BICYCLIC 6-ALKYLIDENE-PENEMS AS CLASS-D BETA-LACTAMASES INHIBITORS

This invention relates to certain bicyclic 6–alkylidene penems which act as a inhibitor of class-D enzymes. β–Lactamases hydrolyze β-lactam antibiotics, and as such serve as the primary cause of bacterial resistance. The compounds of the present invention when combined with β–lactam antibiotics will provide an effective treatment against life threatening bacterial infections.

BACKGROUND OF THE INVENTION

Class D β-lactamases are the smallest (27 kDa) amongst the active-site-serine β-lactamases. These enzymes lack overall amino acid sequence (<20% amino acid identity) with the more prevalent and better-understood β-lactamases of classes A and C (Naas, T. and Nordmann, P. *Curr. Pharm. Design*, **1999**, *5*,865). To date, almost 30 class D enzymes are known. Class D β-lactamases are also called oxacillinases because of their ability to hydrolyze oxacillin and cloxacillin two to four times faster than classical penicillins such as penicillin G (Ledent, P., Raquet,X, Joris, B. VanBeemen, J, Frere, J.M. *Biochem. J.***1993**,*292*,555). They are designated OXA-1, OXA-2, etc., and fall into at least five subgroups on the basis of phylogeny analysis (Barlow, M, Hall, B.G. *J. Mol. Evol.* **2002**, 55,314.).OXA-1 is the most common of the class D enzymes and is found in up to 10% of *Escherichia coli* isolates, in *Pseudomonas aeruginosa* and in epidemic strains of *salmonellae* (Medeiros, A.A. *Brit. Med. J.* **1984**,*40*,18. The genes for most of these enzymes are borne either as chromosomal or plasmid-mediated, which facilitate their dissemination among various organisms. The current knowledge about the catalytic mechanism of the class D β-lactamases is rather limited (Golemi,D, Maveyraud,L, Vakulenko,S, Tranier,S, Ishiwata, A, Kotra, L.P.,Samana, J-P., Mobashery, S. *J. Am. Chem. Soc.* **2000**,*122*, 6132).

Class D enzymes are dimeric, however, OXA-1 from *Escherichia coli* is found to be monomeric in solution and in the crystal, (Sun, T, Nukuga, M, Mayama, K, Braswell, E.H., Knox. J.R. *Protein Sci.*, **2003**, *12*,82.). As a result of point mutations and plasmid transfer, natural OXA variants (e.g. OXA-15, OXA-18, OXA-19) have arisen with an expanded substrate spectrum that includes imipenem and third-generation cephalosporins such as cefotaxime, ceftriaxone, and aztreonam while new variants such as OXA-11 and OXA-14 to OXA-20, show an extended-spectrum profile (ESBLs). These aspects make them important clinically (Buynak, J, *Curr. Med. Chem.*, **2004**, *11*, 1951).

Penicillins, cephalosporins, and carbapenems are the most frequently and widely used β–lactam antibiotics in the clinic. However, the development of resistance to β–lactam antibiotics by different pathogens has had a damaging effect on maintaining the effective treatment of bacterial infections. (Coleman, K. *Expert Opin. Invest. Drugs* **1995,** 4, 693; Sutherland, R. *Infection* **1995,** 23 191; Bush, K, *Cur. Pharm. Design* **1999,** 5, 839) The most significant known mechanism related to the development of bacterial resistance to the β–lactam antibiotics is the production of class-A, class-B, class-C and class-D β-lactamases. These enzymes degrade the β–lactam antibiotics, resulting in the loss of antibacterial activity. Class-A enzymes preferentially hydrolyze penicillins, class-B hydrolyze all B-lactams including carbapenems, class-C lactamases have a substrate profile favoring cephalosporin hydrolysis, whereas substrate preference for class D β-lactamases include oxacillin. (Bush, K.; Jacoby, G.A.; Medeiros, A.A. *Antimicrob. Agents Chemother.* **1995,** 39, 1211). To date over 250 different β-lactamases have been reported (Payne, D.J,: Du, W and Bateson, J.H. *Exp. Opin. Invest. Drugs* **2000,** 247.) and there is a need for a new generation of broad spectrum β-lactamase inhibitors. Bacterial resistance to these antibiotics could be greatly reduced by administering the β–lactam antibiotic in combination with a compound which inhibits these enzymes.

The commercially available β–lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam are all effective against class-A producing pathogens. Clavulanic acid is clinically used in combination with amoxicillin and ticarcillin;

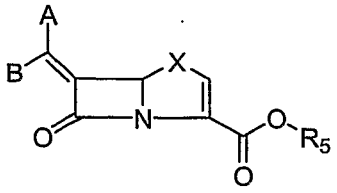
similarly sulbactam with ampicillin and tazobactam with piperacillin. However, these compounds are ineffective against class C producing organisms. The mechanism of inactivation of class-A β–lactamases (such as PCI and TEM-1) has been elucidated. (Bush, K.; *Antimicrob. Agents Chemother.* **1993**, 37, 851; Yang, Y.; Janota, K.; Tabei, K.; Huang, N.; Seigal, M.M.; Lin, Y.I.; Rasmussen, B.A. and Shlaes, D.M. *J. Biol. Chem.* **2000**, 35, 26674-26682). To date there are no reported inhibitors of class D enzymes in clinical use.

Recently certain 6-methylidene penems bearing a bicyclic heterocycle as class-A, class-B and class-C β-lactamse inhibitors have been disclosed in US 2004-0077622 A1, which is hereby incorporated by reference thereto. In addition a number of 6-methylidene penems bearing a tricyclic heterocycle as class-A, class-B, and class-C β-lactamase inhibitors have been disclosed in US 2004-00043978A1.

DETAILED DESCRIPTION OF THE INVENTION

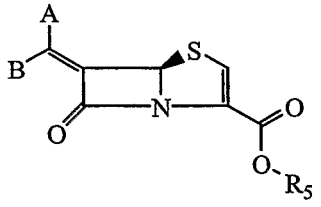
The present invention relates to novel, low molecular weight broad spectrum β–lactam compounds, and in particular to a class of bicyclic heteroaryl substituted 6-alkylidene penems which have β–lactamase inhibitory activity that when used in combination with a β-lactam antibiotic enhance the activity against class-D producing organisms and thereby enhance the antibacterial properties. The compounds are therefore useful in the treatment of antibacterial infections in humans or animals, either alone or in combination with other antibiotics. The compounds may be prepared in accordance with US 2004-0077622A1 which is hereby incorporated by reference thereto.

