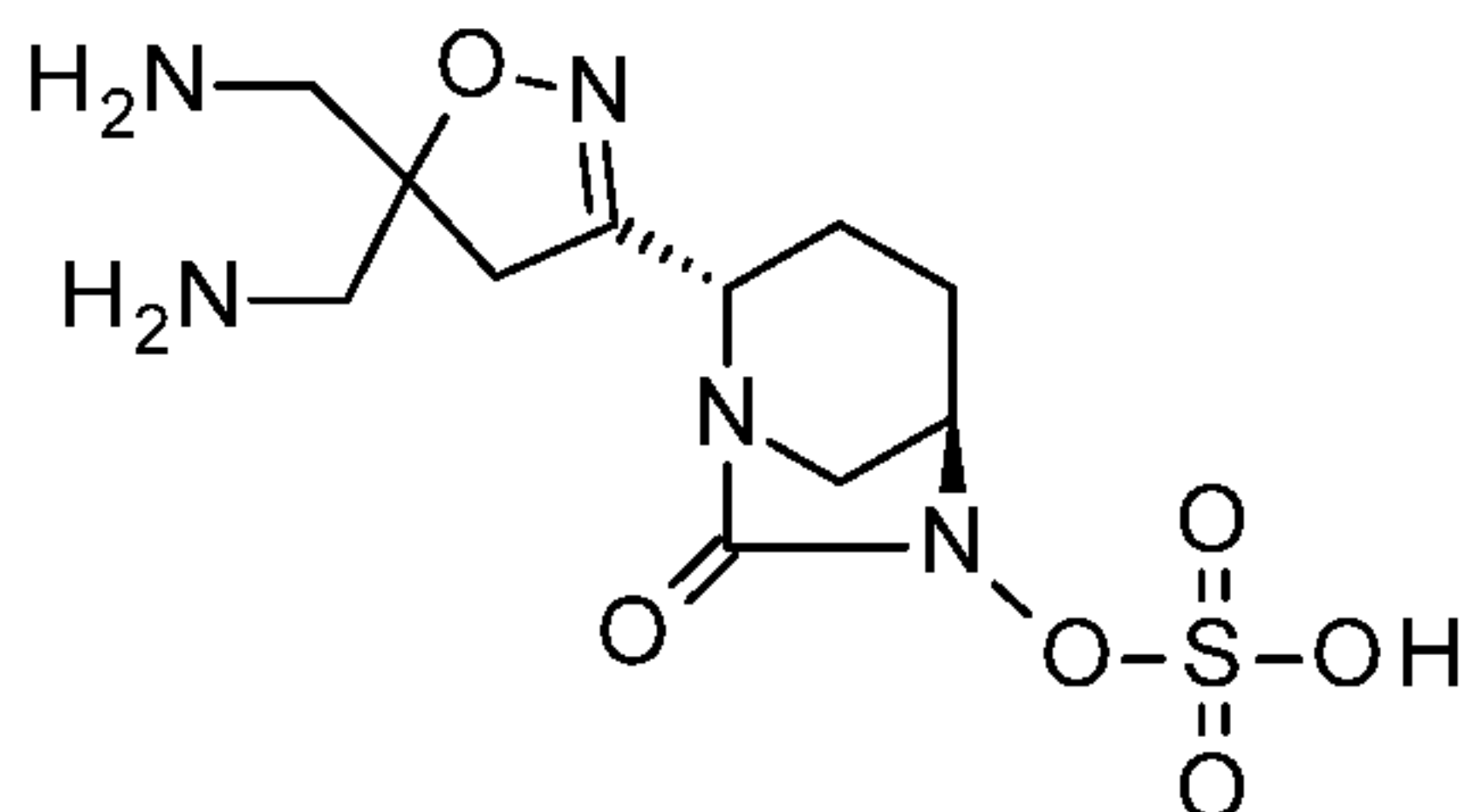


- 15 [(2S,5R)-2-[5-(guanidinooxymethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate is prepared following steps 5-7 in Example 1 using *tert*-butyl *N*-allylcarbamate for the olefin.

¹H NMR (400 MHz, DMSO-*d*₆) δ 7.64 (br, 3H), 4.88 (m, 1H), 4.07 (d, *J* = 6.4 Hz, 1H), 4.01 (br, 1H), 3.92-3.83 (m, 2H), 3.12 (m, 1H), 2.95-2.83 (m, 2H), 2.75 (d, *J* = 15.6 Hz, 1H), 2.02-1.69 (m, 4H).

Example 5:

[(2S, 5R)-2-[5,5-bis(aminomethyl)-4H-isoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate

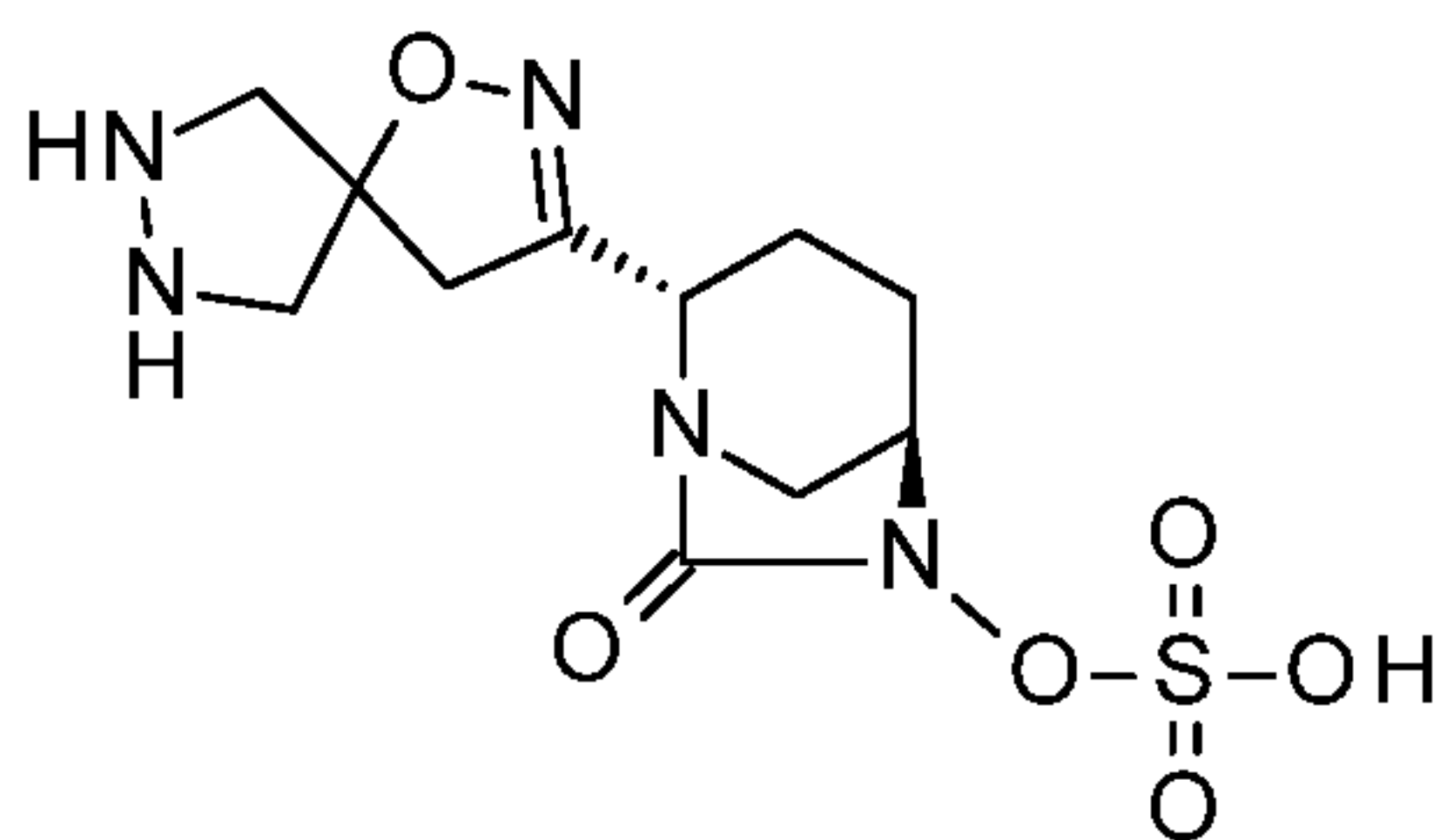


5 [(2S, 5R)-2-[5,5-bis(aminomethyl)-4H-isoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate was prepared following steps 5-7 in Example 1 using *tert*-butyl *N*-[2-[(*tert*-butoxycarbonylamino)methyl]allyl]carbamate for the olefin.

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.13 (br, 3H), 8.03 (br, 3H), 4.14 (d, *J* = 6.8 Hz, 1H),
10 4.03 (m, 1H), 3.28-3.07 (m, 6H), 2.95-2.83 (m, 2H), 2.03-1.66 (m, 4H).

Example 6:

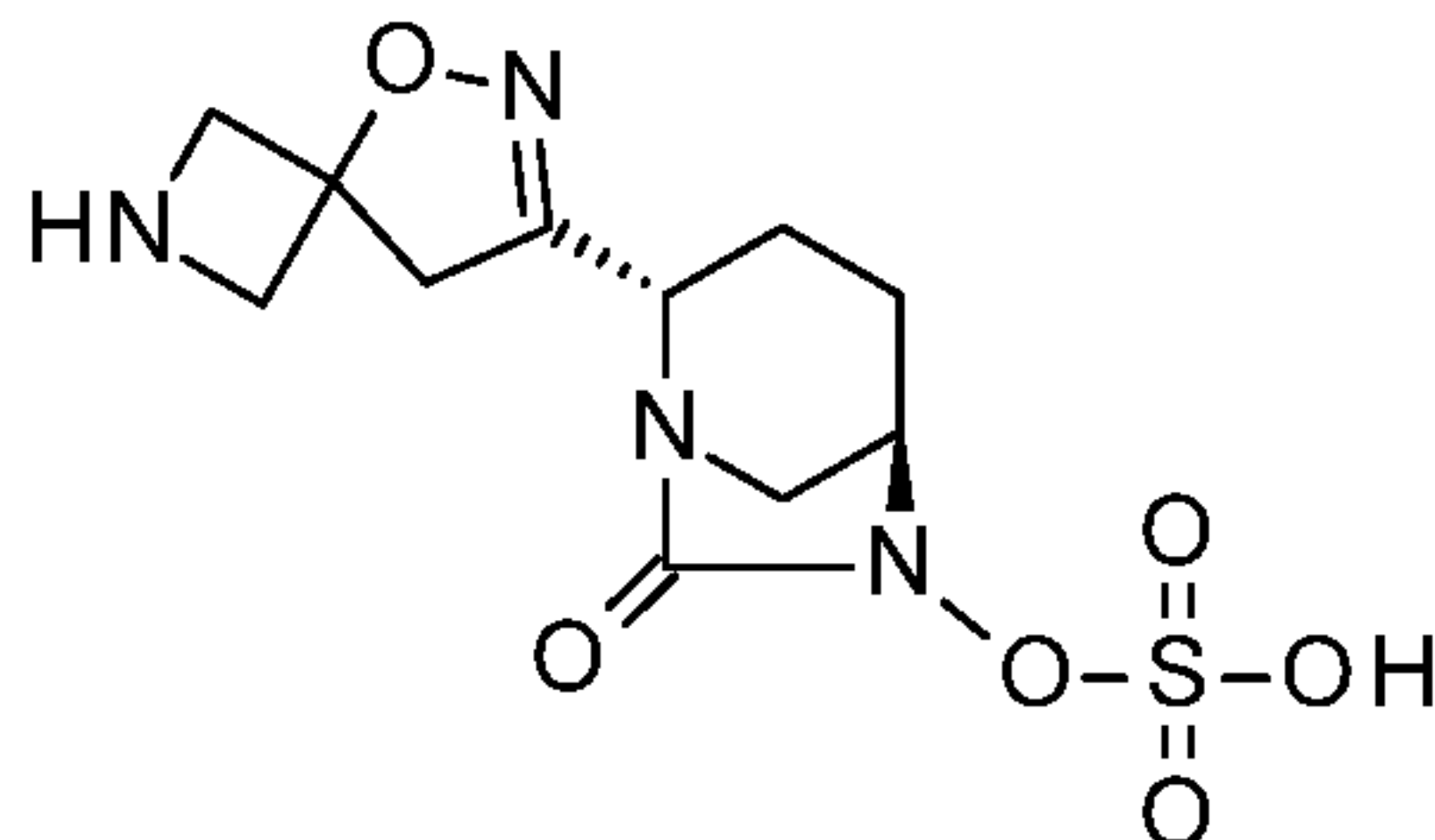
[(2S, 5R)-2-(1-oxa-2,7,8-triazaspiro[4.4]non-2-en-3-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate



15 [(2S, 5R)-2-(1-oxa-2,7,8-triazaspiro[4.4]non-2-en-3-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate was prepared following steps 5-7 in Example 1, using *ditert*-butyl 4-methylenepyrazolidine-1,2-dicarboxylate for the olefin.
¹H NMR (400 MHz, DMSO-*d*₆) δ 4.10 (d, *J* = 6.8 Hz, 1H), 4.01 (br, 1H), 3.50-3.22 (m,
20 6H), 2.99-2.77 (m, 2H), 2.02-1.68 (m, 4H).

Example 7:

[(2S, 5R)-2-(5-oxa-2,6-diazaspiro[3.4]oct-6-en-7-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate



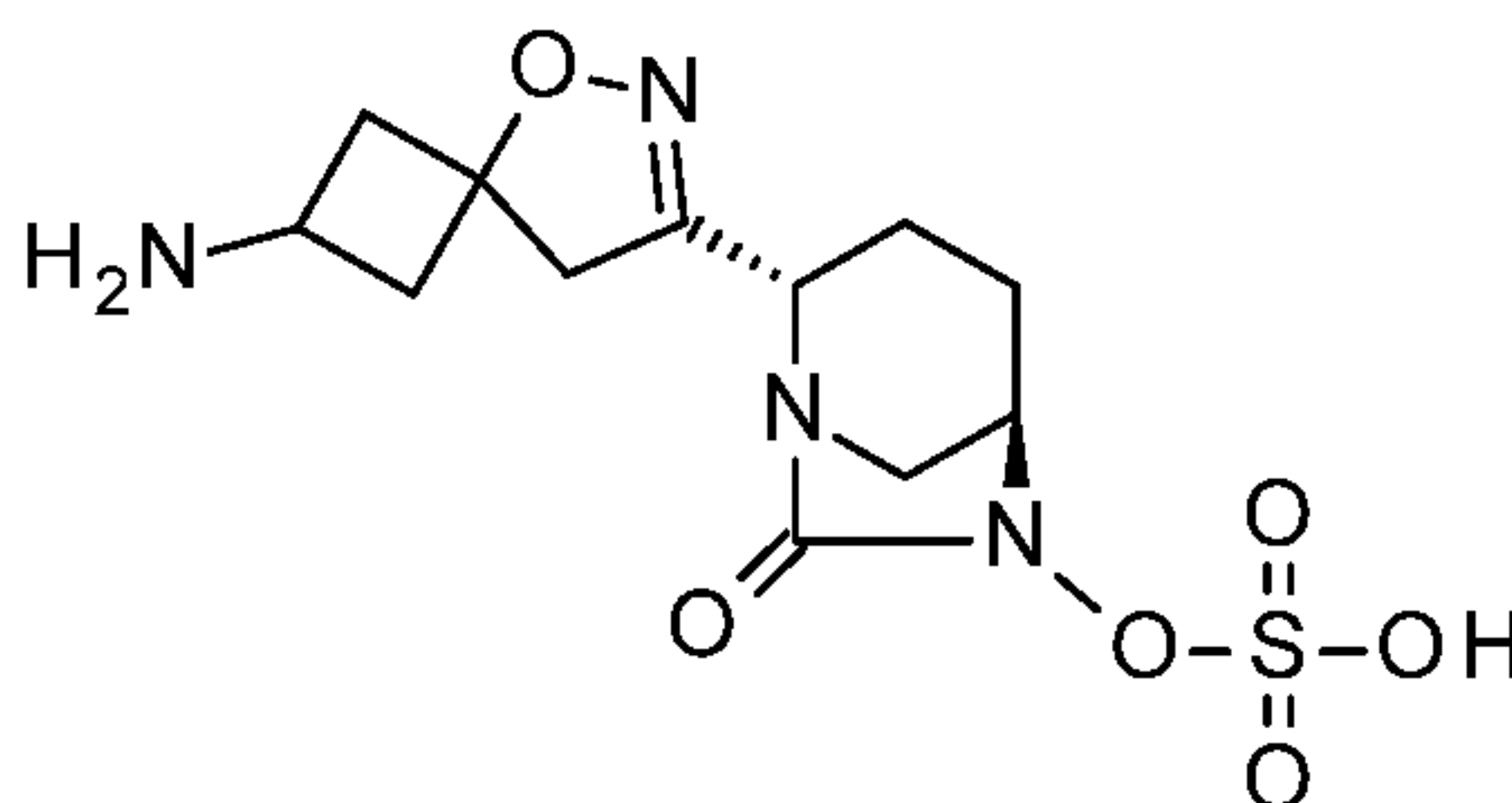
5 [(2S, 5R)-2-(5-oxa-2,6-diazaspiro[3.4]oct-6-en-7-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate was prepared following steps 5-7 in Example 1, using *tert*-butyl 3-methyleneazetidine-1-carboxylate for the olefin.

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.85 (br, 1H), 8.62 (br, 1H), 4.31-4.13 (m, 4H), 4.05 (d, *J* = 7.6Hz, 1H), 3.99 (m, 1H), 3.37 (s, 2H), 2.95-2.72 (m, 2H), 1.97-1.50 (m, 4H).

10

Example 8:

[(2S, 5R)-2-(2-amino-5-oxa-6-azaspiro[3.4]oct-6-en-7-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate



15 [(2S, 5R)-2-(2-amino-5-oxa-6-azaspiro[3.4]oct-6-en-7-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate was prepared following steps 5-7 in Example 1 using *tert*-butyl *N*-(3-methylenecyclobutyl)carbamate for the olefin.