In accordance with the present invention there are provided compounds of general formula **I** or a pharmaceutically acceptable salt or in vivo hydrolyzable ester R_5 thereof:



I

and preferred compounds of the formula:



wherein:

One of A and B denotes hydrogen and the other an optionally substituted fused bicyclic heteroaryl group. The expression 'fused bicyclic heteroaryl group' is used in the specification and claims to mean:

A group comprising two fused rings in which one has aromatic character [i.e. Huckel's rule (4n+2)] and the other ring is non-aromatic;

The fused bicyclic heteroaryl group contains one to six heteroatoms selected from the group O, S, N and N-R_1;

The fused bicyclic heteroaryl group is bonded to the remainder of the molecule through a carbon atom in the aromatic ring as shown in the formula I;

The aromatic ring of the fused bicyclic heteroaryl group contains five or six ring atoms (including bridgehead atoms) selected from CR_2 , N, O, S or N-R_1. The aromatic ring of the fused bicyclic heteroaryl group contains 0 to 3 heteroatoms selected from the group O, S, N and N-R_1;

The non-aromatic ring of the fused bicyclic heteroaryl group contains five to eight ring atoms (including bridgehead atoms) selected from CR_4R_4, N, N-R_1, O, $S(O)_n$ where n= 0-2. The non-aromatic ring of the fused bicyclic heteroaryl group contains 0 to 4 heteroatoms selected from N, N-R_1, O or $S(O)_n$ where n = 0 to 2 .

X is O or S, preferably S;

R_5 is H, an in vivo hydrolyzable ester such as C1 –C6 alkyl, C5 – C6 cycloalkyl, CHR_3OCOC1-C6 or salts such as Na, K, Ca; preferably R_5 is H or a salt;

R_1 is H, optionally substituted -C1-C6 alkyl, optionally substituted -aryl, optionally substituted -heteroaryl or mono or bicyclic saturated heterocycles, optionally substituted –C3-C7 cycloalkyl, optionally substituted -C3-C6 alkenyl, optionally substituted -C3-C6 alkynyl with the proviso that both the double bond and the triple bond should not be present at the carbon atom which is directly linked to N; optionally substituted -C1-C6 per fluoro alkyl, -$S(O)_p$ optionally substituted alkyl or aryl where p is 2, optionally substituted -C=Oheteroaryl, optionally substituted -C=Oaryl, optionally substituted -C=O (C1-C6) alkyl, optionally substituted -C=O (C3-C6) cycloalkyl, optionally substituted -C=O mono or bicyclic saturated heterocycles, optionally substituted C1-C6 alkyl aryl, optionally substituted C1-C6 alkyl heteroaryl, optionally substituted aryl-C1-C6 alkyl, optionally substituted heteroaryl-C1-C6 alkyl, optionally substituted C1-C6 alkyl mono or bicyclic saturated heterocycles, optionally substituted arylalkenyl of 8 to 16 carbon atoms, -$CONR_6R_7$, -$SO_2NR_6R_7$, optionally substituted arylalkyloxyalkyl, optionally substituted -alkyl-O-alkyl-aryl, optionally substituted -alkyl-O-alkyl-heteroaryl, optionally substituted aryloxyalkyl, optionally substituted heteroaryloxyalkyl, optionally substituted aryloxyaryl, optionally substituted aryloxyheteroaryl, optionally substituted C1-C6alkyl aryloxyaryl, optionally substituted C1-C6 alkyl aryloxyheteroaryl, optionally substituted alkyl aryloxy alkylamines, optionally substituted alkoxy carbonyl, optionally substituted aryloxy carbonyl, optionally substituted heteroaryloxy carbonyl. Preferred R_1 groups are H, optionally substituted alkyl, optionally substituted aryl, -C=O(C1-C6)alkyl, C3-C6alkenyl, C3-C6alkynyl, optionally substituted cycloalkyl, SO_2alkyl, SO_2aryl, optionally substituted heterocycles, -$CONR_6R_7$, and optionally substituted heteroaryl.

R_2 is hydrogen, optionally substituted C1-C6 alkyl, optionally substituted C2-C6 alkenyl having 1 to 2 double bonds, optionally substituted C2-C6 alkynyl having 1 to 2 triple bonds, halogen, cyano, N-R_6R_7, optionally substituted C1-C6 alkoxy, hydroxy; optionally substituted aryl, optionally substituted heteroaryl, $COOR_6$, optionally substituted alkyl aryloxy alkylamines, optionally substituted aryloxy, optionally substituted heteroaryloxy, optionally substituted C3-C6 alkenyloxy, optionally substituted C3 –C6 alkynyloxy, C1-C6 alkylamino-C1-C6 alkoxy, alkylene dioxy, optionally substituted aryloxy-C1-C6 alkyl amine, C1-C6 perfluoro alkyl, $S(O)_q$- optionally substituted C1-C6 akyl, $S(O)_q$- optionally substituted aryl where q is 0, 1 or

2, $CONR_6R_7$, guanidino or cyclic guanidino, optionally substituted C1-C6 alkylaryl, optionally substituted arylalkyl, optionally substituted C1-C6 alkylheteroaryl, optionally substituted heteroaryl-C1-C6 alkyl, optionally substituted C1-C6 alkyl mono or bicyclic saturated heterocycles, optionally substituted arylalkenyl of 8 to 16 carbon atoms, $SO_2NR_6R_7$, optionally substituted arylalkyloxyalkyl, optionally substituted aryloxyalkyl, optionally substituted heteroaryloxyalkyl, optionally substituted aryloxyaryl, optionally substituted aryloxyheteroaryl, optionally substituted heteroaryloxyaryl, optionally substituted C1-C6alkyl aryloxyaryl, optionally substituted C1-C6 alkylaryloxyheteroaryl , optionally substituted aryloxyalkyl, optionally substituted heteroaryloxyalkyl, optionally substituted alkylaryloxyalkylamines. Preferred R_2 groups are H, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted heteroaryl, halogen, CN, hydroxy, optionally substituted heterocycle, $-CONR_6R_7$, $COOR_6$, optionally substituted aryl, $S(O)_q$-alkyl, and $S(O)_q$-aryl.

R_3 is hydrogen, C1-C6 alkyl, C5 – C6 cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl; preferred R_3 groups are H or C1-C6 alkyl;

R_4 is H, optionally substituted C1-C6 alkyl, one of R_4 is OH, C1-C6 alkoxy, -S-C1-C6 alkyl, $COOR_6$, $-NR_6R_7$, $-CONR_6R_7$; or R_4R_4 may together be =O or R_4R_4 together with the carbon to which they are attached may form a spiro system of five to eight members with or without the presence of heteroatoms selected N, O, S=(O)n (where n =0 to 2), N-R_1; preferred R_4 groups are H, C1-C6 alkyl, NR_6R_7 or R_4R_4 together with the carbon to which they are attached may form a spiro system of five to eight members with or without the presence of heteroatoms, eg one or two of oxygen, nitrogen and sulfur;

R_6 and R_7 are independently H, optionally substituted C1-C6 alkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted C1-C6 alkyl aryl, optionally substituted arylalkyl, optionally substituted heteroarylalkyl, optionally substituted C1-C6 alkyl heteroaryl, R_6 and R_7 can be together to form a 3-7 membered saturated ring system optionally having one or two heteroatoms such as N-R_1, O, $S=(O)_n$ n = 0-2. Preferred R_6 and R_7 groups are H, C1-C6 alkyl, arylalkyl, heteroarylalkyl or R_6 and R_7 together forming a 3-7 membered saturated ring system optionally having one or two heteroatoms.

Chemical Definitions

The term alkyl means both straight and branched chain alkyl moieties of 1-12 carbons, preferably of 1-6 carbon atoms.