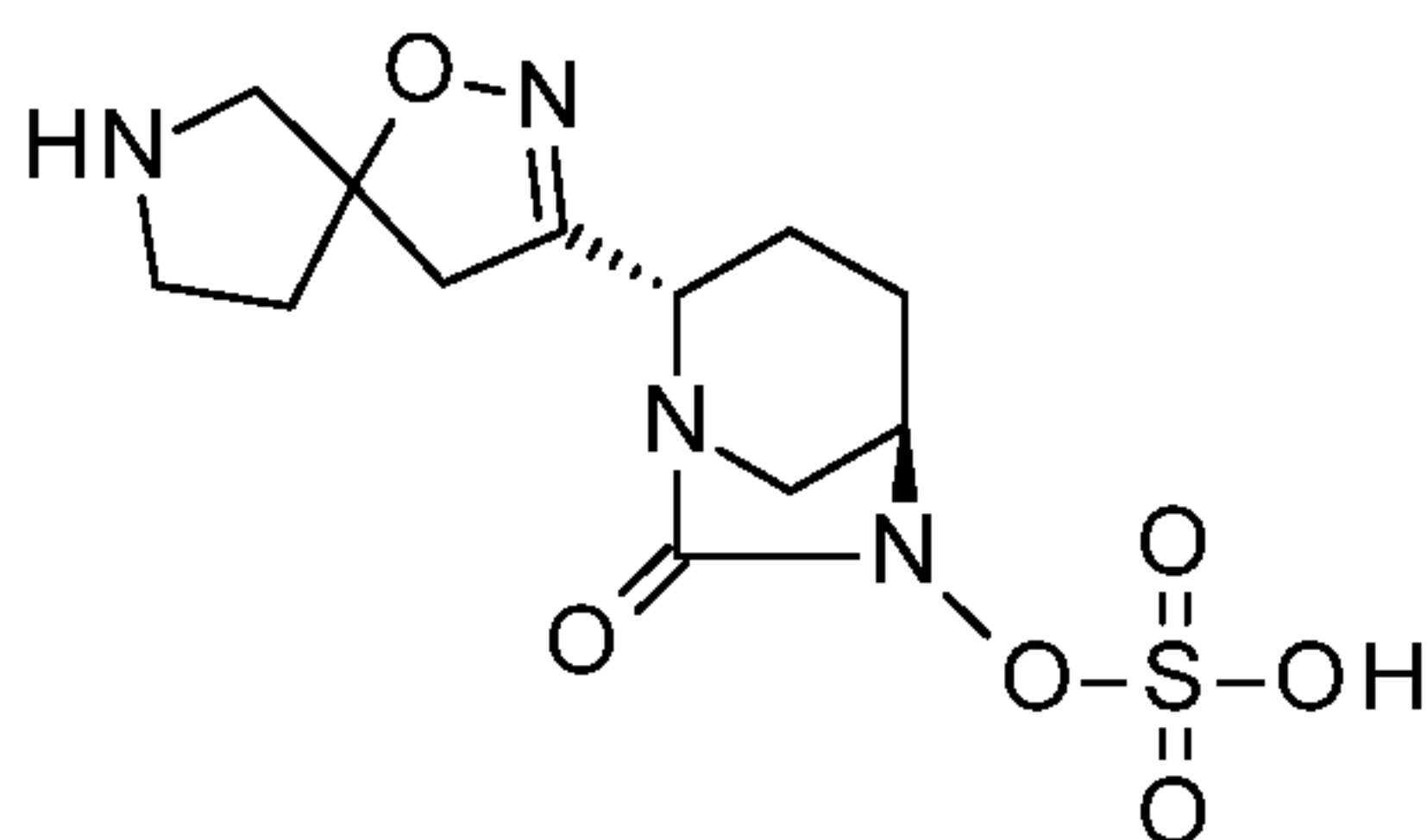
¹H NMR (400 MHz, DMSO-*d*₆) δ 8.02 (br, 3H), 4.05 (d, *J* = 6.8 Hz, 1H), 4.01 (m, 1H), 3.78-3.11 (m, 3H), 2.96-2.54 (m, 6H), 2.03-1.66 (m, 4H).

20

Example 9:

[(2S, 5R)-2-(1-oxa-2,7-diazaspiro[4.4]non-2-en-3-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-

6-yl] hydrogen sulfate

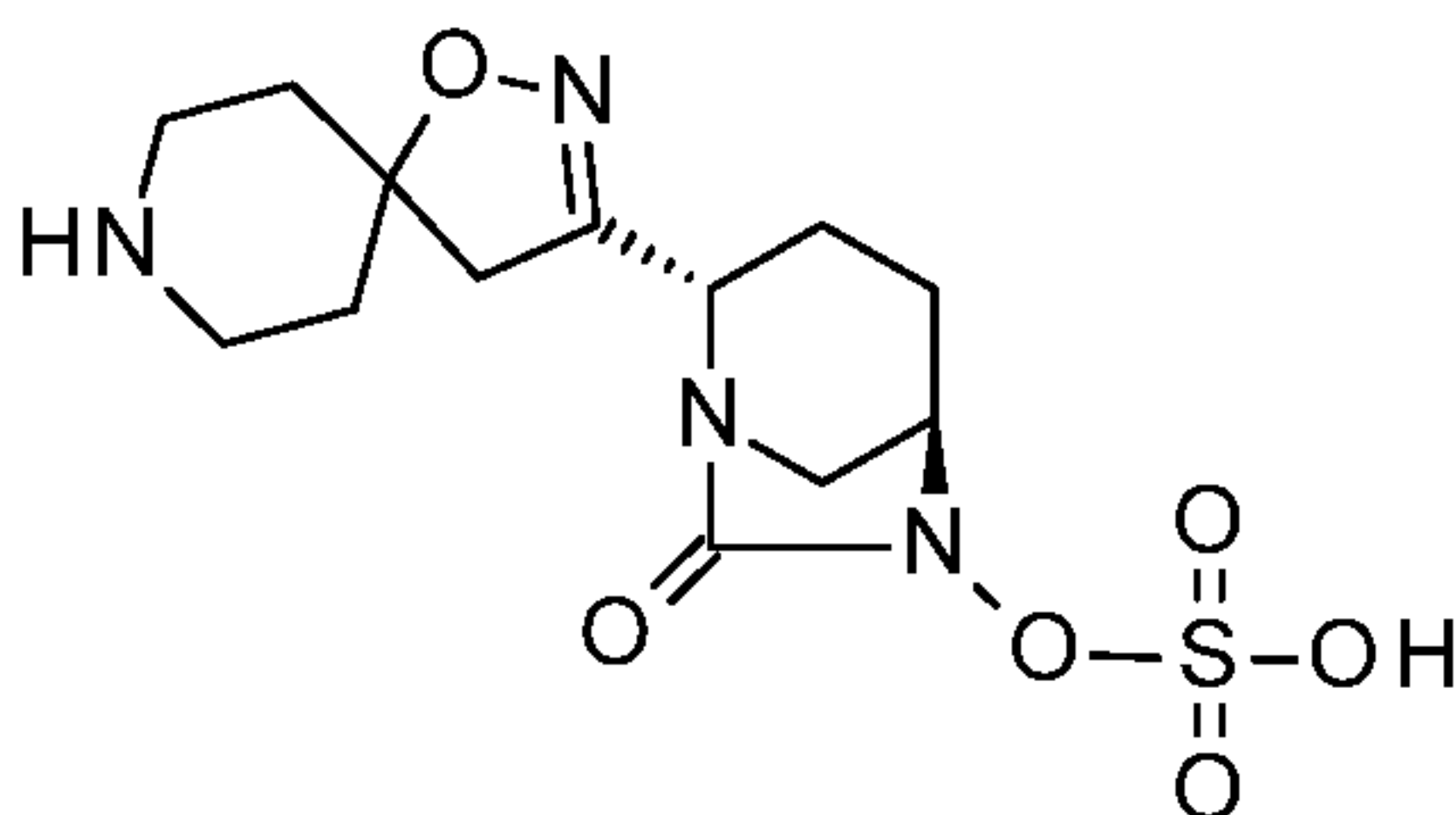


[(2S, 5R)-2-(1-oxa-2,7-diazaspiro[4.4]non-2-en-3-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate was prepared following steps 5-7 in Example 1 using *tert*-butyl 3-methylenepyrrolidine-1-carboxylate for the olefin.

¹H NMR (400 MHz, DMSO-*d*₆) δ 9.13 (br, 1H), 8.97 (br, 1H), 4.10 (br, 1H), 4.03 (br, 1H), 3.49-3.15 (m, 6H), 2.98-2.76 (m, 2H), 2.24-1.70 (m, 6H).

Example 10:

[(2S, 5R)-2-(1-oxa-2,8-diazaspiro[4.5]dec-2-en-3-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate

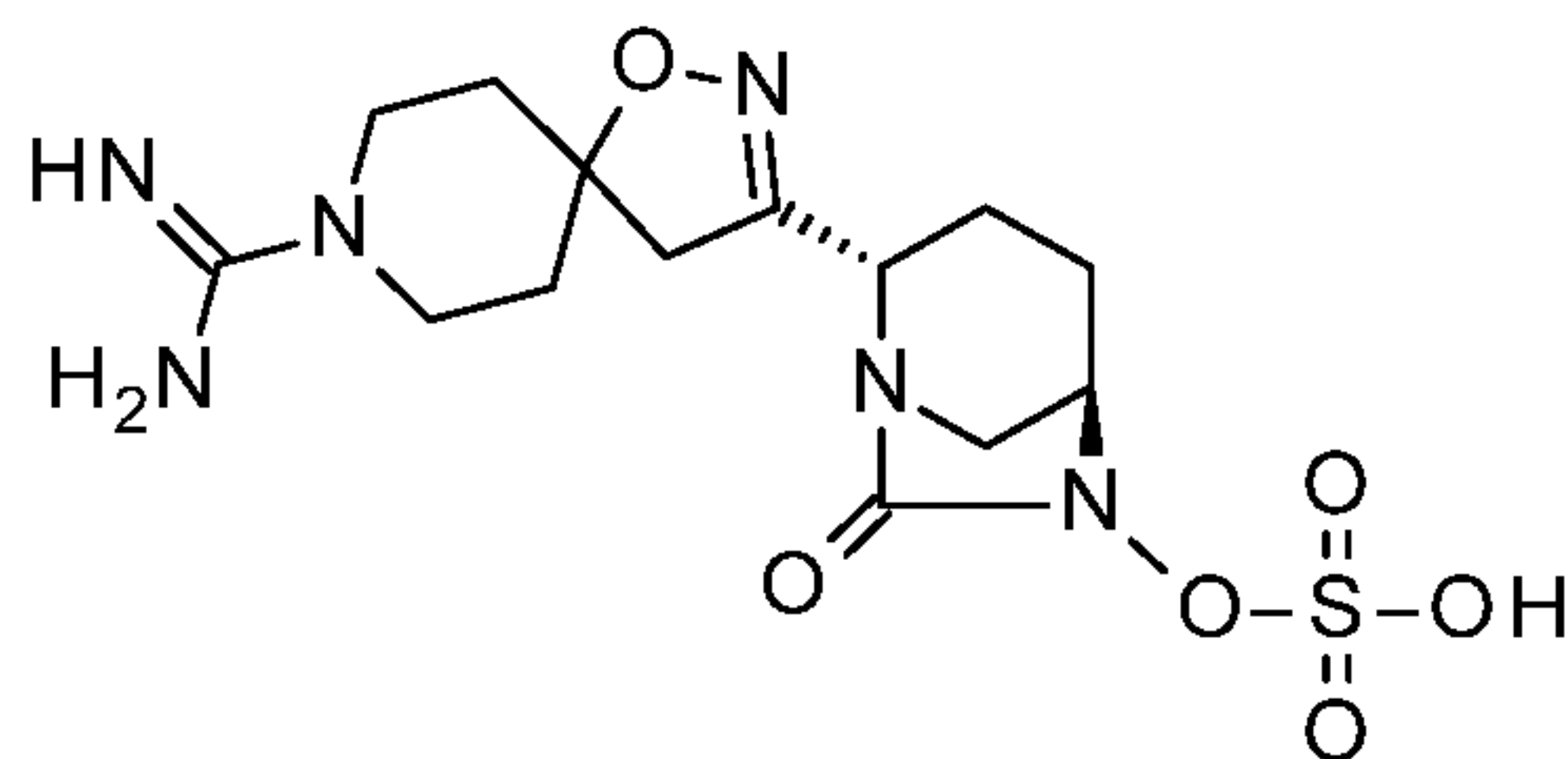


[(2S, 5R)-2-(1-oxa-2,8-diazaspiro[4.5]dec-2-en-3-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate was prepared following steps 5-7 in Example 1 using *tert*-butyl 4-methylenepiperidine-1-carboxylate for the olefin.

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.55 (br, 2H), 4.06 (m, 1H), 4.02 (br, 1H), 3.16 (m, 4H), 2.94 (m, 3H), 2.75 (d, 1H), 2.04-1.69 (m, 8H).

Example 11:

[(2S,5R)-2-(8-carbamimidoyl-1-oxa-2,8-diazaspiro[4.5]dec-2-en-3-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate

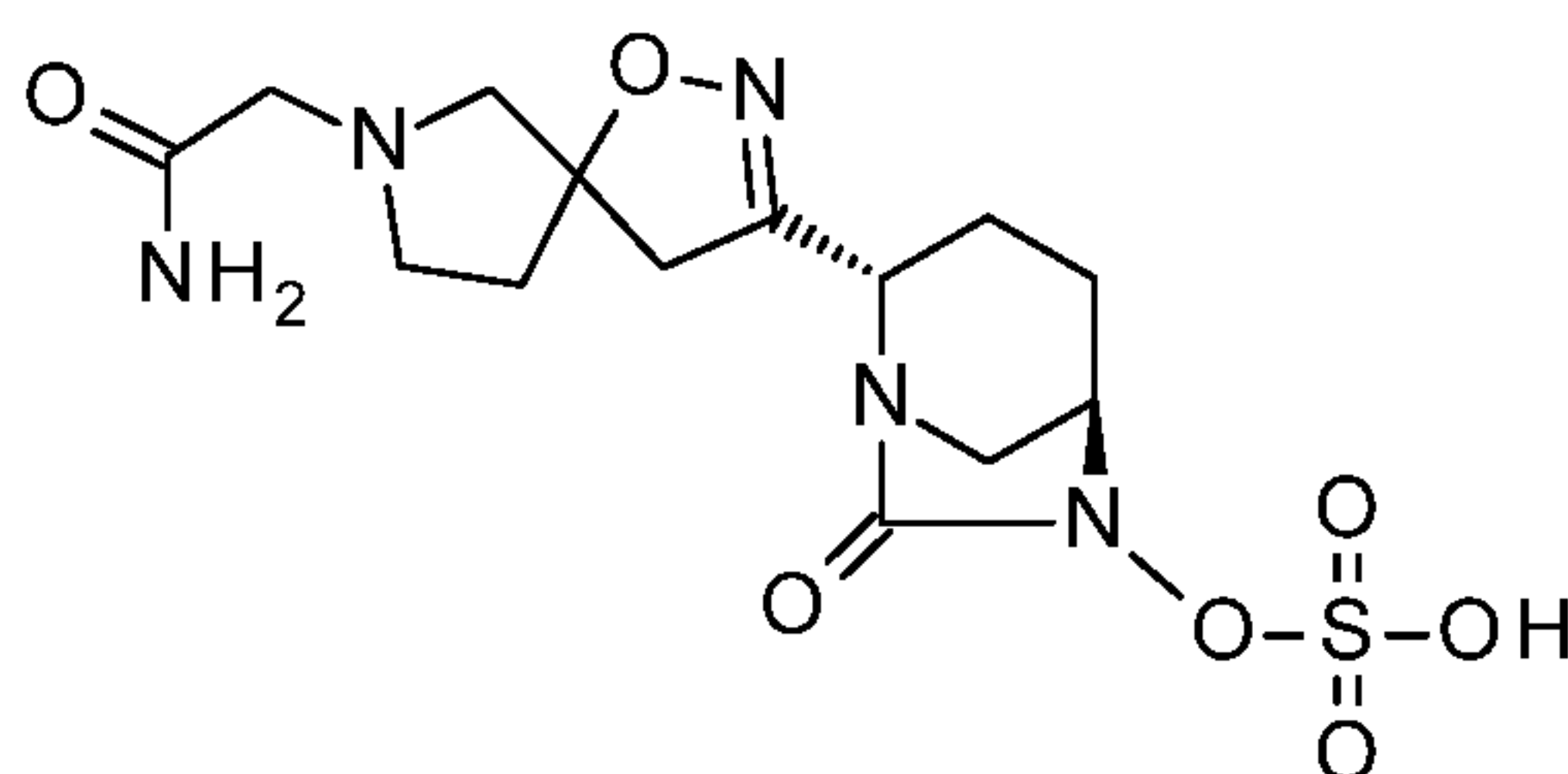


[(2S,5R)-2-(8-carbamimidoyl-1-oxa-2,8-diazaspiro[4.5]dec-2-en-3-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate was prepared following steps 5-7 in Example 1 using *tert*-butyl (NE)-N-[(*tert*-butoxycarbonylamino)-(4-methylene-1-piperidyl)methylene]carbamate for the olefin.

¹H NMR (400 MHz, D₂O) δ 4.32 (d, 1H), 4.25 (s, 1H), 3.36-3.55 (m, 4H), 3.29 (m, 1H), 3.04 (m, 3H), 2.16 (m, 2H), 1.94 (m, 6H).

Example 12:

10 [(2S,5R)-2-[7-(2-amino-2-oxo-ethyl)-1-oxa-2,7-diazaspiro[4.4]non-2-en-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate

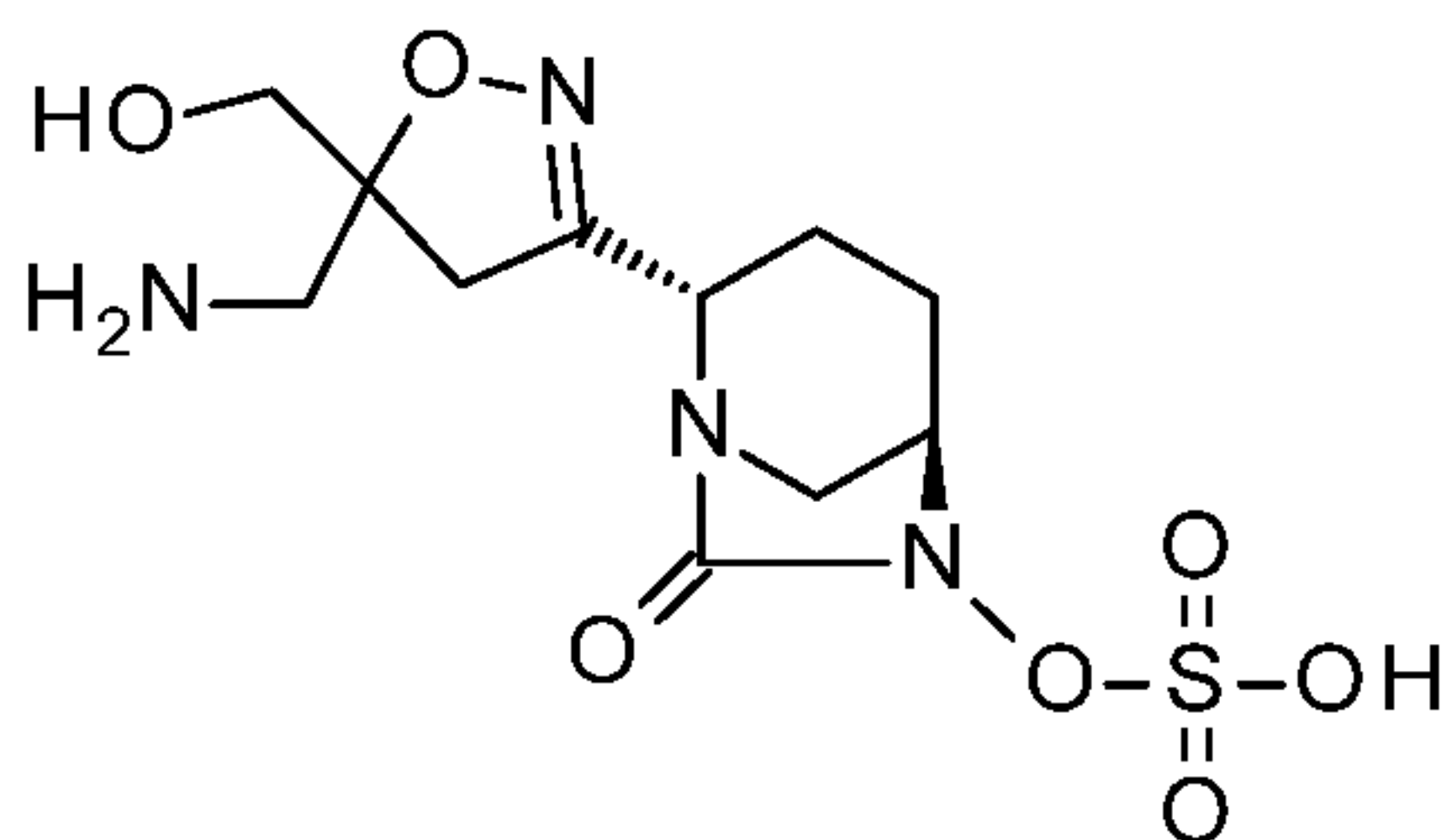


15 [(2S,5R)-2-[7-(2-amino-2-oxo-ethyl)-1-oxa-2,7-diazaspiro[4.4]non-2-en-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate was prepared following steps 5-6 in Example 1 using 2-(3-methylenepyrrolidin-1-yl)acetamide for the olefin.

¹H NMR (400 MHz, DMSO-*d*₆) δ 7.89 (d, *J* = 18.0 Hz, 1H), 7.65 (d, *J* = 18.0 Hz, 1H), 4.02 (m, 4H), 3.75 (m, 2H), 3.46-3.12 (m, 4H), 2.95 (m, 1H), 2.73 (m, 1H), 2.33 (m, 2H), 2.02-1.70 (m, 4H)

20 **Example 13:**

[(2S,5R)-2-[5-(aminomethyl)-5-(hydroxymethyl)-4H-isoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate

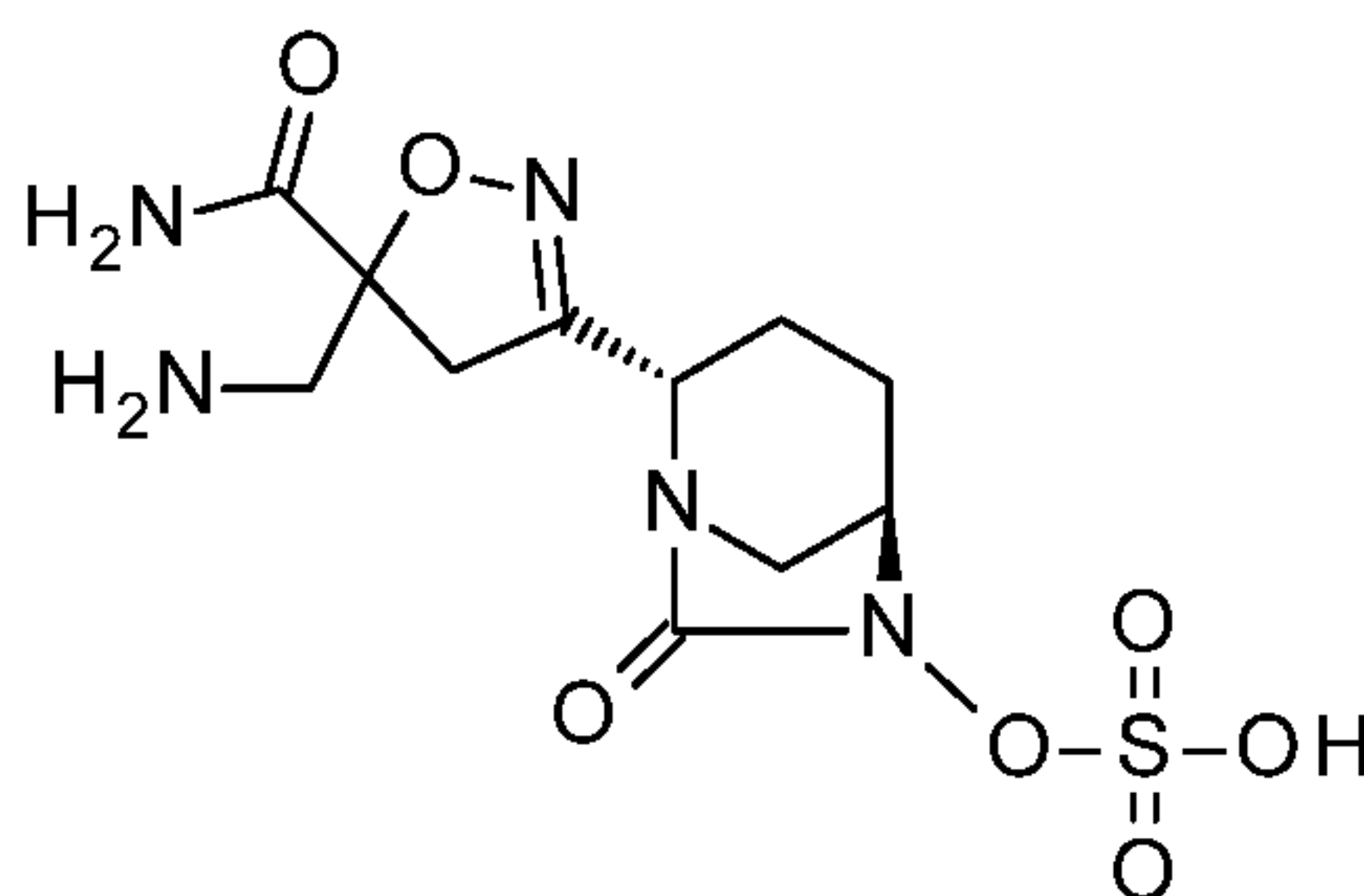


[(2S,5R)-2-[5-(aminomethyl)-5-(hydroxymethyl)-4H-isoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate was prepared following steps 5-7 in Example 1 using *tert*-butyl N-[2-[[*tert*-butyl(dimethyl)silyl]oxymethyl]allyl]carbamate for the olefin.

¹H NMR (400 MHz, D₂O) δ 4.15 (m, 1H), 4.08 (br, 1H), 3.60 (m, 2H), 3.29-3.07 (m, 4H), 2.92 (m, 2H), 2.12- 1.74 (m, 4H).

Example 14:

10 [(2S,5R)-2-[5-(aminomethyl)-5-carbamoyl-4H-isoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate



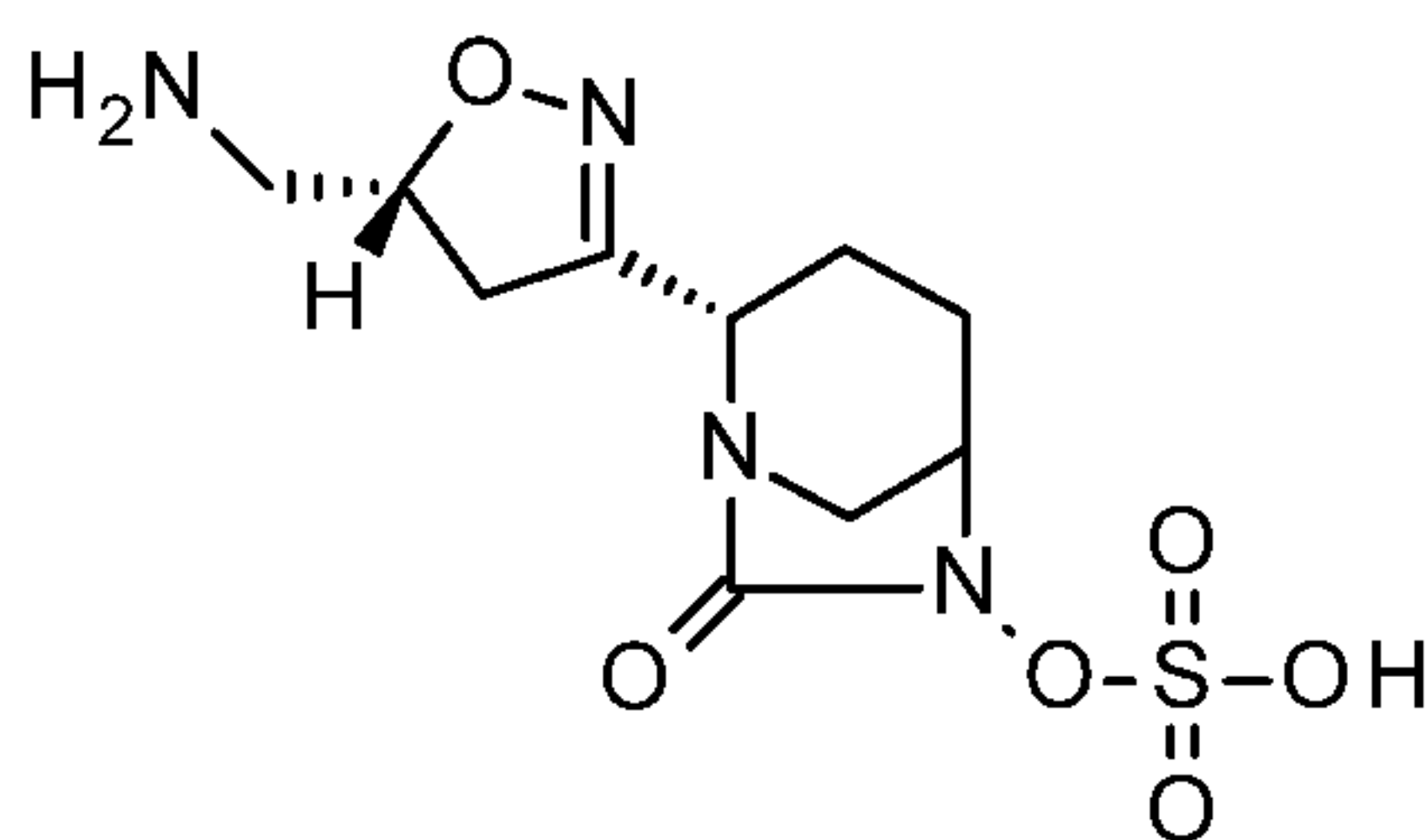
15 [(2S,5R)-2-[5-(aminomethyl)-5-carbamoyl-4H-isoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate was prepared following steps 5-7 in Example 1 using *tert*-butyl N-(2-carbamoylallyl)carbamate for the olefin.

¹H NMR (400MHz, D₂O) δ 4.18 (d, *J* = 6.4 Hz, 1H), 4.10 (s, 1H), 3.57-3.51 (m, 1H), 3.45-3.41 (m, 1.6H), 3.32-3.25 (m, 2.4H), 3.13 (t, *J* = 12.4 Hz, 1H), 2.94 (d, *J* = 12.4 Hz, 0.6H), 2.83 (d, *J* = 11.6Hz, 0.4H), 2.06-1.77 (m, 4H)

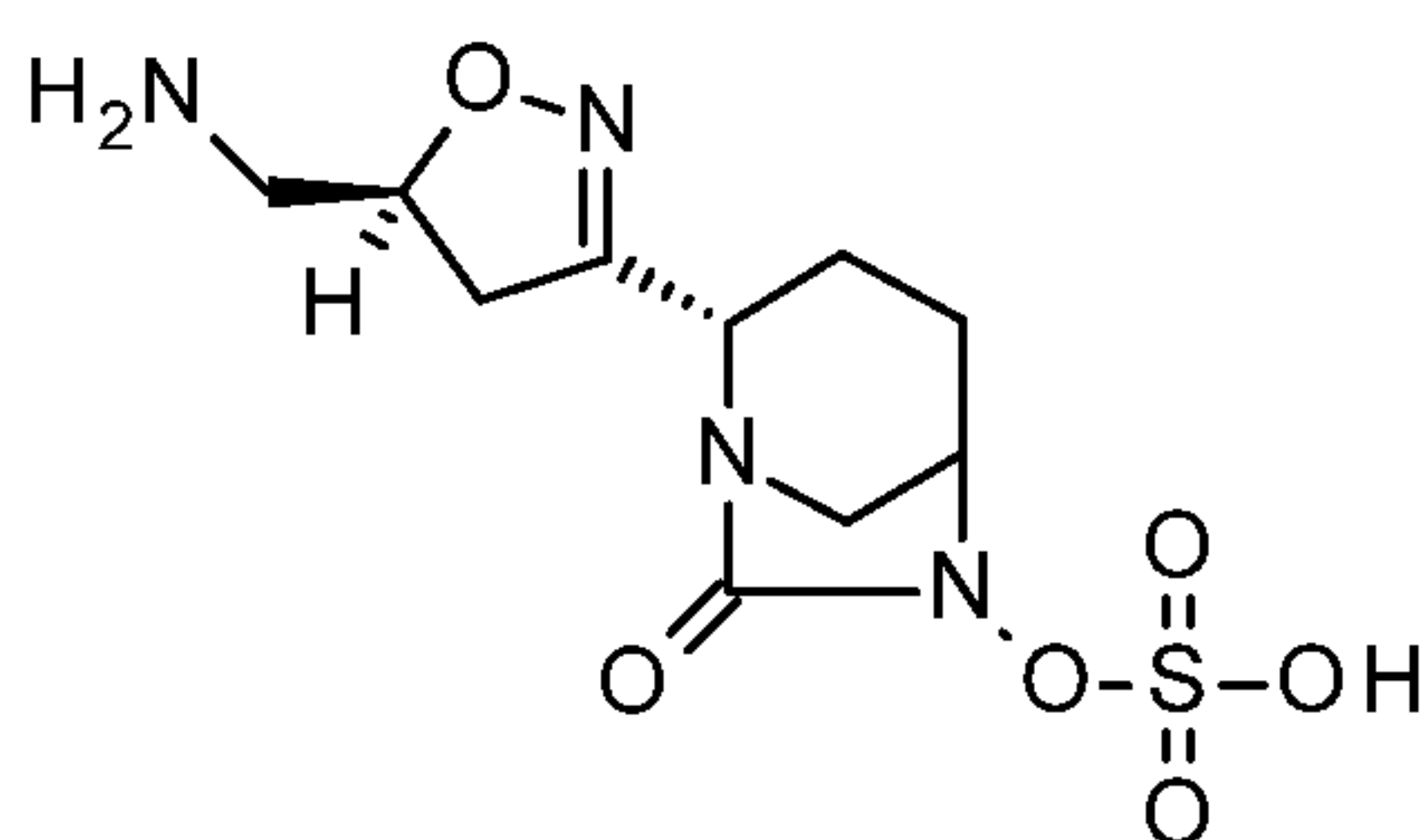
20

Examples 15 and 16

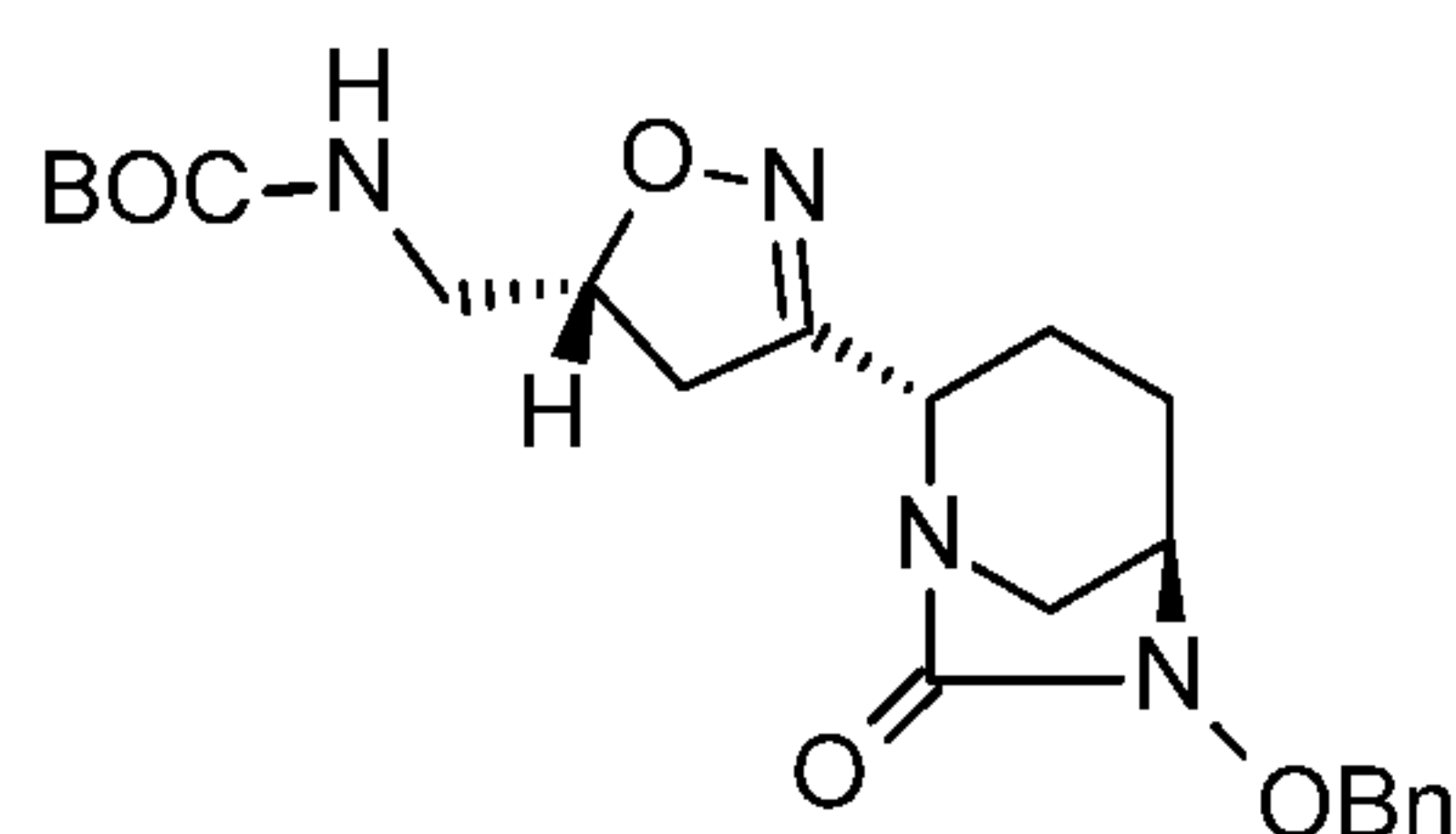
[(2S)-2-[(5R)-5-(aminomethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate



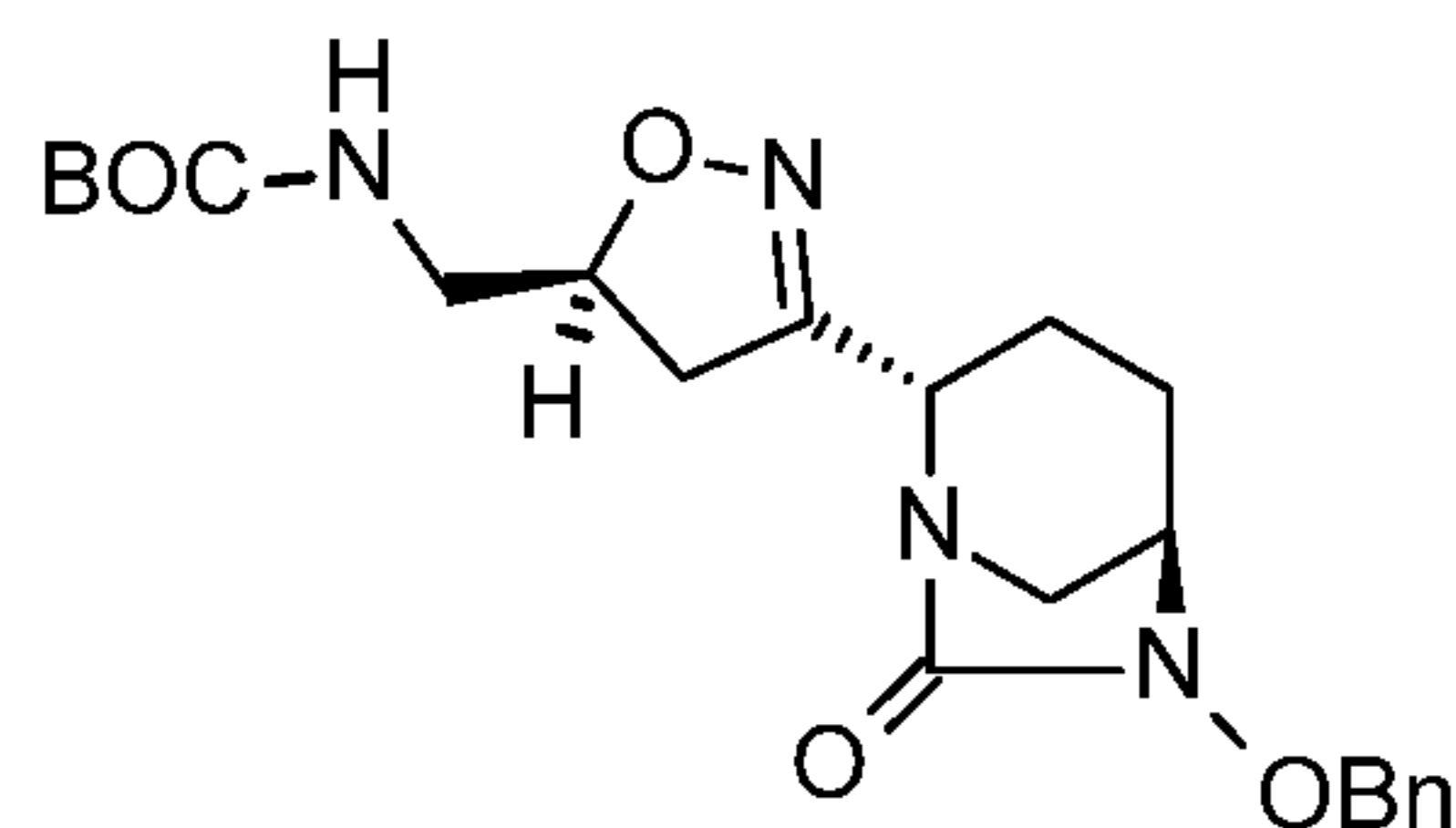
5 [(2S)-2-[(5S)-5-(aminomethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate



Step 1: Separation of stereoisomers of intermediate for example 2 with chiral column
 10 *tert*-butyl N-[[[(5R)-3-[(2S,5R)-6-benzyloxy-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]-4,5-dihydroisoxazol-5-yl]methyl]carbamate



tert-butyl N-[[[(5S)-3-[(2S,5R)-6-benzyloxy-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]-4,5-dihydroisoxazol-5-yl]methyl]carbamate



15

Chromatographic Conditions

column: Chiralpak[®] IB, 5 μ m, 20 x 250mm

Column Temperature: 25 °C

Mobile Phase: 2-Propanol (10%)/n-Hexane(90%)

Flow Rate: 20mL/min

Detection: 254nm

5 Injection Volume: 2mL

Sample preparation: Intermediate-A(100mg) was dissolved in methylene dichloride (2mL)

Retention Time: Less polar isomer 22 minutes, More polar isomer 27 minutes

10 Less polar isomer - ¹H NMR (400 MHz CDCl₃) δ 7.44-7.35 (m, 5H), 5.08 (d, *J* = 11.2Hz, 1H), 4.09 (d, *J* = 11.2Hz, 1H), 4.82 (s, 1H), 4.74 (m, 1H), 4.13 (d, *J* = 6.8Hz, 1H), 3.37-3.26 (m, 3H), 3.05-2.70 (m, 4H), 2.20-1.75 (m, 4H), 1.42 (s, 9H).