The term alkenyl means both straight and branched alkenyl moieties of 2-8 carbon atoms containing at least one double bond, and no triple bond, preferably the alkenyl moiety has 1 or two double bonds. Such alkenyl moieties may exist in the E or Z conformations; the compounds of this invention include both conformations. In the case of alkenyl, heteroatoms such as O, S or N-R_1 should not be present on the carbon that is bonded to a double bond;

The term alkynyl includes both straight chain and branched alkynyl moieties containing 2-6 carbon atoms containing at least one triple bond, preferably the alkynyl moiety has one or two triple bonds. In the case of alkynyl, hetero atoms such as O, S or N-R_1 should not be present on the carbon that is bonded to a double or triple bond;

The term cycloalkyl refers to a alicyclic hydrocarbon group having 3-7 carbon atoms.

The term perfluoroalkyl is used herein to refer to both straight- and branched-chain saturated aliphatic hydrocarbon groups having at least one carbon atom and two or more fluorine atoms. Examples include CF_3, CH_2CF_3, CF_2CF_3 and $CH(CF_3)_2$.

The term halogen is defined as Cl, Br, F, and I.

If alkyl, alkenyl, alkynyl, or cycloalkyl is "optionally substituted", one or two of the following are possible substituents: nitro, -aryl, -heteroaryl, alkoxycarbonyl-, -alkoxy, -alkoxy-alkyl, alkyl-O-C2-C4alkyl-O-, -cyano, -halogen, -hydroxy, -N-R_6R_7, -COOH, -COO-alkyl, -trifluoromethyl, -trifluoromethoxy, arylalkyl, alkylaryl, R_6R_7N-alkyl-, HO-C1-C6-alkyl-, alkoxyalkyl-, alkyl-S-, -SO_2N-R_6R_7, -SO_2NHR_6, -CO_2H, $CONR_6R_7$, aryl-O-, heteroaryl-O-, -$S(O)_s$-aryl (where s = 0 –2), -alkyl-O-alkyl-NR_6R_7, -alkyl-aryl-O-alkylN-R_6R_7, C1-C6alkyl, alkenyl, alkynyl, cycloalkyl, alkoxy-alkyl-O-, R_6R_7N-alkyl-, and -$S(O)_s$-heteroaryl (where s = 0 –2); Preferred

substitutents for alkyl, alkenyl, alkynyl, and cycloalkyl include: halogen, nitro, aryl, heteroaryl, -COOH, -COO-alkyl, alkoxycarbonyl-, alkoxy, -alkoxy-alkyl, -cyano, hydroxy, and -N-R_6R_7.

Aryl is defined as an aromatic hydrocarbon moiety selected from the group: phenyl, α-naphthyl, β-naphthyl, biphenyl, anthryl, tetrahydronaphthyl, fluorenyl, indanyl, biphenylenyl, acenaphthenyl, groups. The preferred aryl groups are phenyl and biphenyl.

Heteroaryl is defined as a aromatic heterocyclic ring system (monocyclic or bicyclic) where the heteroaryl moieties are selected from: (1) furan, thiophene, indole, azaindole, oxazole, thiazole, isoxazole, isothiazole, imidazole, N-methylimidazole, pyridine, pyrimidine, pyrazine, pyrrole, N-methylpyrrole, pyrazole, N-methylpyrazole, 1,3,4-oxadiazole, 1,2,4-triazole, 1-methyl-1,2,4-triazole, 1H-tetrazole, 1-methyltetrazole, benzoxazole, benzothiazole, benzofuran, benzisoxazole, benzimidazole, N-methylbenzimidazole, azabenzimidazole, indazole, quinazoline, quinoline, and isoquinoline; (2) a bicyclic aromatic heterocycle where a phenyl, pyridine, pyrimidine or pyridizine ring is: (a) fused to a 6-membered aromatic (unsaturated) heterocyclic ring having one nitrogen atom; (b) fused to a 5 or 6-membered aromatic (unsaturated) heterocyclic ring having two nitrogen atoms; (c) fused to a 5-membered aromatic (unsaturated) heterocyclic ring having one nitrogen atom together with either one oxygen or one sulfur atom; or (d) fused to a 5-membered aromatic (unsaturated) heterocyclic ring having one heteroatom selected from O, N or S. Preferred heteroaryl groups are furan, oxazole, thiazole, isoxazole, isothiazole, imidazole, N-methylimidazole, pyridine, pyrimidine, pyrazine, pyrrole, N-methylpyrrole, pyrazole, N-methylpyrazole, 1,3,4-oxadiazole, 1,2,4-triazole, 1-methyl-1,2,4-triazole, 1H-tetrazole, 1-methyltetrazole, quinoline, isoquinoline, and naphthyridine.

If aryl or heteroaryl is 'optionally substituted', one or two of the following are possible substituents: nitro, -aryl, -heteroaryl, alkoxycarbonyl-, -alkoxy, -alkoxy-alkyl, alkyl-O-C2-C4alkyl-O-, -cyano, -halogen, -hydroxy, -N-R_6R_7, -trifluoromethyl, -trifluoromethoxy, arylalkyl, alkylaryl, R_6R_7N-alkyl-, HO-C1-C6-alkyl-, alkoxyalkyl-,

alkyl-S-, $-SO_2N-R_6R_7$, $-SO_2NHR_6$, $-CO_2H$, $CONR_6R_7$, aryl-O-, heteroaryl-O-, $-S(O)_s$-aryl (where s = 0 –2), -alkyl-O-alkyl-NR_6R_7, -alkyl-aryl-O-alkyl$N-R_6R_7$, C1-C6alkyl, alkenyl, alkynyl, cycloalkyl, alkoxy-alkyl-O-, R_6R_7N-alkyl-, and $-S(O)_s$-heteroaryl (where s = 0 –2); Preferred substituents for aryl and heteroaryl include: alkyl, halogen, $-N-R_6R_7$, trifluoromethyl, -trifluoromethoxy, arylalkyl, and alkylaryl.

Arylalkyl is defined as Aryl-C1-C6alkyl---; Arylalkyl moieties include benzyl, 1-phenylethyl, 2-phenylethyl, 3-phenylpropyl, 2-phenylpropyl and the like. The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents on the alkyl or aryl moiety as defined above.

Alkylaryl is defined as C1-C6alkyl-aryl-. The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents on the aryl or alkyl moiety as defined above.

Heteroaryl-C1-C6- alkyl is defined as a heteroaryl substituted alkyl moiety wherein the alkyl chain is 1-6 carbon atoms (straight or branched). Alkyl heteroaryl moieties include Heteroaryl-$(CH_2)_{1-6}$-- and the like. The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents on the alkyl or heteroaryl moiety as defined above;

C1-C6 alkylheteroaryl is defined an alkyl chain of 1-6 carbon atoms (straight or branched) attached to a heteroaryl moiety, which is bonded to the rest of the molecule. Ex. C1-C6-alkyl-Heteroaryl--. The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents on the alkyl or heteroaryl moiety as defined above;

Saturated or partially saturated heterocycles groups are defined as heterocyclic rings selected from the moieties; aziridinyl, azetidinyl, 1,4-dioxanyl, hexahydroazepinyl, piperazinyl, piperidinyl, pyrrolidinyl, morpholinyl, thiomorpholinyl, dihydrobenzimidazolyl, dihydrobenzofuranyl, dihydrobenzothienyl, dihydrobenzoxazolyl, dihydrofuranyl, dihydroimidazolyl, dihydroindolyl, dihydroisooxazolyl, dihydroisothiazolyl, dihydrooxadiazolyl, dihydrooxazolyl,

dihydropyrrazinyl, dihydropyrazolyl, dihydropyridinyl, dihydropyrimidinyl, dihydropyrrolyl, dihydroquinolinyl, dihydrotetrazolyl, dihydrothiadiazolyl, dihydrothiazolyl, dihydrothienyl, dihydrotriazolyl, dihydroazetidinyl, dihydro-1,4-dioxanyl, tetrahydrofuranyl, tetrahydrothienyl, tetrahydroquinolinyl, and tetrahydroisoquinolinyl. Preferred saturated or partially saturated heterocycles are aziridinyl, azetidinyl, 1,4-dioxanyl, hexahydroazepinyl, piperazinyl, piperidinyl, pyrrolidinyl, morpholinyl, thiomorpholinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, dihydroimidazolyl, and dihydroisooxazolyl.

C1-C6 alkyl mono or bicyclic saturated or partially saturated heterocycles is defined as an alkyl group (straight or branched) of C1-C6 attached to a heterocycles (which is defined before) through a carbon atom or a nitrogen atom and the other end of the alkyl chain attached to the rest of the molecule. The terms 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present on the alkyl or heterocyclic portion of the molecule, as defined before;

Arylalkyloxyalkyl is defined as Aryl-C1-C6alkyl-O-C1-C6alkyl---.The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present on the alkyl and/or aryl portions as defined before;

Alkyloxyalkyl is defined as C1-C6 alkyl-O-C1-C6alkyl---. The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the alkyl moiety as defined before;

Aryloxyalkyl is defined as Aryl-O-C1-C6 alkyl---. The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the alkyl or aryl moiety as defined before;

Heteroarylalkyloxyalkyl is defined as Heteroaryl-C1-C6alkyl-O-C1-C6alkyl---.The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present on the alkyl or heteroaryl moiety as defined before;

Aryloxyaryl is defined as Aryl-O-Aryl---.. The term 'optionally substituted' refers to unsubstituted or substituted with1 or 2 substituents present on the aryl moiety as defined before;

Aryloxyheteroaryl is defined as Aryl-O-Heteroaryl- or –Aryl-O-Heteroaryl; In this definition either the aryl moiety or the heteroaryl moiety can be attached to the remaining portion of the molecule; The term 'optionally substituted' refers to unsubstituted or substituted with1 or 2 substituents present on the aryl moiety or on the heteroaryl moiety as defined before;

Alkyl aryloxyaryl is defined as Aryl-O-Aryl-C1-C6alkyl----; The term 'optionally substituted' refers to unsubstituted or substituted with1 or 2 substituents present at the aryl moiety as defined before;

Alkylaryloxyheteroaryl is defined as Heteroaryl-O-Aryl-C1-C6alkyl--; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present on the aryl moiety or on the hetroaryl moiety as defined before;

Alkylaryloxyalkylamine is defined as R_6R_7N-C1-C6alkyl-O-Aryl-C1C6alkyl---; The terms 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present on the alkyl or aryl moiety as defined before; R_6 and R_7 as defined before;

Alkoxycarbonyl is defined as C1-C6alkyl-O-C=O--; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present on the alkyl portion of the alkoxy moiety as defined before;

Aryloxycarbonyl is defined as Aryl-O-C=O--; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the aryl moiety as defined before;

Heteroaryloxy carbonyl is defined as Heteroaryl-O-C=O--; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the heteroaryl moiety as defined before;

Alkoxy is defined as C1-C6alkyl-O--; The terms 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the alkyl moiety as defined before;

Aryloxy is defined as Aryl-O--; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the aryl moiety as defined before;

Heteroaryloxy is defined as Heteroaryl-O--; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the heteroaryl moiety as defined before;

Alkenyloxy is defined as C3-C6 alkene-O--; Example allyl-O--, bute-2-ene-O like moieties; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the alkene moiety as defined before, with the proviso that no hetero atom such as O, S or N-R_1 is present on the carbon atom, which is attached to a double bond;

Alkynyloxy is defined as C3-C6alkyne-O--; Example CH triple bond C-CH_2-O- , like moieties; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the alkyne moiety as defined before, with the proviso that no hetero atom such as O, S or N-R_1 is present on a carbon atom which is attached to a double or triple bond;

Alkylaminoalkoxy is defined as R_6R_7N-C1-C6-alkyl-O-C1-C6-alkyl---, where the terminal alkyl group attached to the oxygen is connected to the rest of the molecule; The terms R_6 and R_7 are defined above; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the alkyl moiety as defined before;

Alkylenedioxy is defined as –O-CH_2-O- or –O—$(CH_2)_2$---O---;

Aryloxyalkylamine is defined as R_6R_7N-C1-C6-alkyl-O-Aryl--, where the aryl is attached to the rest of the molecule; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the alkyl or aryl moiety as defined before;

Arylalkenyl is defined as Aryl-C2-C8alkene--, with the proviso that no hetero atom such as O, S or N-R_1 is present on the carbon atom, which is attached to a double bond; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present on the alkene or aryl moiety as defined before;

Heteroaryloxyalkyl is defined as Heteroaryl-O-C1-C6alkyl---; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the heteroaryl moiety as defined before;

Heteroaryloxyaryl is defined as Heteroaryl-O-aryl---, where the aryl moiety is attached to the rest of the molecule; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the heteroaryl moiety or the aryl moiety as defined before;

Alkoxy, alkoxyalkyl, alkoxyalkyloxy and alkylthioalkyloxy are moieties wherein the alkyl chain is 1-6 carbon atoms (straight or branched). Aryloxy, heteroaryloxy, arylthio and heteroarylthio are moieties wherein the aryl and heteroaryl groups are as herein before defined. Arylalkyloxy, heteroarylalkyloxy, arylalkylthio and heteroarylalkylthio are moieties wherein the aryl and heteroaryl groups are as herein before defined and wherein the alkyl chain is 1-6 carbons (straight or branched). Aryloxyalkyl, heteroaryloxyalkyl, aryloxyalkyloxy and heteroaryloxyalkyloxy are substituents wherein the alkyl radical is 1-6 carbon atoms. The terms monoalkylamino and dialkylamino refer to moieties with one or two alkyl groups wherein the alkyl chain is 1-6 carbons and the groups may be the same or different. The terms monoalkylaminoalkyl and dialkylaminoalkyl refer to monoalkylamino and dialkylamino moieties with one or two alkyl groups (the same or different) bonded to the nitrogen atom which is attached to an alkyl group of 1-3 carbon atoms.