More polar isomer - ¹H NMR (400 MHz CDCl₃) δ 7.52-7.35 (m, 5H), 5.07 (d, *J* = 11.6Hz, 1H), 4.89 (d, *J* = 11.6Hz, 1H), 4.72 (m, 1H), 4.14 (d, *J* = 7.2Hz, 1H), 3.37-3.08 (m, 4H), 2.92-2.68 (m, 3H), 2.20-1.72 (m, 4H), 1.44 (s, 9H).

15 Step 2: Isolated chiral isomers were treated according to same procedure as step 6 and 7 in Example 1 to give Example 15 (from LP) and 16 (from MP).

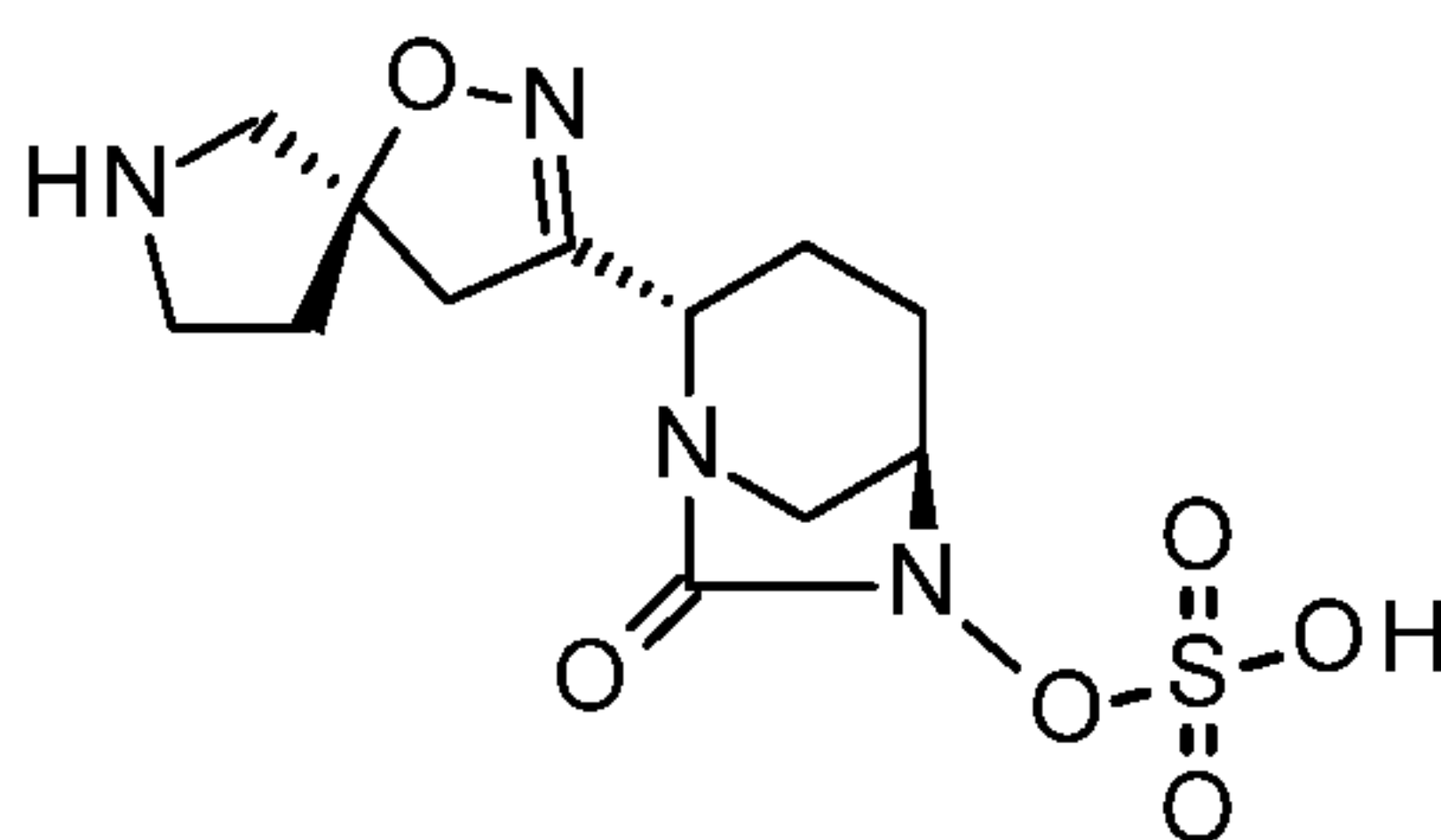
From LP isomer (Example 15) - ¹H NMR (400MHz, D₂O) δ 5.04 (m, 1H), 4.30 (d, *J* = 6.8Hz, 1H), 4.23 (s, 1H), 3.42-2.96 (m, 6H), 2.19-1.87 (m, 4H).

From MP isomer (Example 16) - ¹H NMR (400MHz, D₂O) δ 5.04 (m, 1H), 4.30(d, *J* = 6.4Hz, 1H), 4.22 (s, 1H), 3.47-2.90 (m, 6H), 2.05-1.87 (m, 4H).

20

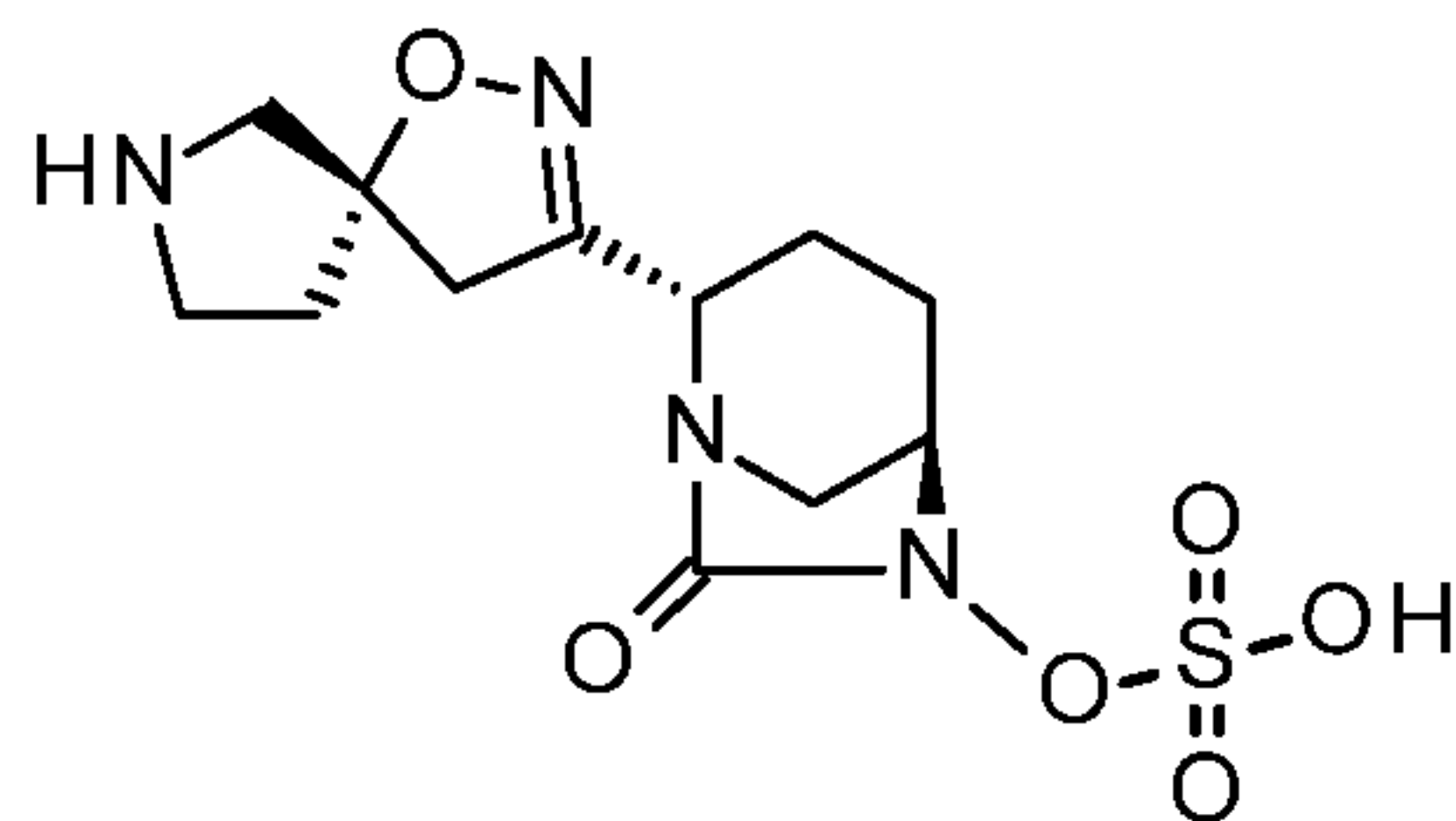
Example 17 and 18:

[(2S,5R)-2-[(5R)-1-oxa-2,7-diazaspiro[4.4]non-2-en-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate

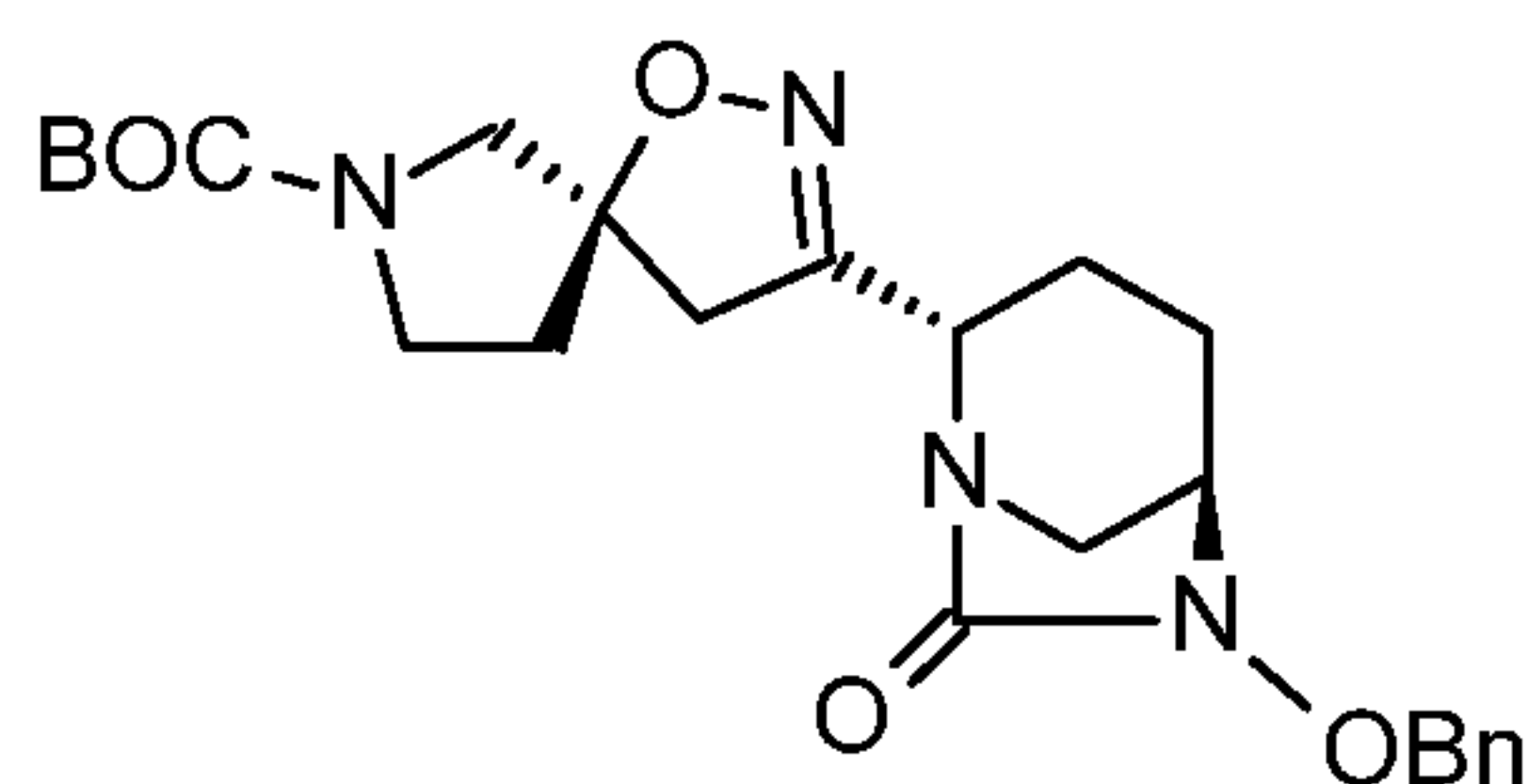


25

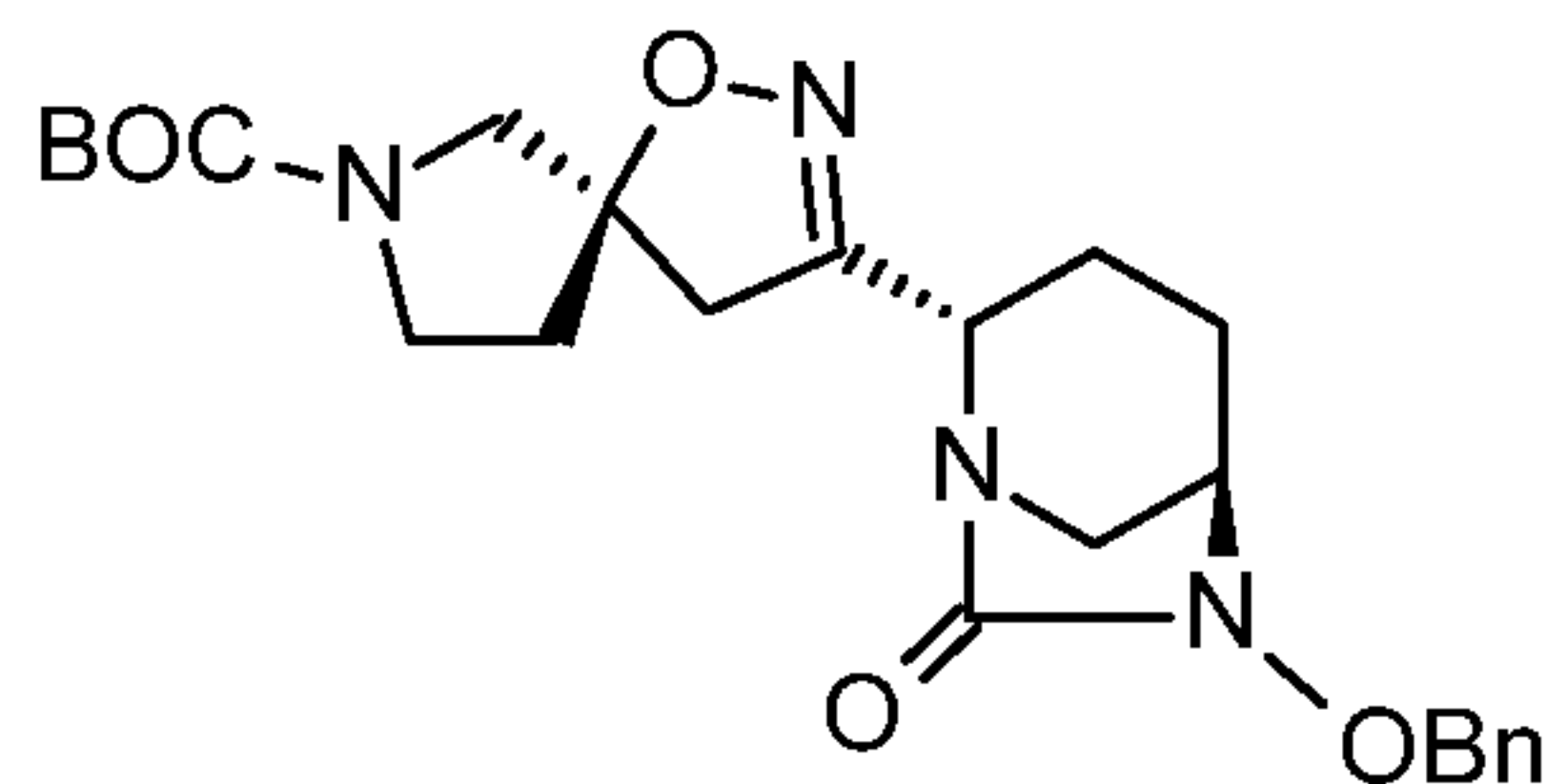
[(2S,5R)-2-[(5S)-1-oxa-2,7-diazaspiro[4.4]non-2-en-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate



Step 1: Separation of stereoisomers of intermediate for example 9 with chiral column *tert*-butyl (5R)-3-[(2S,5R)-6-benzyloxy-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]-1-oxa-2,7-diazaspiro[4.4]non-2-ene-7-carboxylate



tert-butyl (5S)-3-[(2S,5R)-6-benzyloxy-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]-1-oxa-2,7-diazaspiro[4.4]non-2-ene-7-carboxylate



Column: Chiralpak[®] IB, 5 μ m, 20 x 250mm

Column Temperature: 25 °C

15 Mobile Phase: Ethanol (20%)/n-Hexane(80%)

Flow Rate: 20mL/min

Detection: 254nm

Injection Volume: 2mL

Sample preparation: Intermediate-A (50mg) was dissolved in methylene dichloride (2mL)

20 Retention Time: less polar isomer at 10 minutes, more polar isomer at 13 minutes

Less Polar isomer - ^1H NMR (400 MHz CDCl_3) δ 7.42-7.37 (m, 5H), 5.07 (d, $J = 11.2\text{Hz}$, 1H), 4.91 (d, $J = 11.2\text{Hz}$, 1H), 4.16 (s, 1H), 3.71-3.33 (m, 5H), 3.11-2.71 (m, 4H), 2.21-1.76 (m, 6H), 1.46 (s, 9H).