Examples of fused bicyclic heteroaryl groups are optionally substituted ring systems such as one of the following:

4,5,6,7-tetrahydrothieno[3,2-c]pyridine, optionally substituted by e.g., arylalkyl such as benzyl; by alkoxyarylalkyl such as 4-methoxybenzyl; by C1-C6alkyl such as methyl; by heteroarylalkyl such as pyridin-3-ylmethyl; by arylalkylCO- such as phenylacetyl; or heteroarylCO- such as pyridin-3-ylcarbonyl; e.g. by alkylCO- such as acetyl;

5,6,7,8-tetrahydroimidazo[1,2-a]pyrazine, optionally substituted e.g., by C1-C6alkyl such as methyl;

5,6-dihydro-8*H*-imidazo[2,1-c][1,4]thiazine;

6,7-Dihydro-5*H*-pyrrolo[1,2-a]imidazole

5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]oxazine

5,6-Dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole

4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridine

6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazine, optionally substituted e.g., by C1-C6alkyl such as methyl;

6,7-Dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazine;

4*H*-5-thia-1,6a-diazapentalene;

7*H*-Imidazo[1,2-c]thiazole;

4-oxo-6,7-dihydro-4H-pyrazolo[5,1-c][1,4]oxazine;

6,7-Dihydro-4H-thieno[3,2-c]pyran;

6,7-Dihydro-4H-thieno[3,2-c]thiopyran;

6,7-dihydro-4H-thieno[3,2-c]pyridine, optionally substituted by C2-C7alkoxycarbonyl;

6,7,8,9-tetrahydro-5H-imidazo[1,2-a]azepine;

5,6,7,8-tetrahydroimidazo[1,2-a]pyrazine, optionally substituted by arylalkyl such as benzyl;

5,5-Dioxo-4,5,6,7-tetrahydro-5λ^6-pyrazolo[5,1-*c*][1,4]thiazine;

4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine;

5,6-Dihydro-4*H*-cyclopenta[*b*]furan;

4,5-Dihydro-6-thia-1,7a-diazaindene;

5,6-Dihydro-8-*H*-imidazo[2,1-c][1,4]thiazine;

4*H*-5-thia-1,6a-diazapentalene;

2,3-Dihydropyrazolo[5,1-*b*]thiazole;

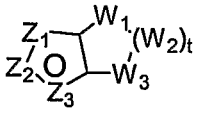
2,3-Dihydropyrazolo[5,1-*b*]oxazole;

6,7-dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazine;

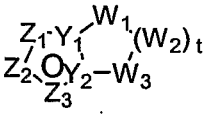
6,7-5H-Dihydropyrazolo[5,1-*b*]oxazine; and

4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine optionally substituted, .e.g, by alkoxyalkylCO- such as 2-methoxyacetyl; or by alkyloxyalkylCO- such as methoxyacetyl.

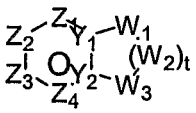
Examples of bicyclic heteroarylgroup:



1-A



1-B



1-C

In formula **1-A** Z1, Z2 and Z3 are independently CR_2, N, O, S or N-R_1 and one of Z1 –Z3 is carbon and is bonded to the remainder of the molecule as shown in formula **I**. When one of Z's is CR_2 the other two Zs can be either two N or one N and O, S, N-R_1 in any combinations with out disrupting the aromaticity; when two Z,s = CR_2 the other Z can be optionally selected from one N, O, S or N-R_1 in any combination with out disrupting the aromaticity;

W_1, W_2 and W_3 are independently CR_4R_4, S, SO, SO_2, O, N-R_1, C=O; with the proviso that no S-S or O-O or S-O bond formation can occur to form the saturated ring system; t= 1 to 4.

In formula **1-B** Z1, Z2 and Z3 are independently CR_2, N, O, S or N-R_1 and one of Z1 –Z3 is carbon and is bonded to the remainder of the molecule as shown in formula **I**.

When one of Z's = CR_2 , then the other two Z's can be independently CR_2, N, O, S or N-R_1 in any combinations with out disrupting the aromaticity;

When two Z's =N, then the other carbon in the ring is bonded to the penem portion of the molecule as shown in formula **I**.

W_1, W_2 and W_3 are independently CR_4R_4, S, SO, SO_2, O, N-R_1,

t= 1 to 4;

Y_1 and Y_2 = N or C; with the proviso that when the aromatic heterocycle is imidazole, the saturated ring may not contain a S adjacent to the bridgehead carbon.

In formula **1-C** Z1, Z2, Z3 and Z4 are independently CR_2 or N and one of Z1 –Z4 is carbon and is bonded to the remainder of the molecule.

W_1, W_2 and W_3 are independently CR_4R_4, S, SO, SO_2, O, or N-R_1; with the proviso that no S-S or O-O or S-O bond formation can occur to form the saturated ring system; t= 1 to 4.

Y_1 and Y_2 are independently C or N.

The more preferred embodiment of the formula **1-A**:

1. t= 1 to 3.
2. In formula **1-A** Z1 is N, S, N-R_1 or O and one of Z2 or Z3 is CR_2 and the other of Z2 or Z3 is carbon and is bonded to the remainder of the molecule as shown in formula **I**.
3. In formula **1-A** Z3 is N, S, N-R_1 or O and one of Z2 or Z1 is CR_2 and the other of Z2 or Z1 is carbon and is bonded to the remainder of the molecule as shown in formula **I**.
4. In formula **1-A** Z2 is N, S, N-R_1 or O and one of Z1 or Z3 is CR_2 and the other of Z1 or Z3 is carbon bonded to the remainder of the molecule as shown in formula **I**.
5. In formula **1-A** Z1 is N, N-R_1, O or S and Z2 is N, O or S and Z3 is a carbon bonded to the penem portion of the molecule as shown in formula **I**.
6. In formula **1-A** Z3 is N, N-R_1, O or S and Z2 is N, O or S and Z1 is a carbon bonded to the penem portion of the molecule as shown in formula **I**.
7. In formula **1-A** Z1 is N, N-R_1, O or S and Z3 is N, O or S and Z2 is a carbon bonded to the penem portion of the molecule as shown in formula **I**.
8. In formula **1-A** Z1 is N, S, N-R_1 or O and Z2 or Z3 is CR_2 and the other of Z2 or Z3 is carbon and is bonded to the remainder of the molecule; W_1, W_2 and W_3 are independently CR_4R_4.

9. In formula **1-A** Z3 is N, S, N-R_1 or O and one of Z2 or Z1 is CR_2 and the other of Z2 or Z1 is carbon and is bonded to the remainder of the molecule; W_1, W_2, and W_3 are independently CR_4R_4.
10. In formula **1-A** Z2 is N, S, N-R_1 or O and one of Z1 or Z3 is CR_2 and the other of Z1 or Z3 is carbon and is bonded to the remainder of the molecule; W_1, W_2, and W_3 are independently CR_4R_4.
11. In formula **1-A** Z1 is N, N-R_1, O or S and Z2 is N, O or S; Z3 is a carbon bonded to the penem portion of the molecule; W_1, W_2, W_3 are independently CR_4R_4.

12. In formula **1-A** Z3 is N, N-R_1, O or S; Z2 is N, O or S; Z1 is a carbon bonded to the penem portion of the molecule; W_1, W_2, W_3 are independently CR_4R_4.

13. In formula **1-A** Z1 is N, N-R_1, O or S ; Z3 is N, O or S; Z2 is a carbon bonded to the penem portion of the molecule; W_1, W_2, W_3 are independently CR4R4.

14. In formula **1-A** Z3 is N, N-R_1, O or S; Z1 is N, O or S; Z2 is a carbon bonded to the penem portion of the molecule; W_1, W_2, W_3 are independently CR4R4.

15. In formula **1-A** Z1 is N, S, N-R_1 or O; one of Z2 or Z3 is CR_2 and the other of Z2 or Z3 is carbon and is bonded to the remainder of the molecule, t = 1 – 3; one W_2 is N-R_1, O or $S(O)_n$ n = 0-2 and another W_2 is CR4R4.

16. In formula **1-A** Z3 is N, S, N-R_1 or O; one of Z2 or Z1 is CR_2 and the other of Z2 or Z1 is carbon and is bonded to the remainder of the molecule, t = 1 – 3; one W_2 is N-R_1, O or $S(O)_n$ n = 0-2 and another W_2 =CR4R4.

17. In formula **1-A** Z2 is N, S, N-R_1 or O; one of Z1 or Z3 is CR_2 and the other of Z1 or Z3 is carbon and is bonded to the remainder of the molecule; t = 1 – 3; one W_2 is N-R_1, O or $S(O)_n$ n = 0-2 and another W_2 is CR4R4.

18. In formula **1-A** when Z1 = N, N-R_1, O or S and Z2 = N, O or S and Z3 = a carbon bonded to the penem portion of the molecule where t = 1 - 3 then one W_2 = N-R_1, O or $S(O)_n$ n = 0-2 and other W_2 = CR4R4.

19. In formula **1-A** Z3 = N, N-R_1, O or S and Z2 = N, O or S and Z1 = a carbon bonded to the penem portion of the molecule where t = 1 - 3 then one W_2 = N-R_1, O or $S(O)_n$ n = 0-2 and other W_2 = CR4R4.

20. In formula **1-A** when Z1 = N, N-R_1, O or S and Z3 = N, O or S and Z2 = a carbon bonded to the penem portion of the molecule where t = 1 - 3 then one W_2 = N-R_1, O or $S(O)_n$ n = 0-2 and other W_2 = CR4R4.

21. In formula **1-A** Z1 =N, S, N-R_1 or O and Z2 or Z3 = CR_2 and the other of Z2 or Z3 is carbon and is bonded to the remainder of the molecule; then W_1 and W_3 = CH_2 or both hydrogens on the methylene linkage can be substituted to form a spiro system with or without the presence of hetero atoms selected from O, $S=(O)_n$ (n= 0 to 2), N-R_1 to form five to eight membered cyclic system; t = 1 – 3; one W_2 = N-R_1, O or $S(O)_n$ n = 0-2 and other W_2 = CR4R4.

22. In formula **1-A** Z3 =N, S, N-R_1 or O and Z2 or Z1 = CR_2 and the other of Z2 or Z1 is carbon and is bonded to the remainder of the molecule; then W_1 and W_3 = CR4R4; where t = 1 - 3 then one W_2 = N-R_1, O or $S(O)_n$ n = 0-2 and other W_2 = CR4R4.

23. In formula **1-A** Z2 =N, S, N-R_1 or O and Z1 or Z3 = CR_2 and the other of Z1 or Z3 is carbon and is bonded to the remainder of the molecule; then W_1 and W_3 = CR4R4, where t = 1 - 3 then one W_2 = N-R_1, O or $S(O)_n$ n = 0-2 and other W_2 = CR4R4.

24. In formula **1-A** when Z1 = N, N-R_1, O or S and Z2 = N, O or S then Z3 = a carbon bonded to the penem portion of the molecule; then W_1 and W_3 = CR4R4, where t = 1 - 3 then one W_2 = N-R_1, O or $S(O)_n$ n = 0-2 and other W_2 = CR4R4.

25. In formula **1-A** Z3 = N, N-R_1, O or S and Z2 = N, O or S then Z1 = a carbon bonded to the penem portion of the molecule; then W_1 and W_3 = CR4R4, where t = 1 - 3 then one W_2 = N-R_1, O or $S(O)_n$ n = 0-2 and other W_2 = CR4R4.

26. In formula **1-A** when Z1 = N, N-R_1, O or S and Z3 = N, O or S then Z2 = a carbon bonded to the penem portion of the molecule; then W_1 and W_3 =CR4R4 ; t = 1 – 3; one W_2 is N-R_1, O or $S(O)_n$ n = 0-2 and another W_2 is CR4R4.

27. In formula **1-A** Z3 is N, N-R_1, O or S; Z1 is N, O or S; Z2 is a carbon bonded to the remainder of the molecule; W_1 and W_3 are independently CR4R4 ; t = 1 – 3; one W_2 is N-R_1, O or $S(O)_n$ n = 0-2 and another W_2 is CR4R4.

The more preferred embodiments of the formula **1-B**:

28. In formula **1-B** t=3.

29. In formula **1-B** Z1 and Z3 are N; Y1 is N ; Y2 is C and Z2 is carbon and is bonded to the remainder of the molecule as shown in formula **I**.

30. In formula **1-B** Z2 and Z3 are N; Y1 is N; Y2 is C and Z1 is carbon and is bonded to the remainder of the molecule as shown in formula **I**.

31. In formula **1-B** Z1 is N, Y1 is N, Y2 is C, one of Z_2 or Z_3 is CR_2 and the other of Z2 or Z3 is a carbon and is bonded to the remainder of the molecule as shown in formula **I**.

32. In formula **1-B** Z1 is N, Y1 is C, Y2 is N, one of Z_2 or Z_3 is CR_2 and the other of Z2 or Z3 is a carbon and is bonded to the remainder of the molecule as shown in formula **I**.

33. In formula **1-B** Z1 is N, Y1 is N, Y2 is C, one of Z_2 or Z_3 is CR_2 and the other of Z2 or Z3 is a carbon and is bonded to the remainder of the molecule as shown in formula **I**, W_1 and W_3 are independently CR4R4; t = 1-3; one W_2 is N-R_1, O, $S=(O)_n$ (n = 0-2), and another W_2 is CR4R4.

34. In formula **1-B** Z1 is N, Y1 is C, Y2 is N, one of Z_2 or Z_3 is CR_2 and the other of Z2 or Z3 is a carbon and is bonded to the remainder of the molecule as shown in formula **I**; W_1 and W_3 are independently CR4R4; t = 1-3; one W_2 is N-R_1, O, $S=(O)_n$ (n = 0-2), and another W_2 is CR4R4.

35. In formula **1-B** Z3 is N; Y1 is N; Y2 is C; one of Z1 or Z2 is CR_2 and the other of Z_1 or Z_2 is carbon and is bonded to the remainder of the molecule as shown in formula **I**.

36. In formula **1-B** Z2 is N; Y1 is N; Y2 is C; one of Z1 or Z3 is CR_2 and the other of Z_1 or Z_3 is carbon and is bonded to the remainder of the molecule as shown in formula **I**.

37. In formula **1-B** Z1 and Z2 are N; Y1 is N; Y2 is C; and Z3 is carbon and is bonded to the remainder of the molecule as shown in formula **I**.