More Polar isomer - ^1H NMR (400 MHz CDCl_3) δ 7.42-7.37 (m, 5H),), 5.06 (d, $J = 11.2\text{Hz}$, 1H), 4.91 (d, $J = 11.2\text{Hz}$, 1H), 3.68-3.33 (m, 5H), 3.07-2.74 (m, 4H), 2.20-1.78 (m, 6H), 1.46 (s, 9H).

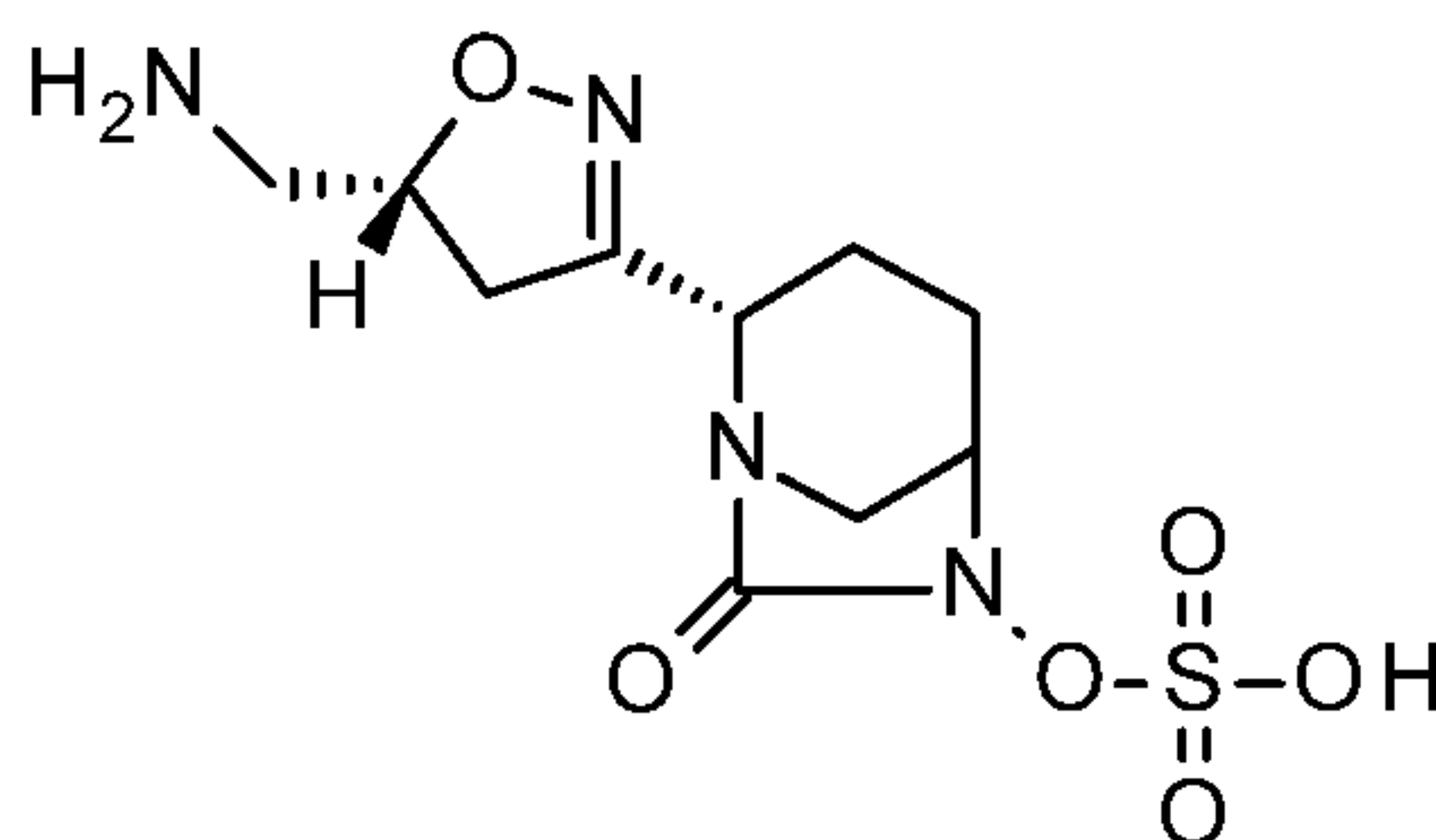
Step 2: Isolated chiral isomers were treated according to the same procedure as step 6 and 7 in Example 1 to give Example 17 (from LP) and 18(from MP).

From LP isomer (Example 17) - ^1H NMR (400MHz, D_2O) δ 4.26(d, $J = 7.2\text{Hz}$, 1H), 4.16 (s, 1H), 3.64-2.96 (m, 8H), 2.41-1.77(m, 6H).

From MP isomer (Example 18) - ^1H NMR (400MHz, D_2O) δ 4.32 (d, $J = 6.4\text{Hz}$, 1H), 4.24 (s, 1H), 3.69-3.04 (m, 8H), 2.47-1.89(m, 6H).

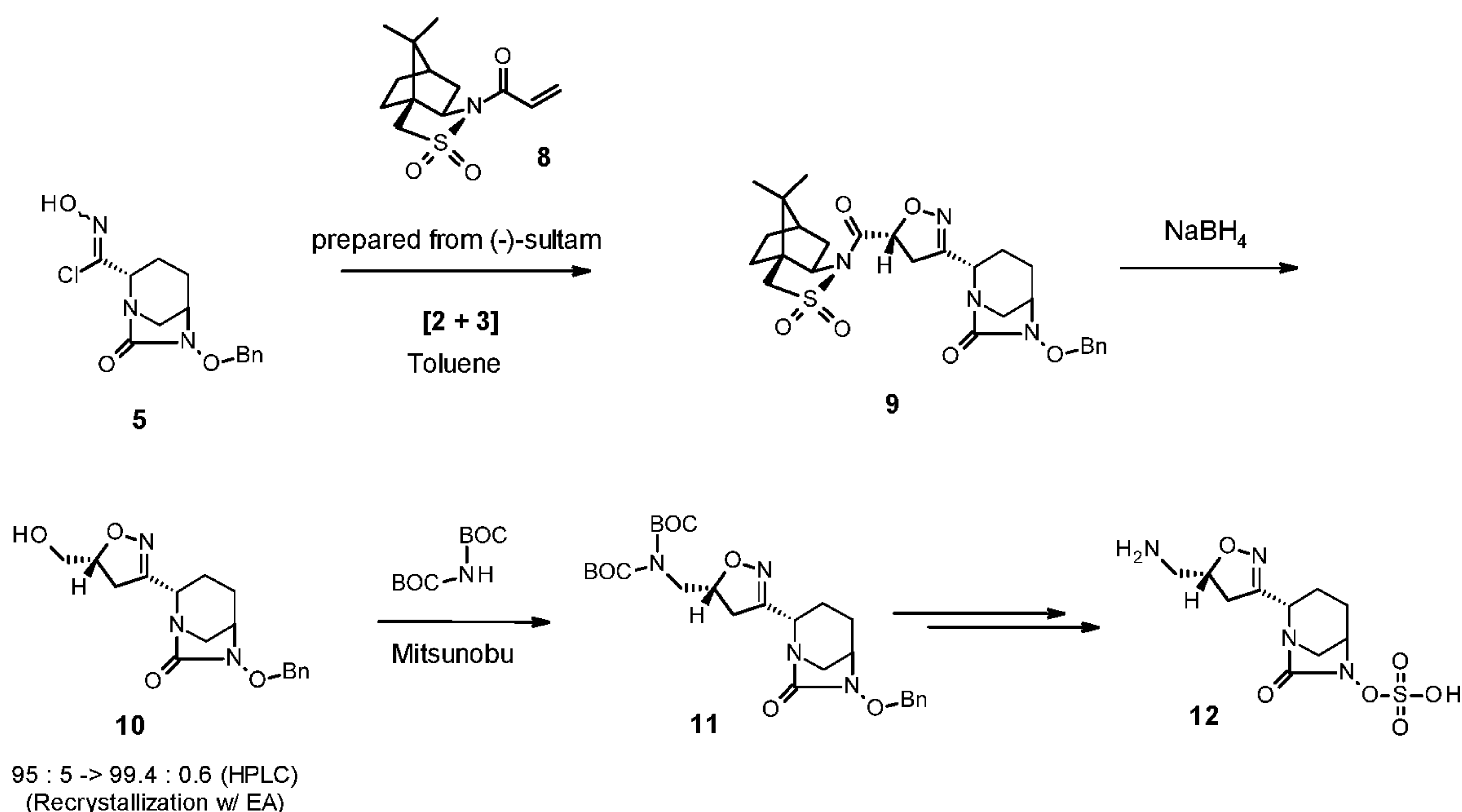
Example 19: Chiral synthesis of Example 15

15 [(2S)-2-[(5R)-5-(aminomethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate

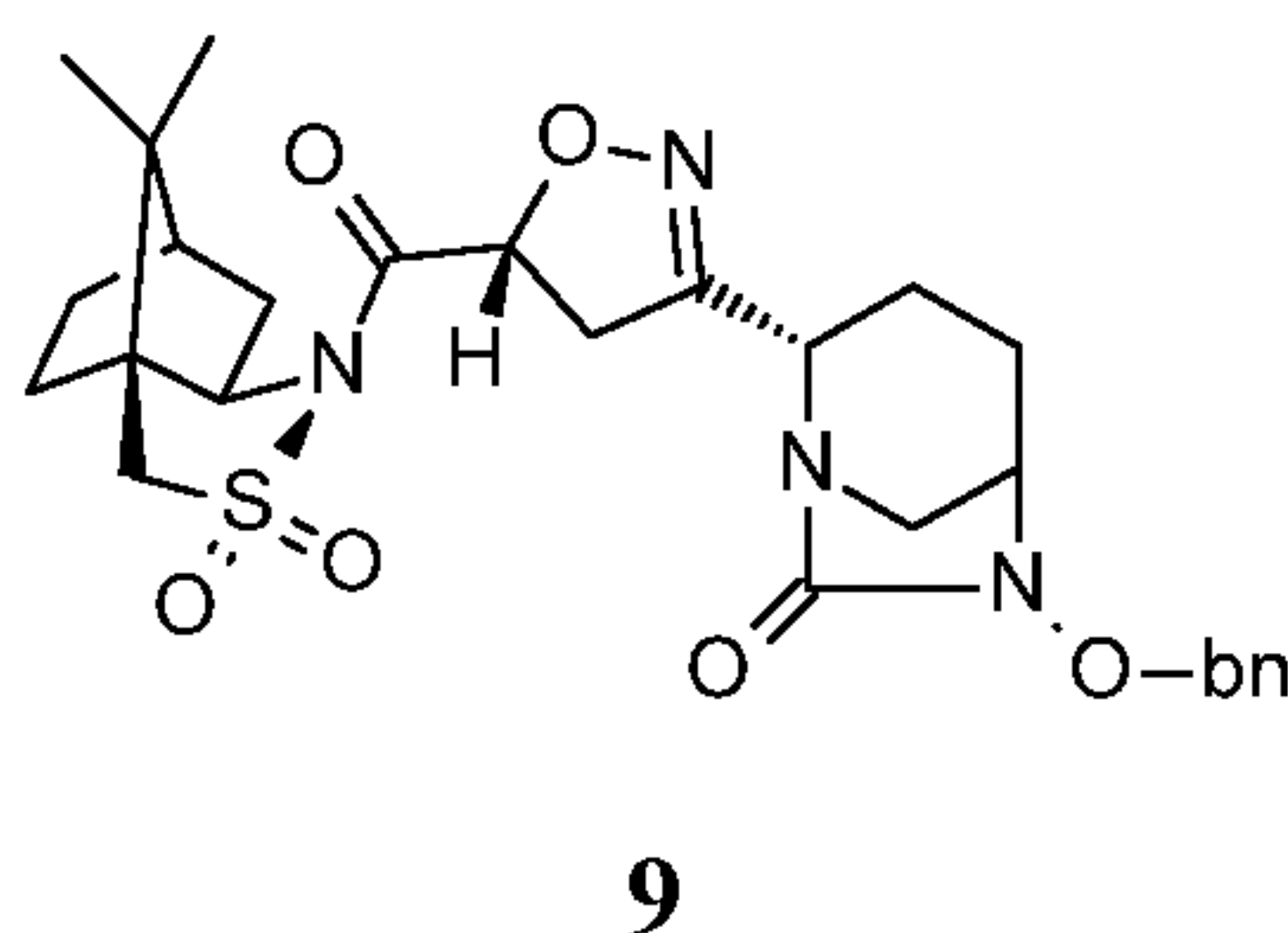


20 The synthesis of [(2S)-2-[(5R)-5-(aminomethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate is carried out according to Scheme 4 and as outlined in the steps below. Chiral center at 5 was assigned as (R) by the known publications; 1) Tetrahedron Letters, 29, (1988), 3555-3558, 2) European Journal of Medicinal Chemistry 42 (2007) 1044-1048.

Scheme 4

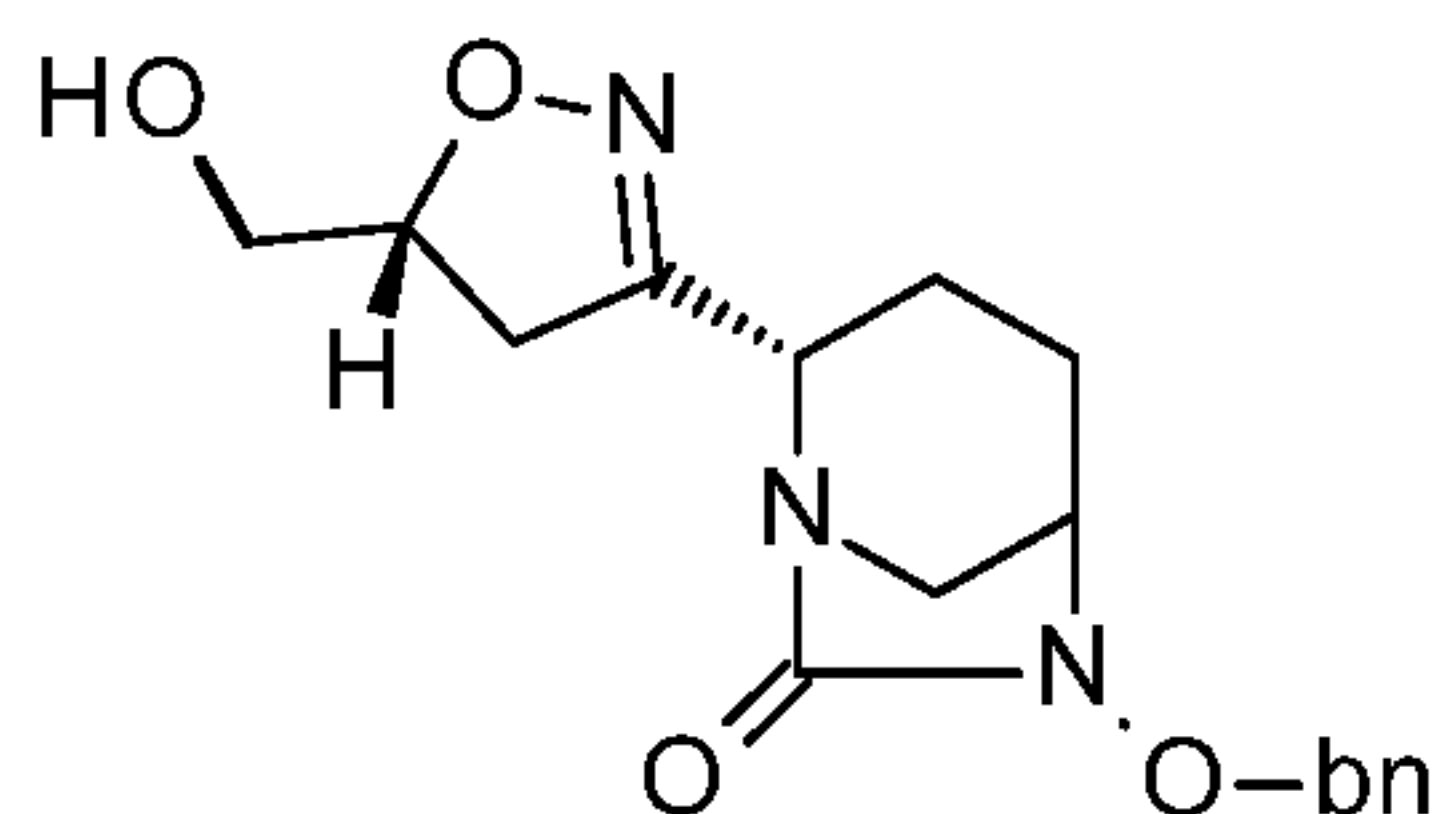


Step 1: (2S)-6-(benzyloxy)-2-((R)-5-((3aS,6R,7aR)-8,8-dimethyl-2,2-dioxidohexahydro-3H-3a,6-methanobenzo[c]isothiazole-1-carbonyl)-4,5-dihydroisoxazol-3-yl)-1,6-diazabicyclo[3.2.1]octan-7-one



To a solution of **Compound-5** (3.3g, 10.6 mmol) and **compound-8**, (R)-2-propenoyl-2,10-camphorsultam (4.28 g, 15.9 mmol, prepared from (1S)-(-)-2,10-camphorsultam and 2-propenoyl chloride) in methylene chloride (10 mL) was added TEA (2.2 mL, 15.9 mmol) dropwise at room temperature. The mixture was stirred overnight at room temperature. The mixture was washed with saturated NaHCO₃ (100 mL) and dried over anhydrous sodium sulfate. The combined organic layer was concentrated under reduced pressure and purified by silica gel column chromatography (30% EA/Hexane) to give **Compound-9** (2.7 g, 47 %). ¹H NMR (400 MHz, CDCl₃) δ 7.44-7.36 (m, 5H), 5.62 (m, 1H), 5.06 (d, *J* = 11.2Hz, 1H), 4.90 (d, *J* = 11.2Hz, 1H), 4.14 (d, *J* = 6.8Hz, 1H), 3.89 (m, 1H), 3.56-3.20 (m, 5H), 2.88 (m, 2H), 2.22-1.77 (m, 10H), 1.44-1.25 (m, 4H), 1.87 (s, 3H), 0.98 (s, 3H).