38. In formula **1-B** Z1, Z2 and Z3 are independently CR_2; Y1 is C; Y2 is N; except one of Z1-Z3 is carbon and is bonded to the remainder of the molecule as shown in formula **I**.

39. In formula **1-B** Z1 and Z3 are N; Y1 is N; Y2 is C; Z2 is carbon and is bonded to the remainder of the molecule as shown in formula **I**; and t = 1-3.

40. In formula **1-B** Z2 and Z3 are N ; Y1 is N; Y2 is C; and Z1 is carbon and is bonded to the remainder of the molecule; and t = 1-3;

41. In formula **1-B** Z2 and Z3 are N , Y1 is C and Y2 =N and Z1 is carbon and is bonded to the remainder of the molecule and t = 1-3;

42. In formula **1-B** Z2 and Z3 are N , Y1 is N; Y2 is C; Z1 is carbon and is bonded to the remainder of the molecule; W_1 and W_3 are independently CH_2 or both hydrogens on the methylene linkage can be substituted to form a spiro system with or without the presence of hetero atoms selected from O, $S(O)_n$ n= 0-2, $N\text{-}R_1$ to form five to eight membered cyclic system; t = 1-3 and W_2 is CH_2, $N\text{-}R_1$, O, $S(O)_n$ where n = 0 -2.

43. In formula **1-B** Z3 is N; Y1 is N; Y2 is C; Z1 is CR_2 and Z2 is the carbon atom bonded to the remainder of the molecule.

44. In formula **1-B** Z3 is N; Y1 is N; Y2 is C; Z1 is CR_2 ; Z2 is the carbon atom bonded to the remainder of the molecule; W_1, W_2 and W_3 are independently CR4R4; t = 1 to 3.

45. In formula **1-B** Z3 is N; Y1 is N; Y2 is C; Z1 is CR_2; Z2 is the carbon atom bonded to the remainder of the molecule; W_1 and W_3 are independently CR4R4; and one of W_2 is $N\text{-}R_1$, O or $S(O)_n$, and another W_2 is CR4R4; t = 1-3.

46. In formula **1-B** Z3 is N; Y1 is N; Y2 is C; Z1 is CR_2 ; Z2 is the carbon atom bonded to the remainder of the molecule; W_1 and W_2 are independently CR4R4; W_3 is $N\text{-}R_1$, O or $S(O)_n$; and t = 2.

47. In formula **1-B** Z3 is N; Y1 is N; Y2 is C; Z1 is CR_2 ; Z2 is the carbon atom bonded to the remainder of the molecule; W_1 and W_3 are independently CR4R4; W_2 is N-R_1, O or $S(O)_n$; and t = 1.

48. In formula **1-B** Z2 is N; Y1 is N; Y2 is C; Z3 is CR_2; Z1 is the carbon bonded to the remainder of the molecule; W_1 and W_2 is CH_2 or both hydrogens on the methylene linkage can be substituted to form a spiro system with or without the presence of hetero atoms selected from O, $S(O)_n$ n= 0-2, N-R_1 to form five to eight membered cyclic system; W_3 is N-R_1, O or $S(O)_n$; and t = 3.

49. In formula **1-B** Z2 is N; Y1 is N; Y2 is C, Z3 is CR_2 ; Z1 is the carbon bonded to the remainder of the molecule; W_1 and W_3 are independently CH_2 or both hydrogens on the methylene linkage can be substituted to form a spiro system with or without the presence of hetero atoms selected from O, $S(O)_n$ n= 0-2, N-R_1 to form five to eight membered cyclic system; and one W_2 is N-R_1, O or $S(O)_n$ and another W_2 is CR4R4; and t=2.

50. In formula **1-B** Z2 is N; Y1 is N; Y2 is C; Z3 is CR_2 ; Z1 is the carbon bonded to the remainder of the molecule; W_1 and W_3 are independently CH_2 or both hydrogens on the methylene linkage can be substituted to form a spiro system with or without the presence of hetero atoms selected from O, $S(O)_n$ n= 0-2, N-R_1 to form five to eight membered cyclic system; W_2 is N-R_1, O or $S(O)_n$; and t = 1.

51. In formula **1-B** Z2 is N; Y1 is N; Y2 is C; Z1 is CR_2 ; Z3 is the carbon bonded to the remainder of the molecule; W_1 and W_3 are independently CR4R4; one of W_2 is N-R_1, O or $S(O)_n$ and another W_2 is CR4R4; and t = 3.

52. In formula **1-B** Z2 is N; Y1 is N; Y2 is C; Z1 is CR_2 ; Z3 is the carbon bonded to the remainder of the molecule; W_1 and W_3 are independently CR4R4; one W_2 is N-R_1, O or $S(O)_n$, and another W_2 is CR4R4; and t = 2.

53. In formula **1-B** Z2 is N; Y1 is N; Y2 is C; Z1 is CR_2 ; Z3 is the carbon bonded to the remainder of the molecule; W_1 and W_3 are independently CR4R4; W_2 is N-R_1, O or $S(O)_n$; and t = 1.

54. In formula **1-B** Z1 and Z2 are N; Y1 is N; Y2 is C; Z3 is carbon and is bonded to the remainder of the molecule; W_1 and W_3 are independently CR4R4 ; one of W_2 is N-R_1, O or $S(O)_n$ and another W_2 is CR4R4; and t = 3.

55. In formula **1-B** Z1 and Z2 are N; Y1 is N; Y2 is C; Z3 is carbon and is bonded to the remainder of the molecule; W_1 and W_3 are independently CR4R4; one of W_2 is N-R_1, O or $S(O)_n$ and another W_2 is CR4R4; and t = 2.

56. In formula **1-B** Z1 and Z2 are N; Y1 is N; Y2 is C; Z3 is carbon and is bonded to the remainder of the molecule; W_1 and W_3 are independently CR4R4; W_2 is N-R_1, O or $S(O)_n$; and t = 1

57. In formula **1-B** Z1 and Z2 are independently CR_2 ; Y1 is C; Y2 is N; Z3 is carbon and is bonded to the remainder of the molecule; W_1 and W_3 are independently CR4R4; one of W_2 is N-R_1, O or $S(O)_n$; another W_2 is CR4R4; and t = 3 .

58. In formula **1-B** Z1 and Z2 are independently CR_2; Y1 is C and Y2 is N and Z3 is carbon and is bonded to the remainder of the molecule; W_1 and W_3 are independently CR4R4; and one W_2 is N-R_1, O or $S(O)_n$ and the other W_2 is CR4R4; and t = 2.

59. In formula **1-B** Z1 and Z2 are independently CR_2; Y1 is C; Y2 is N; Z3 is carbon and is bonded to the remainder of the molecule; W_1 and W_3 are independently CR4R4; W_2 is N-R_1, O or $S(O)_n$; and t = 1.

60. In formula **1-B** Z1 and Z3 are independently CR_2; Y1 is C; Y2 is N; Z2 is carbon and is bonded to the remainder of the molecule; W_1 and W_3 are independently CR4R4; one W_2 is N-R_1, O or $S(O)_n$; another W_2 is CR4R4; and t = 3.