Step 2: (2S)-6-benzyloxy-2-[(5R)-5-(hydroxymethyl)-4,5-dihydroisoxazol-3-yl]-1,6-diazabicyclo[3.2.1]octan-7-one

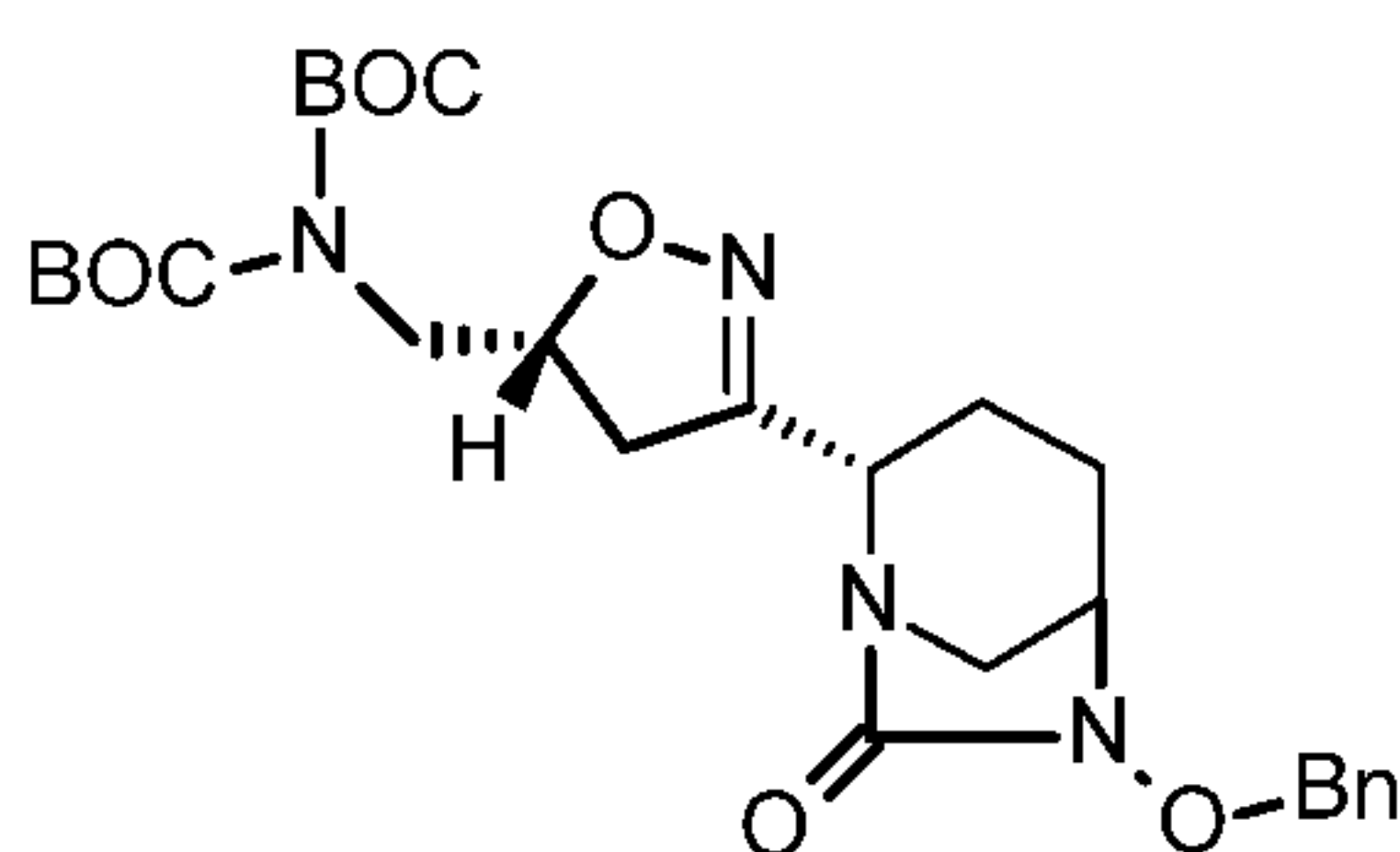


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To a solution of NaBH₄ (140mg, 3.69 mmol) and water (2 mL) in THF (6mL) was added **Compound-9** (1g, 1.84 mmol) in THF(3mL) dropwise at 0 °C. The mixture was stirred for 1h at 0 °C. The reaction mixture was carefully quenched by addition of saturated NaH₂PO₄ solution (30 mL) at 0 °C. The reaction mixture was diluted with water and
 10 extracted with EA (50mL x 2). The combined organic layer was concentrated and purified by silica gel column chromatography (50-100% EtOAc/n-Hexane) to give **Compound-10** (500mg, 82%). After recrystallization with EtOAc, ¹H NMR (400 MHz, CDCl₃) δ 7.42-7.36 (m, 5H), 5.07 (d, *J* = 11.2Hz, 1H), 4.91 (d, *J* = 11.2Hz, 1H), 4.78 (m, 1H), 4.14 (d, *J* = 9.0Hz, 1H), 3.85 (m, 1H), 3.55(m, 1H), 3.29 (s, 1H), 3.20 (m, 2H), 2.90 (d, *J* = 12Hz, 1H),
 15 2.78 (d, *J* = 19.2Hz, 1H), 2.21-1.73 (m, 4H).

Step 3: *tert*-butyl N-[[[(5R)-3-[(2S)-6-benzyloxy-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl]-4,5-dihydroisoxazol-5-yl]methyl]-N-*tert*-butoxycarbonyl-carbamate



20

11

To a solution of **Compound-10** (300mg, 0.9 mmol), Di-*tert*-butyliminodicarboxylate (0.4g, 1.8mmol) and triphenylphosphine (0.47g, 1.8mmol) in THF (5mL) was added Diisopropyl diazocarboxylate (0.36mL, 1.84 mmol) at 0 °C. The mixture

was stirred overnight at room temperature. The reaction mixture was concentrated and purified by silica gel column chromatography (20-50% EtOAc/n-Hexane) to give **Compound-11** (280mg, 59%). ¹H NMR (400 MHz, CDCl₃) δ 7.43-7.37 (m, 5H), 5.05 (m, 1H), 4.93-4.86 (m, 2H), 4.14 (dd, *J* = 16.0, 6.8Hz, 1H), 3.81-3.66 (m, 2H), 3.32(s, 1H), 3.07-2.72 (m, 4H), 2.22-1.76 (m, 4H), 1.58 (s, 18H).

Step 4 : **Compound -11** was treated according to the same procedure as step 6 and 7 in Example 1 to give Example 15.

B. Biological Assays

The following assay methods were used to evaluate antibacterial activity of compounds of Formula I.

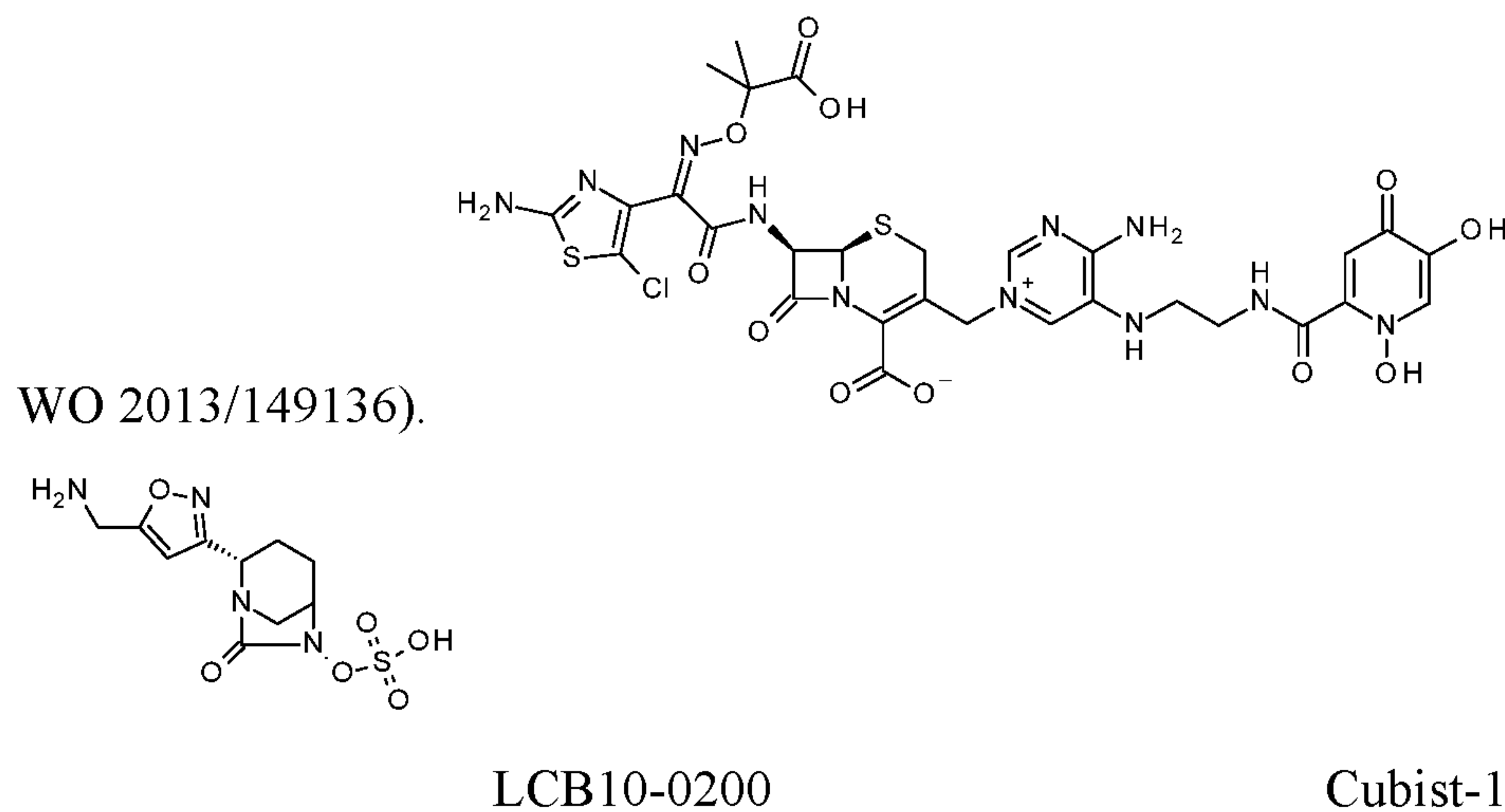
Example 20: *in vitro* Antibacterial activity assay

The *in vitro* antibacterial activity was evaluated by measuring MIC₉₀ (μg/mL) of each of the compounds of Examples 1 to 10. MIC₉₀ (μg/mL) is the lowest concentration of an antibiotic that will inhibit the visible growth of 90% of microorganisms after incubation as compared with a control group to which the antibiotic is not treated.

MIC values were measured by the broth microdilution method developed by the Clinical and Laboratory Standards Institute (CLSI) (see CLSI M07-A9, Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically; Approved Standard-Ninth Edition (2012): CLSI, Villanova, PA). The *in vitro* antibacterial activity was measured with respect to the following 9 clinical isolates: *P. aeruginosa* (3 strains); *K. pneumoniae* (6 strains). The *in vitro* antibacterial activity of selected compounds was measured with respect to the following 15 clinical isolates: *K. pneumoniae* (15 strains).

Briefly, compounds (Ceftazidime, Meropenem, and LCB10-0200) were dissolved in DMSO at the concentration of 5,120 μg/mL, were diluted by two fold with DMSO, and then were diluted by twenty fold with sterilized distilled water. The final concentration in the antibacterial activity test was in the range of 0.03125 μg/mL to 64 μg/mL, and the final concentration of DMSO used as an adjuvant was 2.5% (v/v). For Ceftazidime, Meropenem, or LCB10-0200 combination studies, doubling dilutions of each compound were utilized in combination with a fixed 4 μg/mL concentration of each compounds of Examples 1 to 18.

The results of the assay for 9 clinical isolates: *P. aeruginosa* (3 strains); *K. pneumoniae* (6 strains) are summarized in Tables 2 and 3 for Ceftazidime, Tables 4, 5 and 6 for LCB10-0200 (LegoChem Biosciences, US 2012 0264727), and Table 7 for Meropenem. The results for 15 clinical isolates: *K. pneumoniae* (15 strains) are summarized in Table 8 and 9 for Meropenem, Table 10 and 11 for LCB10-0200, and Table 12 for Meropenem and LCB10-0200 with a Cubist β -lactamase inhibitor containing an isoxazole ring (Cubist-1,



10

In Table 12, the compound of Example 15 showed much broader coverage on various β -lactamases than Cubist-1 in combination with Meropenem and LCB10-0200.

Table 2: Standard BLI potentiation MIC assay in combination with Ceftazidime against a panel of clinical strains expressing β -lactamases.

strain		β -lactamase content	compound						
incubation for 19 h			Ceftazidime						
with BLI (4ug/ml)			alone	OP-595	ex 1	ex 2	ex 3	ex 4	ex 5
<i>P.aeruginosa</i>	ATCC27853	PoxB, AmpC	2	2	2	2	2	2	2
	ARC3506	VEB-1, OXA-10, OprD , AmpC	>64	32	16	32	32	>64	64
	ARC3514	KPC-2, OprD-, AmpC	>64	8	8	16	ND	ND	32
<i>K.pneumoniae</i>	ATCC700603	SHV-3	32	0.125	1	0.5	1	2	0.25
	NDM-506		>64	0.125	4	1	8	4	0.5
	5576	SHV-11, CMY-2, DHA-1, TEM-1	>64	0.125	16	1	4	8	0.5
	3784	SHV-11, SHV-12, DHA-1	>64	0.5	4	2	ND	ND	1
	4006	SHV-12, CTX-M-15, TEM-1, OXA-9	>64	0.125	4	0.5	ND	ND	0.125
	4248	SHV-11, CTX-M-15, TEM-1, OXA-1	64	0.0625	1	0.125	ND	ND	0.0625

Table 3: Standard BLI potentiation MIC assay in combination with Ceftazidime against a panel of clinical strains expressing β -lactamases.

strain		β -lactamase content	compound						
incubation for 19 h			Ceftazidime						
with BLI (4ug/ml)			alone	OP-595	ex 6	ex7	ex 8	ex 9	ex 10
<i>P.aeruginosa</i>	ATCC27853	PoxB, AmpC	2	2	2	2	2	2	2
	ARC3506	VEB-1, OXA-10, OprD ⁻ , AmpC	>64	32	>64	32	64	32	32
	ARC3514	KPC-2, OprD ⁻ , AmpC	>64	8	64	8	16	16	16
<i>K.pneumoniae</i>	ATCC700603	SHV-3	32	0.125	1	0.125	2	0.125	0.25
	NDM-506		>64	0.125	8	0.25	8	0.25	0.5
	5576	SHV-11, CMY-2, DHA-1, TEM-1	>64	0.125	32	0.25	16	0.25	0.25
	3784	SHV-11, SHV-12, DHA-1	>64	0.5	4	1	4	0.5	1
	4006	SHV-12, CTX-M-15, TEM-1, OXA-9	>64	0.125	4	0.25	4	0.0625	0.25
	4248	SHV-11, CTX-M-15, TEM-1, OXA-1	64	0.0625	1	0.0625	1	0.03125	0.0625

Table 4: Standard BLI potentiation MIC assay in combination with LCB10-0200 against a panel of clinical strains expressing β -lactamases.

strain		β -lactamase content	compound						
incubation for 19 h			LCB10-0200						
with BLI (4ug/ml)			alone	OP-595	ex 1	ex 2	ex 3	ex 4	ex 5
<i>P.aeruginosa</i>	ATCC27853	PoxB, AmpC	0.25	0.25	0.125	0.25	0.125	0.125	0.25
	ARC3506	VEB-1, OXA-10, OprD ⁻ , AmpC	2	0.25	0.5	1	2	8	0.25
	ARC3514	KPC-2, OprD ⁻ , AmpC	0.25	0.125	0.125	0.25	0.0625	0.0625	0.125
<i>K.pneumoniae</i>	ATCC700603	SHV-3	1	0.125	0.125	0.125	0.125	0.25	0.125
	NDM-506		>64	0.125	4	0.25	4	4	0.5
	5576	SHV-11, CMY-2, DHA-1,TEM-1	>64	<0.03125	0.25	0.0625	<0.03125	0.0625	<0.03125
	3784	SHV-11, SHV-12, DHA-1	32	0.0625	8	0.0625	16	16	0.125
	4006	SHV-12, CTX-M-15, TEM-1, OXA-9	32	<0.03125	0.0625	<0.03125	0.0625	0.0625	<0.03125
	4248	SHV-11, CTX-M-15, TEM-1, OXA-1	2	<0.03125	<0.03125	<0.03125	<0.03125	<0.03125	<0.03125

Table 5: Standard BLI potentiation MIC assay in combination with LCB10-0200 against a panel of clinical strains expressing β -lactamases.

strain		β -lactamase content	compound						
incubation for 19 h			LCB10-0200						
with BLI (4ug/ml)			Alone	OP-595	From ex 6	ex7	ex 8	ex 9	ex 10
<i>P.aeruginosa</i>	ATCC27853	PoxB, AmpC	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	ARC3506	VEB-1, OXA-10, OprD ⁻ , AmpC	2	0.25	0.5	2	2	0.25	0.5
	ARC3514	KPC-2, OprD-, AmpC	0.25	0.125	0.25	0.25	0.25	0.25	0.25
<i>K.pneumoniae</i>	ATCC700603	SHV-3	1	0.125	0.25	0.125	0.25	0.125	0.125
	NDM-506		>64	0.125	16	0.125	8	0.125	0.5
	5576	SHV-11, CMY-2, DHA-1, TEM-1	>64	<0.03125	0.5	<0.03125	0.25	<0.03125	<0.03125
	3784	SHV-11, SHV-12, DHA-1	32	0.0625	4	0.0625	16	0.0625	0.25
	4006	SHV-12, CTX-M-15, TEM-1, OXA-9	32	<0.03125	0.125	<0.03125	0.125	<0.03125	<0.03125
	4248	SHV-11, CTX-M-15, TEM-1, OXA-1	2	<0.03125	0.0625	<0.03125	0.125	<0.03125	<0.03125

Table 6: Standard BLI potentiation MIC assay in combination with LCB10-0200 against a panel of clinical strains expressing β -lactamases

strain		β -lactamase content	compound					
incubation for 19 h			LCB10-0200					
with BLI (4ug/ml)			alone	OP-595	ex 11	ex 12	ex 13	ex 14
<i>P.aeruginosa</i>	ATCC27853	PoxB, AmpC	0.5	0.5	0.25	1	0.5	0.5
	ARC3506	VEB-1, OXA-10, OprD ⁻ , AmpC	2	0.25	0.5	2	1	4
	ARC3514	KPC-2, OprD ⁻ , AmpC	0.5	0.25	0.125	0.5	0.25	0.25
<i>K.pneumoniae</i>	ATCC700603	SHV-3	2	0.125	0.25	1	0.25	0.25
	NDM-506		>64	>64	4	>64	>64	>64
	5576	SHV-11,CMY-2,DHA-1,TEM-1	>64	0.0625	1	16	0.0625	1
	3784	SHV-11,SHV-12,DHA-1	64	0.0625	0.25	64	0.125	0.25
	4006	SHV-12,CTX-M-15,TEM-1,OXA-9	8	<0.03125	1	1	0.0625	0.25
	4248	SHV-11,CTX-M-15,TEM-1,OXA-1	4	<0.03125	<0.0312	0.25	<0.03125	<0.03125

Table 7: Standard BLI potentiation MIC assay in combination with Meropenem against a panel of clinical strains expressing β -lactamases

strain		β -lactamase content	compound				
incubation for 19 h			Meropenem				
with BLI (4ug/ml)			alone	OP-595	ex 12	ex 13	ex 14
<i>P.aeruginosa</i>	ATCC27853	PoxB, AmpC	2	2	2	2	2
	ARC3506	VEB-1, OXA-10, OprD ⁻ , AmpC	64	64	64	32	64
	ARC3514	KPC-2, OprD-, AmpC	>64	>64	>64	>64	>64
<i>K.pneumoniae</i>	ATCC700603	SHV-3	0.125	0.125	0.25	0.125	0.125
	NDM-506		>64	>64	>64	>64	>64
	5576	SHV-11,CMY-2,DHA-1,TEM-1	1	0.03125	0.25	0.0625	0.0625
	3784	SHV-11,SHV-12,DHA-1	2	0.25	1	0.25	0.25
	4006	SHV-12,CTX-M-15,TEM-1,OXA-9	0.5	0.0625	1	0.0625	0.0625
	4248	SHV-11,CTX-M-15,TEM-1,OXA-1	0.125	0.0625	0.125	0.0625	0.0625

Table 8: Standard BLI potentiation MIC assay in combination with Meropenem against a panel of *K. pneumoniae* clinical strains expressing β -lactamases.

strain		β -lactamase content	compound				
incubation for 19 h			Meropenem				
with BLI (4ug/ml)			alone	OP-595	ex 12	ex 13	ex 14
<i>K.pneumoniae</i>	ATCC700603		0.25	0.125	0.25	0.125	0.025
	4006	SHV-12,CTX-M-15,TEM-1,OXA-9	1	0.0625	0.5	0.125	0.25
	5216	TEM-1A	64	2	64	32	32
	7586		64	8	64	32	16
	6540		1	0.125	1	0.25	0.125
	6839	SHV-11,SHV-12,DHA-1	4	0.25	4	0.5	0.5
	89		8	4	4	4	4
	100		4	4	8	4	4
	101		>64	0.125	8	0.125	0.125
	23		16	16	16	16	16
	5971(KOX)		64	16	64	16	16
	KU2		>64	>64	>64	>64	>64
	MP14		>64	0.5	>64	1	1
	5117	SHV-11, TEM-1	8	0.5	8	2	2
	5576	SHV-11,CMY-2,DHA-1,TEM-1	1	0.0625	2	0.0625	0.25

Table 9: Standard BLI potentiation MIC assay in combination with Meropenem against a panel of *K. pneumoniae* clinical strains expressing β -lactamases.

strain		β -lactamase content	compound							
incubation for 19 h			Meropenem							
with BLI (4ug/ml)			alone	Avibactam	OP-595	ex 15	ex 16	ex 17	ex 18	ex 5
<i>K.pneumoniae</i>	ATCC700603		0.125	0.0625	0.0625	0.0625	0.0625	0.0625	0.0625	< 0.0312
	4006	SHV-12,CTX-M-15,TEM-1,OXA-9	0.5	0.125	0.0625	0.0625	0.125	0.0625	0.125	< 0.0312
	5216	TEM-1A	32	8	2	2	8	2	8	8
	7586		16	8	2	2	4	2	4	4
	6540		0.5	0.0625	0.0625	0.0625	0.0625	0.0625	0.0625	< 0.0312
	6839	SHV-11,SHV-12,DHA-1	2	0.5	0.0625	0.0625	0.0625	0.0625	0.0625	0.25
	89		4	2	2	1	2	1	1	2
	100		4	4	2	2	2	2	2	2
	101		64	0.125	0.0625	0.0625	0.0625	0.0625	0.0625	< 0.0312
	23		1	1	2	1	1	1	1	4
	5971(KOX)		64	64	4	4	16	4	4	4
	KU2		>64	32	8	32	32	8	16	> 64
	MP14		64	0.125	0.125	0.125	0.125	0.125	0.125	0.125
	5117	SHV-11, TEM-1	4	1	0.25	0.25	1	0.25	1	0.5
	5576	SHV-11,CMY-2,DHA-1,TEM-1	1	0.0625	<0.0312	<0.0312	0.0625	<0.0312	<0.0312	< 0.0312

Table 10: Standard BLI potentiation MIC assay in combination with LCB10-0200 against a panel of *K. pneumoniae* clinical strains expressing β -lactamases.

strain		β -lactamase content	compound				
incubation for 19 h			LCB10-0200				
with BLI (4ug/ml)			alone	OP-595	ex 12	ex 13	ex 14
<i>K.pneumoniae</i>	ATCC700603		0.5	0.125	0.5	0.25	0.125
	4006	SHV-12,CTX-M-15,TEM-1,OXA-9	4	0.03125	4	0.125	0.125
	5216	TEM-1A	>64	0.25	>64	64	>64
	7586		4	0.03125	2	0.5	0.25
	6540		0.5	<0.03125	0.25	0.0625	0.03125
	6839	SHV-11,SHV-12,DHA-1	32	<0.03125	64	0.125	0.125
	89		0.125	0.0625	0.0625	0.0625	0.0625
	100		0.0625	0.0625	0.0625	0.0625	0.125
	101		16	<0.03125	8	0.0625	<0.03125
	23		1	1	1	1	1
	5971(KOX)		32	8	64	64	16
	KU2		32	8	32	32	32
	MP14		0.125	0.03125	0.25	0.125	0.0625
	5117	SHV-11, TEM-1	0.125	0.0625	0.25	0.0625	0.0625
	5576	SHV-11,CMY-2,DHA-1,TEM-1	>64	<0.03125	16	0.125	1

Table 11: Standard BLI potentiation MIC assay in combination with LCB10-0200 against a panel of *K. pneumoniae* clinical strains expressing β -lactamases.

strain		β -lactamase content	compound							
incubation for 19 h			LCB10-0200							
with BLI (4ug/ml)			alone	Avibactam	OP-595	ex 15	ex 16	ex 17	ex 18	ex 5
<i>K.pneumoniae</i>	ATCC700603		0.5	0.25	0.125	0.125	0.25	0.125	0.125	0.125
	4006	SHV-12,CTX-M-15,TEM-1,OXA-9	4	<0.0312	<0.0312	<0.0312	0.25	<0.0312	0.0312	0.0312
	5216	TEM-1A	64	4	0.5	0.5	16	0.25	2	2
	7586		4	0.125	0.0625	0.0625	0.5	<0.0312	0.25	0.25
	6540		4	0.125	0.0625	0.0625	0.125	<0.0312	<0.0312	<0.0312
	6839	SHV-11,SHV-12,DHA-1	16	8	<0.0312	<0.0312	8	<0.0312	0.0625	0.0625
	89		0.125	0.0625	0.0625	0.0625	0.125	<0.0312	0.0312	0.0312
	100		0.0625	0.0625	0.0625	0.0625	0.125	0.0625	<0.0312	<0.0312
	101		32	1	0.25	0.25	0.25	<0.0312	<0.0312	<0.0312
	23		0.125	0.125	<0.0312	<0.0312	<0.0312	<0.0312	0.0312	0.0312
	5971(KOX)		64	64	16	16	64	8	16	16
	KU2		32	16	2	2	16	2	16	16
	MP14		0.125	<0.0312	<0.0312	<0.0312	0.0625	<0.0312	<0.0312	<0.0312
	5117	SHV-11,TEM-1	0.125	0.125	<0.0312	<0.0312	0.0625	<0.0312	0.0625	0.0625
	5576	SHV-11,CMY-2,DHA-1,TEM-1	64	0.25	0.0625	0.0625	0.25	<0.0312	<0.0312	<0.0312

Table 12: Standard BLI potentiation MIC assay in combination with Meropenem / LCB10-0200 against a panel of *K. pneumoniae* clinical strains expressing β -lactamases. (isoxazoline vs. isoxazole)

strain		β -lactamase content	compound							
incubation for 19 h			Meropenem				LCB10-0200			
with BLI (4ug/ml)			alone	OP-595	ex 15	Cubist-1	alone	OP-595	ex 15	Cubist-1
<i>K.pneumoniae</i>	ATCC700603		0.125	0.0625	0.0625	0.0625	0.5	0.125	0.125	0.25
	4006	SHV-12,CTX-M-15,TEM-1,OXA-9	0.5	<0.0312	0.0625	0.125	8	<0.0312	<0.0312	0.25
	5216	TEM-1A	32	2	2	8	32	0.25	0.5	1
	7586		16	1	2	16	2	<0.0312	0.0625	0.5
	6540		0.5	0.0625	0.0625	0.0625	2	<0.0312	0.0625	0.0625
	6839	SHV-11,SHV-12,DHA-1	2	0.125	0.0625	0.25	32	0.0625	<0.0312	16
	89		2	1	1	0.125	0.0625	<0.0312	0.0625	<0.0312
	100		4	1	2	0.5	0.0625	0.0625	0.0625	0.0625
	101		32	<0.0312	0.0625	0.0625	32	<0.0312	0.25	4
	23		2	2	1	2	0.25	<0.0312	<0.0312	0.125
	5971(KOX)		32	2	4	32	32	8	16	32
	KU2		>64	8	32	16	32	2	2	16
	MP14		64	0.125	0.125	0.125	0.25	<0.0312	<0.0312	0.0625
	5117	SHV-11,TEM-1	4	0.25	0.25	4	0.25	<0.0312	<0.0312	0.125
	5576	SHV-11,CMY-2,DHA-1,TEM-1	1	<0.0312	<0.0312	<0.0625	64	<0.0312	0.0625	0.25

Example 21 – Inhibitory activity against β -lactamases (IC₅₀ determination)

Four compounds (Avibactam, OP-595, Ex15 and Ex17) were dissolved in T.D.W at 10 mM. More diluted stocks were prepared as necessary. Enzyme activity was determined by monitoring the change of absorbance at 492 nm using the characteristic molecular extinction coefficient ($\Delta\epsilon_{492} = 17,400 \text{ M}^{-1} \text{ cm}^{-1}$) of nitrocefin by a Shimazu UV-1650PC spectrophotometer. The nitrocefin was purchased from OXOID. Kinetic measurements were run at 30 °C in 10 mM MES[2-(N-morpholino)ethanesulfonic acid] buffer (pH 6.8) or 10 mM MES[2-(N-morpholino)ethanesulfonic acid] buffer (pH 6.8), 20mg/ml BSA and 50mM sodium bicarbonate. Reactions were performed in 2.5 mL cuvettes with 100 μM nitrocefin and initiated by adding 1.5 nM to 2.5nM enzyme. The first 180 s of each reaction was used to measure initial rates. Data were evaluated using Microsoft Excel. Concentration dependent inhibition of enzyme was measured using different concentrations of inhibitors resulting from two-fold dilution series. Reaction progress at every concentration of inhibitor was measured in triplicates. IC₅₀ values were calculated using 4-parameter log fits using XL Fit curve fitting software (www.idbs.com) for Excel using the following equation:

$$y = A + \frac{B - A}{1 + \left(\frac{x}{\text{IC}_{50}}\right)^{\text{slope}}}$$

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where y is the remaining enzyme activity (in %) and x is the corresponding concentration. the fitted IC₅₀ parameter is the relative IC₅₀, and is defined as the concentration giving a response half way between the fitted top (B) and bottom (A) of the curve.

Table 13: IC₅₀ values (μM) of selected comounds

β-lactamase	Molecular class	Avibactam (μM)	OP-0595	Ex 17	Ex 15
KPC-3	A	3.4715	0.0637	2.74	3.03
CTX-M-15	A	NT	NT	NT	NT
NDM-1	B	NI	NI	1191	1652
CMY-10	C	0.013	42.58	0.185	0.123
OXA-48	D	5.10	44.17	29.54	6.42

Table 14: MICs of bacterial strains used for extraction of enzymes (BLI : 4 ug/mL)

strain		β-lactamase content	<u>Meropenem</u>				<u>LCB10-0200</u>			
with BLI (4 mg/ml)			Alone	Avibactam	OP-595	Ex 17	Alone	Avibactam	OP-595	Ex 17
<i>K. pneumoniae</i>	4006	SHV-12, CTX-M-15, TEM-1, OXA-9	1	0.5	0.0625	0.0625	4	0.25	0.0625	0.0625
	5576	SHV-11, CMY-2, DHA-1, TEM-1	2	0.25	0.0625	0.0625	32	0.125	<0.0312	<0.0312
	CL5761	KPC-3 , TEM-216, SHV-5	>64	1	1	0.5	32	16	0.25	0.125
<i>E. coli</i>	ECO200	NDM-1 , TEM-1, CTX-M-15, EC (AmpC), OXA-2 type	>64	>64	<0.0312	<0.0312	>64	>64	<0.0312	<0.0312
	K0519020	CTX-M-15 , TEM-1, OXA-1, AmpC (NC_000913)	1	0.25	0.125	0.0625	64	0.25	0.0625	<0.0312
	TOPOXA048	OXA-48 , TEM-116, AmpC (NC_000913)	0.25	0.5	<0.0312	<0.0312	0.25	0.25	<0.0312	<0.0312
<i>E. aerogenes</i>	K9911729	CMY-10 , TEM-1, SHV-12, AmpC (DQ478697)	0.5	0.5	<0.0312	<0.0312	2	2	<0.0312	<0.0312

Example 22 - Mouse Pharmacokinetics

The pharmacokinetics values were evaluated in a mouse model. The test compound (50 mg/kg) was injected into the tail vein of 8 week old ICR mouse, weighing approximately 26-30 g. Blood samples were taken from inferior vena cava at 5 min, 15 min, 30 min, 1h, 1.5h, 2h, 3h, 4h, and 8h after administration, plasma was separated, and quantified by using LC-MS/MS. See Figure 1, Panels A-C. Lab conditions were temperature of $22 \pm 3^\circ\text{C}$ and humidity of $50 \pm 20\%$.

The results from the compounds of Example 2, 5, and 17 are summarized in Table 15.

Table 15

Parameter	Mouse (IV, 50 mg/kg)		
	Compound of Example 2	Compound of Example 5	Compound of Example 17
$\text{AUC}_{\text{inf}}(\text{mg} \cdot \text{h/l})$	34.54 ± 3.61	55.29 ± 4.16	30.96 ± 0.50
$\text{AUC}_{\text{norm}}(\text{kg} \cdot \text{h/l})$	0.69 ± 0.07	1.11 ± 0.08	0.62 ± 0.01
$\text{CL}(\text{l/h/kg})$	1.46 ± 0.16	0.91 ± 0.07	1.62 ± 0.03
$\text{V}_{\text{ss}}(\text{l/kg})$	1.57 ± 0.12	2.23 ± 0.61	1.81 ± 0.14
$\text{C}_{\text{max}}(\text{mg/l})$	74.37 ± 3.44	103.2 ± 9.93	64.17 ± 3.88
$\text{C}_{\text{max,norm}}(\text{kg/l})$	1.49 ± 0.07	2.06 ± 0.20	1.28 ± 0.08
$\text{T}_{\text{max}}(\text{h})$	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00
$\text{T}_{1/2}(\text{h})$	1.47 ± 0.16	3.19 ± 1.56	1.25 ± 0.15

Incorporation by Reference

All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

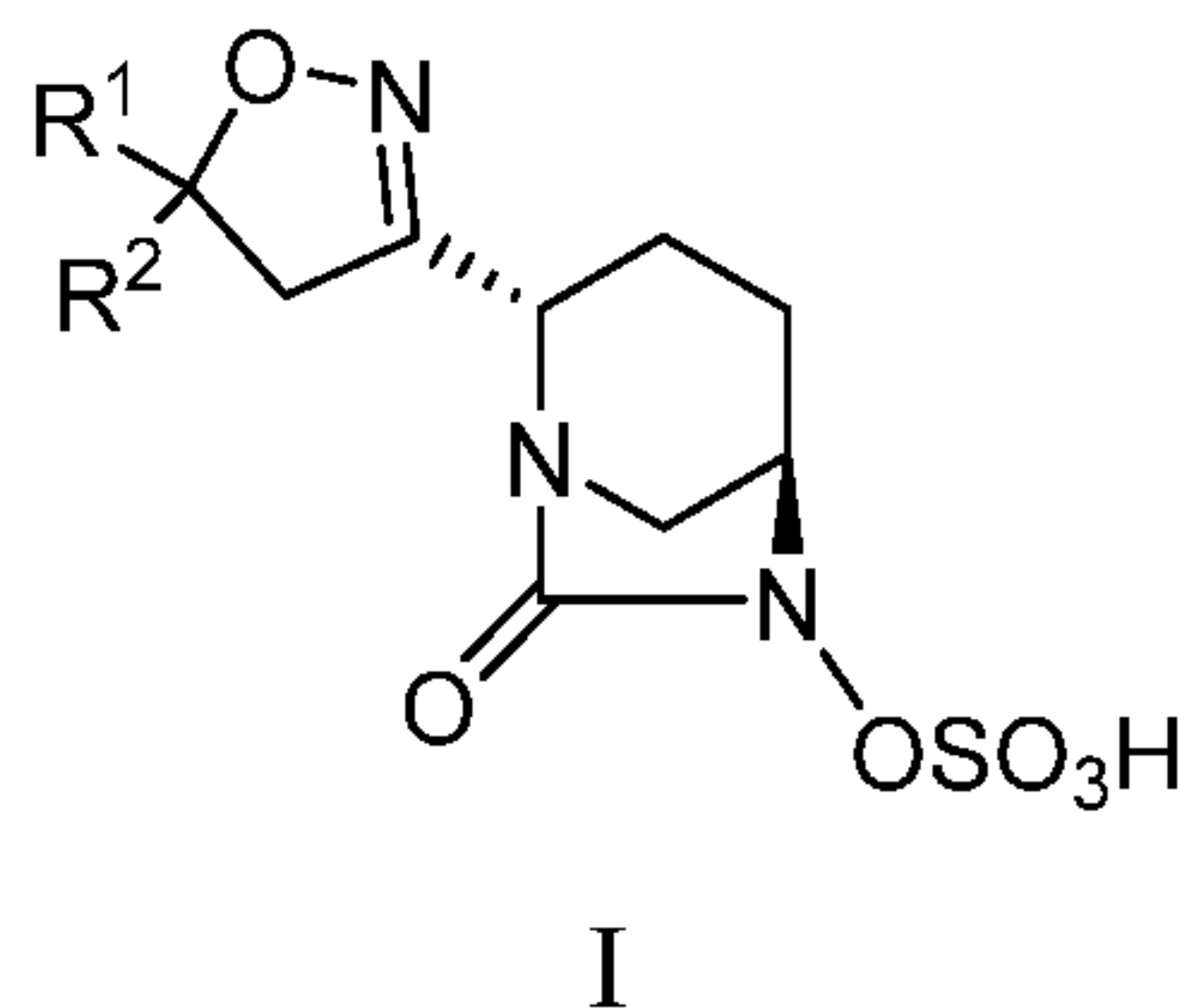
Equivalents

While specific embodiments of the subject invention have 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 and the claims below. 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.

Claims:

1. A compound having a structure of Formula I or a pharmaceutically acceptable salt thereof:



wherein

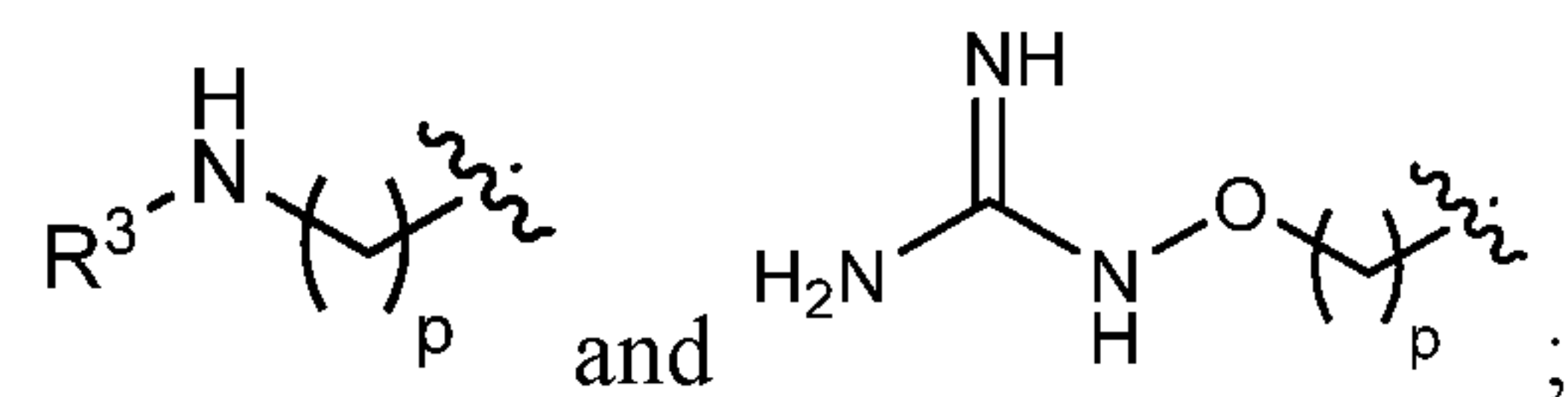
R^1 and R^2 are independently selected from H, hydroxyalkyl, $-C(O)-NH_2$, amido-, amino-, or guanidino-substituted alkyl, amido-, amino-, or guanidino-substituted alkoxyalkyl, and $-(CH_2)_p-O-NHR^3$, or

R^1 and R^2 combine to form an amino-, or guanidino-substituted cycloalkyl ring, or an optionally substituted nitrogen-containing heterocyclyl ring;

p is an integer from 1 to 6; and

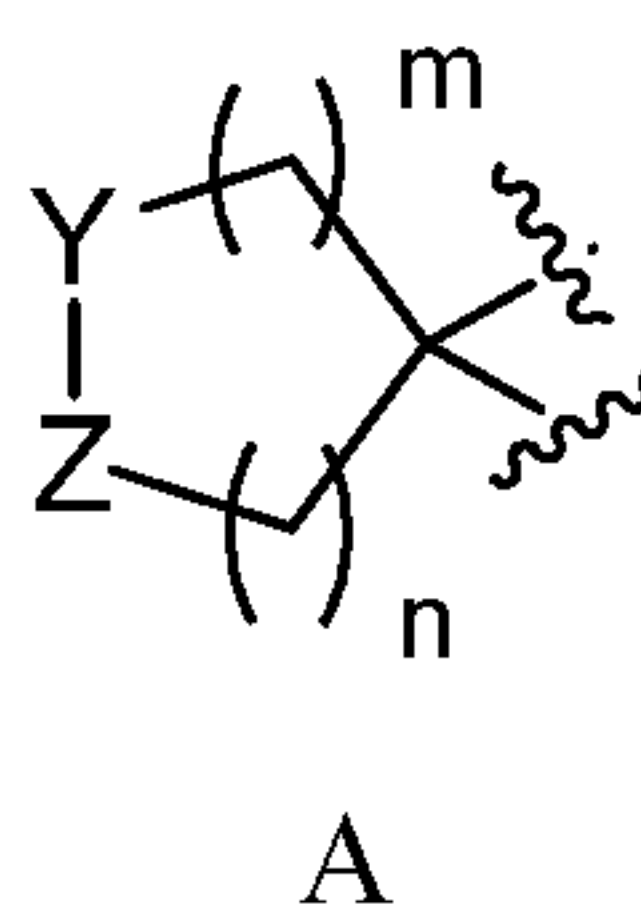
R^3 is, independently for each occurrence, selected from H, lower alkyl, and $-C(=NH)NH_2$.

2. The compound of claim 1, wherein at least one of R^1 and R^2 is independently selected from



wherein p is an integer from 1 to 5.

3. The compound of claim 1, wherein R^1 and R^2 combine to form a structure of Formula A



wherein

Y and Z are each independently CHR^4 , NR^5 , or absent;

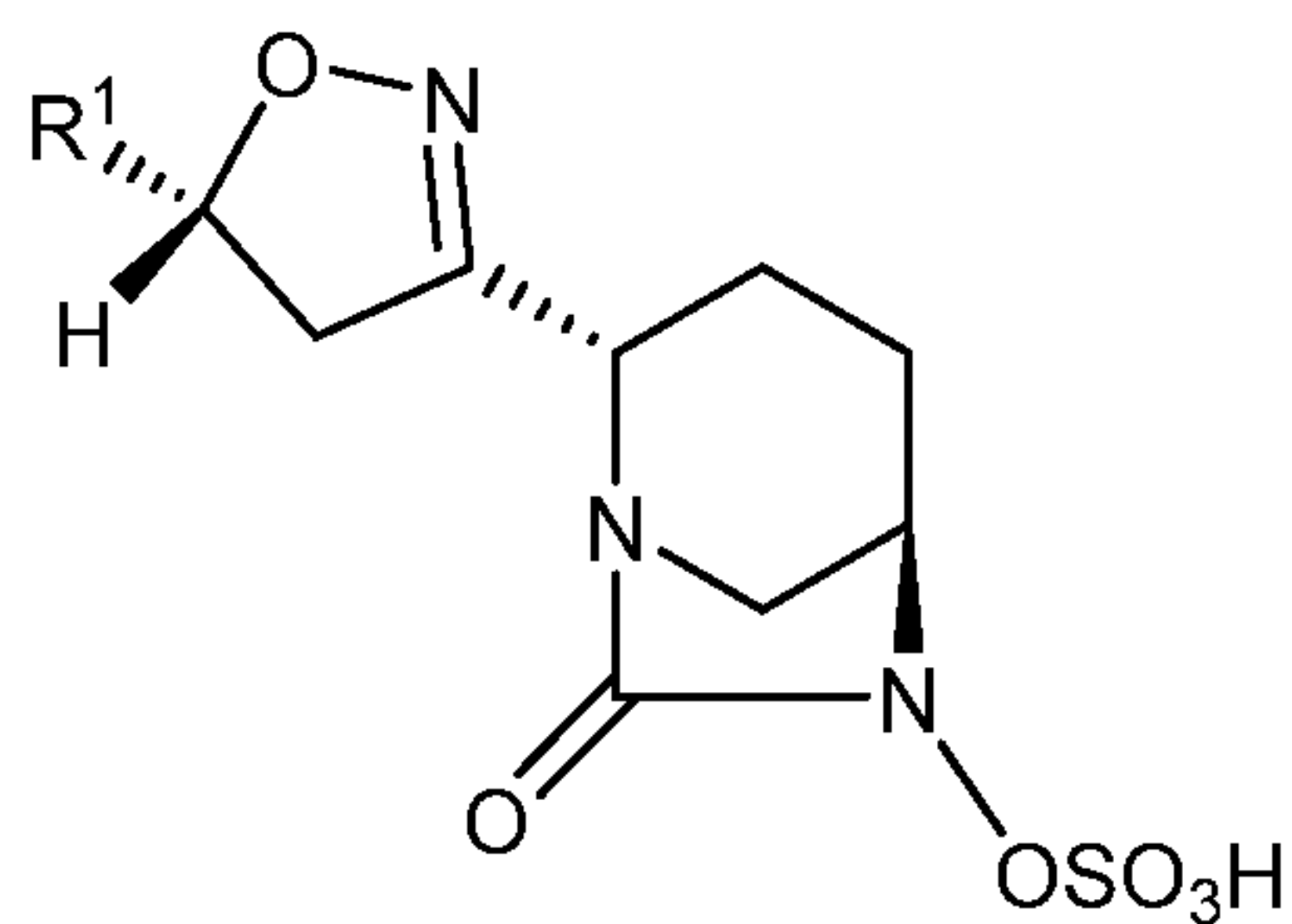
R^4 is, independently for each occurrence, selected from H, amido-, amino-, or guanidino-substituted lower alkyl, and NHR^3 ;

R^5 is, independently for each occurrence, selected from H, amido-, amino-, or guanidino-substituted lower alkyl and $-C(=NH)NH_2$; and

m and n are each independently an integer from 1 to 3;

provided that both Y and Z are not absent.

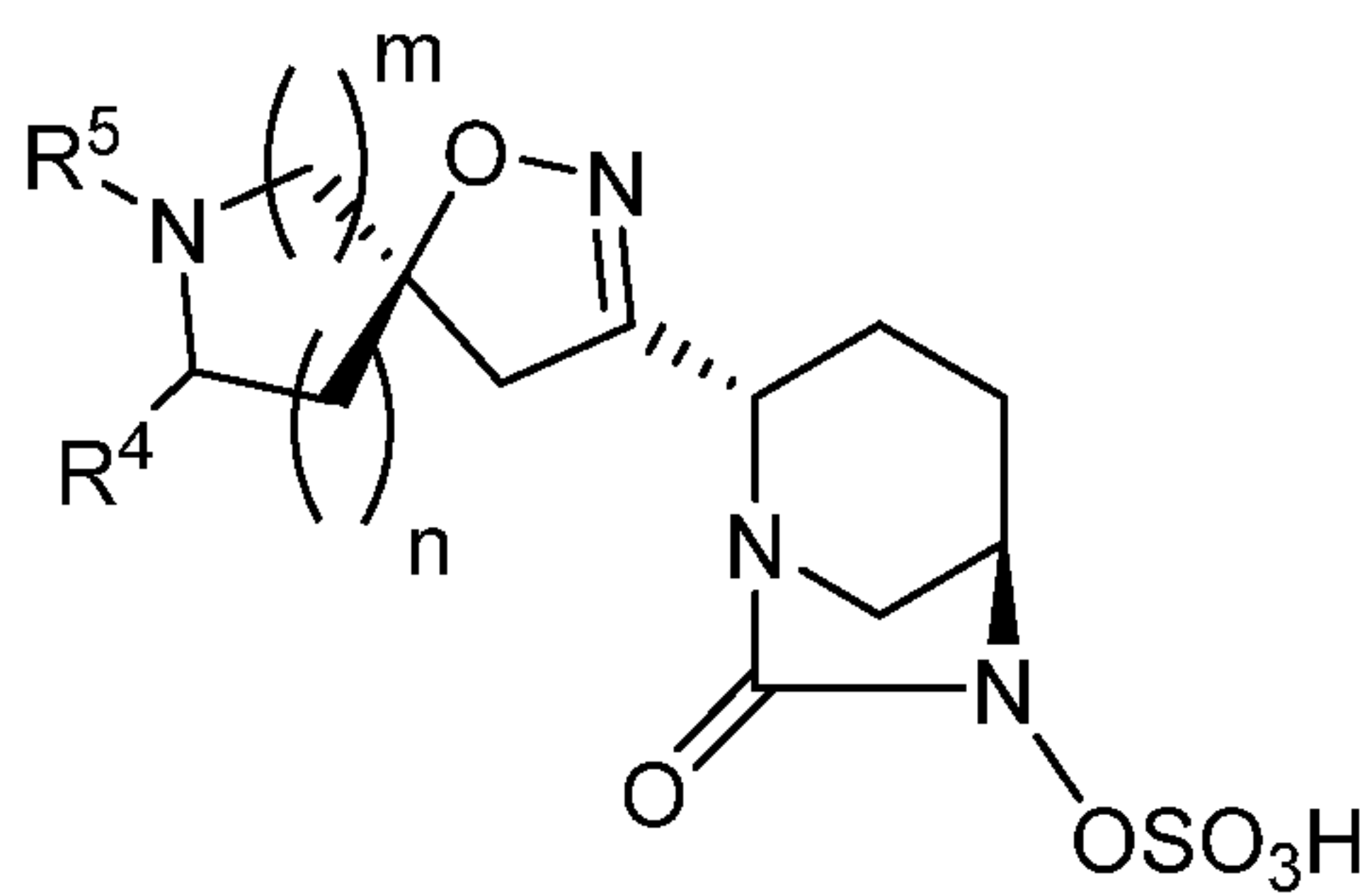
4. The compound of claim 1 or 2, wherein the compound has the structure of formula II or a pharmaceutically acceptable salt thereof:



II

and R^1 is an amido-, amino- or guanidino substituted alkyl, an amido-, amino-, guanidino-substituted alkoxyalkyl, or $-(CH_2)_p-O-NHR^3$.

5. The compound of claim 1 or 3, wherein the compound has the structure of formula III or a pharmaceutically acceptable salt thereof:



III

and m is less than or equal to n.

6. The compound of claim 1, wherein the compound is selected from:

[(2S,5R)-2-[5-(2-aminoethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate,

[(2S, 5R)-2-[5-(aminomethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate,

[(2S, 5R)-2-[5-(3-aminopropyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate,

[(2S,5R)-2-[5-(guanidinooxymethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate,

[(2S, 5R)-2-[5,5-bis(aminomethyl)-4H-isoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate,

[(2S, 5R)-2-(1-oxa-2,7,8-triazaspiro[4.4]non-2-en-3-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate,

[(2S, 5R)-2-(5-oxa-2,6-diazaspiro[3.4]oct-6-en-7-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate,

[(2S, 5R)-2-(2-amino-5-oxa-6-azaspiro[3.4]oct-6-en-7-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate,

[(2S, 5R)-2-(1-oxa-2,7-diazaspiro[4.4]non-2-en-3-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate,

[(2S, 5R)-2-(1-oxa-2,8-diazaspiro[4.5]dec-2-en-3-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate,

[(2S,5R)-2-(8-carbamimidoyl-1-oxa-2,8-diazaspiro[4.5]dec-2-en-3-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate,

[(2S,5R)-2-[7-(2-amino-2-oxo-ethyl)-1-oxa-2,7-diazaspiro[4.4]non-2-en-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate,

[(2S,5R)-2-[5-(aminomethyl)-5-(hydroxymethyl)-4H-isoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate,

[(2S,5R)-2-[5-(aminomethyl)-5-carbamoyl-4H-isoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate,

[(2S)-2-[(5R)-5-(aminomethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate,

[(2S)-2-[(5S)-5-(aminomethyl)-4,5-dihydroisoxazol-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate,

[(2S,5R)-2-[(5R)-1-oxa-2,7-diazaspiro[4.4]non-2-en-3-yl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate

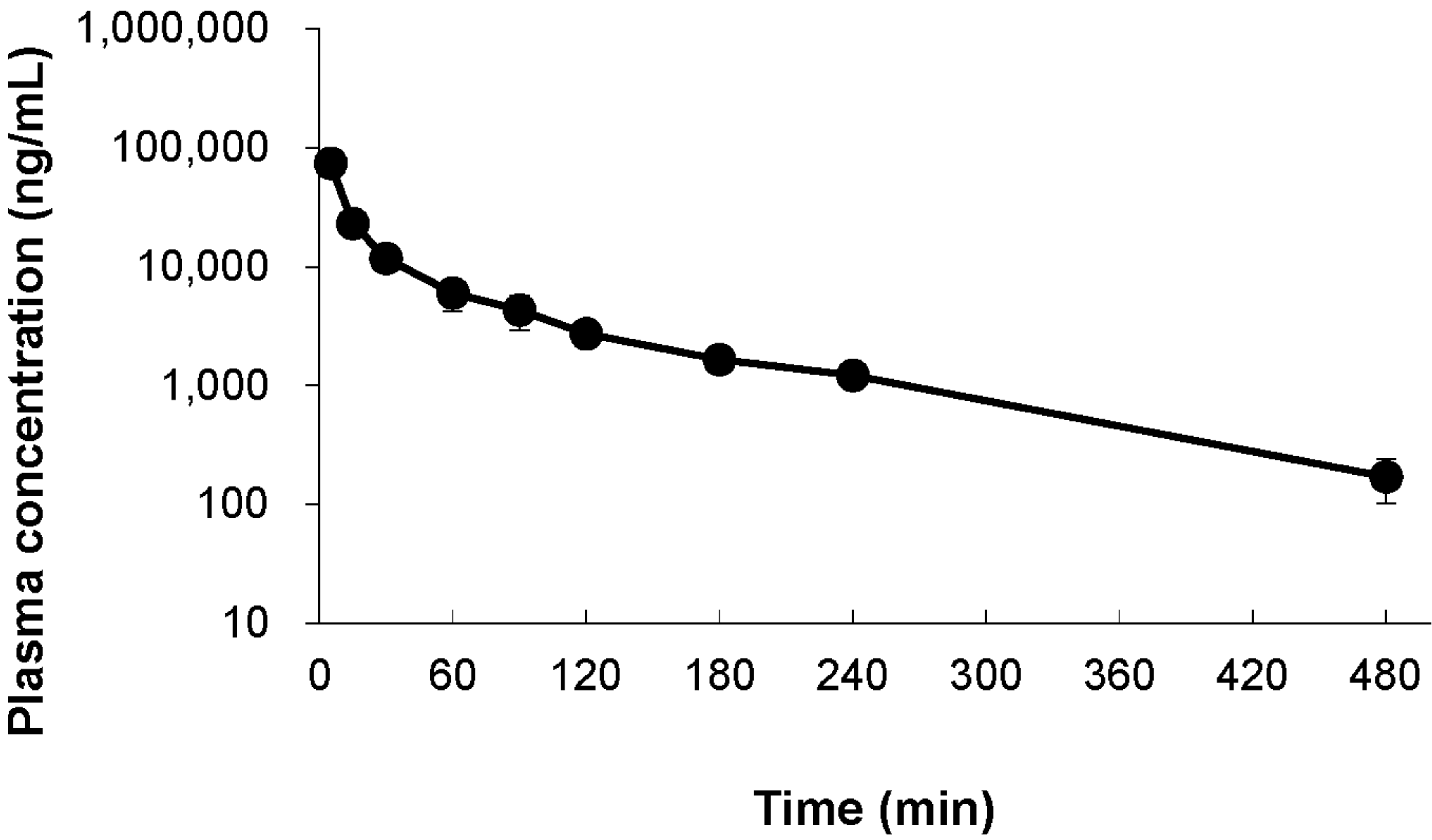
[(2S,5R)-2-[(5S)-1-oxa-2,7-diazaspiro[4.4]non-2-en-3-yl]-7-oxo-1,6-

diazabicyclo[3.2.1]octan-6-yl] hydrogen sulfate or a pharmaceutically acceptable salt thereof.

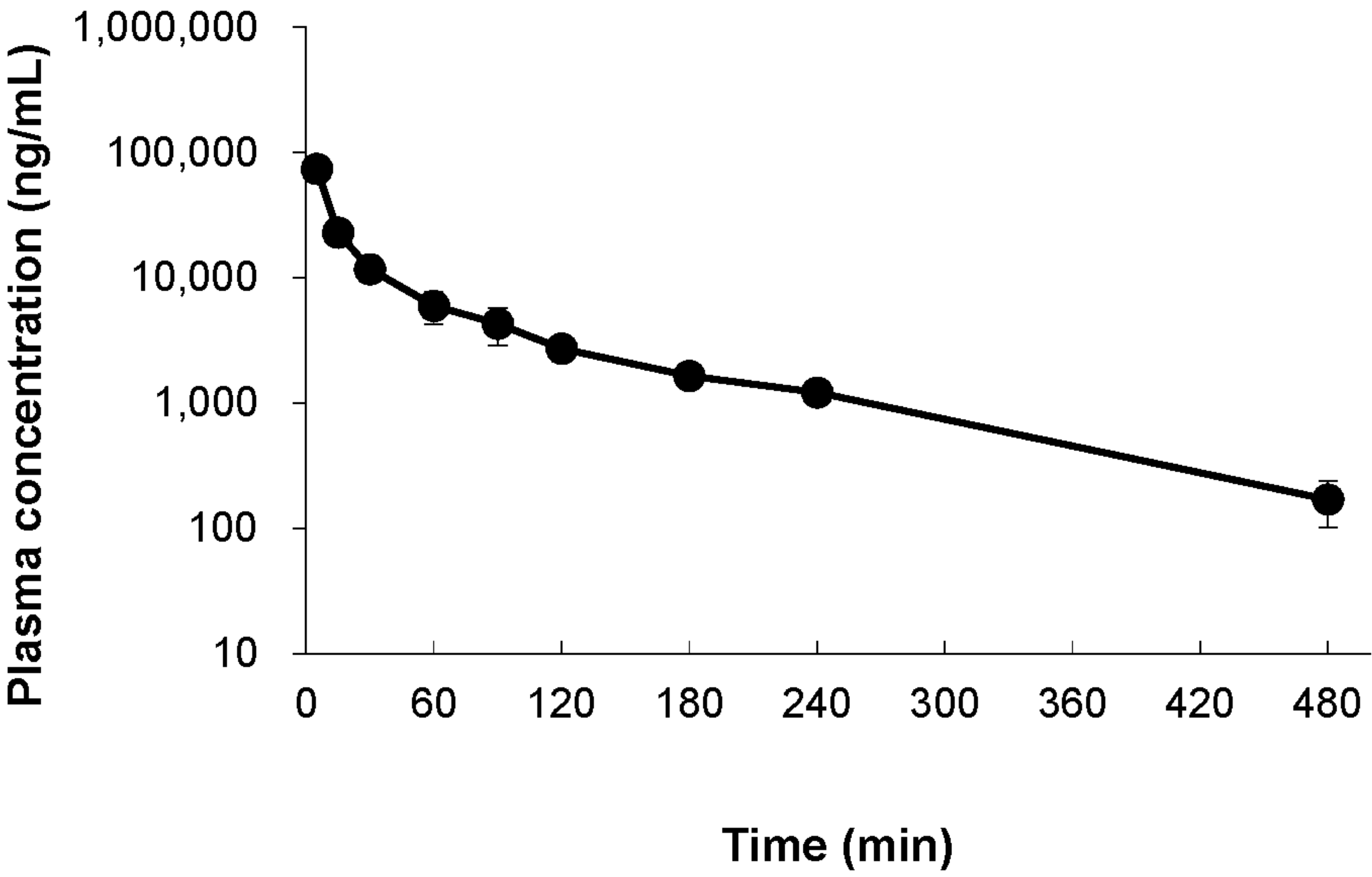
7. A method for inhibiting β -lactamase, the method comprising contacting the β -lactamase with a compound of any preceding claim.
8. A method for inhibiting growth of a bacterium, the method comprising contacting the bacterium with a β -lactam antibiotic and a compound of any one of claims 1-6.
9. A method for treating a bacterial infection, the method comprising administering a compound of any one of claims 1-6 to a subject in need of treatment.
10. The method of claim 9, further comprising administering a β -lactam antibiotic.

Figure 1

A



B



2/2

Figure 1 (cont.)

C