61. In formula **1-B** Z1 and Z3 are independently CR_2; Y1 is C; Y2 is N; Z2 is carbon and is bonded to the remainder of the molecule; W_1 and W_3 are independently CR4R4; and one W_2 is N-R_1, O or $S(O)_n$ and the other W_2 is CR4R4; and t = 2.

62. In formula **1-B** Z1 and Z3 are independently CR_2 ; Y1 is C; Y2 is N; Z2 is carbon and is bonded to the remainder of the molecule; W_1 and W_3 are independently CR4R4; W_2 is N-R_1, O or $S(O)_n$; and t = 1.

63. In formula **1-B** Z3 and Z2 are independently CR_2 ; Y1 is C; Y2 is N; Z1 is carbon and is bonded to the remainder of the molecule; W_1 and W_2 are independently CR4R4; one W_2 is N-R_1, O or $S(O)_n$; another W_2 is CR4R4; and t = 3.

64. In formula **1-B** Z3 and Z2 are independently CR_2 ; Y1 is C; Y2 is N; Z1 is carbon and is bonded to the remainder of the molecule; W_1 and W_3 are independently CR4R4; one W_2 is N-R_1, O or $S(O)_n$; the other W_2 is CR4R4; and t = 2.

65. In formula **1-B** Z3 and Z2 are independently CR_2 ; Y1 is C; Y2 is N; Z1 is carbon and is bonded to the remainder of the molecule; W_1 and W_3 are independently CR4R4; W_2 is N-R_1, O or $S(O)_n$; and t = 1.

66. In formula **1-B** Z3 is N; Y1 is N; Y2 is C; one of Z1 and Z2 is CR_2 and the other is C; W_1 is CR4R4; W_2 is CR4R4; W_3 is CH_2, N-R_1 or O; and t= 1.

67. In formula **1-B** Z3 is N; Y1 is N; Y2 is C; one of Z1 and Z2 is CR_2 and the other is C; W_1 is CR4R4 ; W_2 is C=O; W_3 is N-R_1; and t= 1.

68. In formula **1-B** Z3 is N; Y1 is N; Y2 is C; one of Z1 and Z2 is CR_2 and the other is C; W_1 is N-R_1; W_2 is C=O; W_3 is CR4R4; and t= 1.

69. In formula **1-B** Z3 is N; Y1 is N; Y2 is C; one of Z1 and Z2 is CR_2 and the other is C; W_1 is C=O; W_2 is N-R_1 ; W_3 is CH_2; and t= 1.

The more preferred embodiments of the formula **1-C** are:

70. In formula **1-C** Z1, Z2, Z3 and Z4 are independently CR_2 ;one of Z1-Z4 is carbon and is bonded to the remainder of the molecule; Y_1 and Y_2 are C; t = 1 to 3; and W_1, W_2 and W_3 are independently CR4R4, S, SO, SO_2, O, or N-R_1.

71. In formula **1-C** Z1, Z2, Z3 and Z4 are independently CR_2 and one of Z1-Z4 is carbon and is bonded to the remainder of the molecule; Y_1 and Y_2 = C or N; t = 1 to 3; W_1, W_2 and W_3 are independently CR4R4, S, SO, SO_2, O, or N-R_1.

72. In formula **1-C** Z1, Z2, Z3 and Z4 are independently CR_2; Y_1 and Y_2 are N; t = 1 to 3; W_1, W_2 and W_3 are independently CR4R4, S, SO, SO_2, O, or N-R_1.

73. In formula **1-C** Z1 is N and Z2, Z3 and Z4 are independently CR_2 ; Y_1 and Y_2 are C; t = 1 to 3; W_1, W_2 and W_3 are independently CR4R4, S, SO, SO_2, O, or N-R_1.

74. In formula **1-C** Z1 is N and Z2, Z3 and Z4 are independently CR_2 ; one of Z1-Z4 is carbon and is bonded to the remainder of the molecule; Y_1 is C; Y_2 is N; t = 1 to 3; and W_1, W_2 and W_3 are independently CR4R4, S, SO, SO_2, O, or N-R_1.

75. In formula **1-C** Z2 = N and Z1, Z3 and Z4 are independently CR_2 ; one of Z1-Z4 is carbon and is bonded to the remainder of the molecule; Y_1 and Y_2 are C; t = 1 to 3; and W_1, W_2 and W_3 are independently CR4R4, S, SO, SO_2, O, or N-R_1.

76. In formula **1-C** Z2 is N and Z1, Z3 and Z4 are independently CR_2 ; one of Z1-Z4 is carbon and is bonded to the remainder of the molecule; Y_1 is C; Y_2 is N; t = 1 to 3; and W_1, W_2 and W_3 are independently CR4R4, S, SO, SO2, O, or N-R_1.

77. In formula **1-C** Z3 is N; Z1, Z2 and Z4 are independently CR_2 ; one of Z1-Z4 is carbon and is bonded to the remainder of the molecule; Y_1 and Y_2 are C; t = 1 to 3; and W_1, W_2 and W_3 are independently CR4R4, S, SO, SO2, O, or N-R_1.
78. In formula **1-C** Z3 is N and Z1, Z2 and Z4 are independently CR_2 ; one of Z1-Z4 is carbon and is bonded to the remainder of the molecule; Y_1 is C and Y_2 is N; t = 1 to 3; and W_1, W_2 and W_3 are independently CR4R4, S, SO, SO2, O, or N-R_1.
79. In formula **1-C** Z4 is N and Z1, Z2 and Z3 are independently CR_2 ; one of Z1-Z4 is carbon and is bonded to the remainder of the molecule; Y_1 and Y_2 are C; t = 1 to 3; and W_1, W_2 and W_3 are independently CR4R4, S, SO, SO2, O, or N-R_1.
80. In formula **1-C** Z4 is N and Z1, Z2 and Z3 are independently CR_2; one of Z1-Z4 is carbon and is bonded to the remainder of the molecule; Y_1 is N; Y_2 is C; t = 1 to 3; and W_1, W_2 and W_3 are independently CR4R4, S, SO, SO2, O, or N-R_1.
81. In formula **1-C** Z1 is N and Z2, Z3 and Z4 are independently CR_2; one of Z1-Z4 is carbon and is bonded to the remainder of the molecule; Y_1 and Y_2 are C; t = 1 to 3; and W_1, W_2 and W_3 are independently CR4R4, S, SO, SO2, O, or N-R_1.
82. In formula **1-C** Z1 and Z2 are N and Z3 or Z4 are independently CR_2 ; one of Z1-Z4 is carbon and is bonded to the remainder of the molecule; Y_1 is C; Y_2 is N; t = 1 to 3; and W_1, W_2 and W_3 are independently CR4R4, S, SO, SO2, O, or N-R_1.
83. In formula **1-C** Z1 and Z3 are N and Z2 or Z4 are independently CR_2 ; one of Z1-Z4 is carbon and is bonded to the remainder of the molecule; Y_1 is C; Y_2 is N; t = 1 to 3; and W_1, W_2 and W_3 are independently CR4R4, S, SO, SO2, O, or N-R_1.
84. In formula **1-C** Z1 and Z4 are N and Z2 or Z3 are independently CR_2; one of Z1-Z4 is carbon and is bonded to the remainder of the molecule; Y_1 is N; Y_2 is C; t = 1 to 3; and W_1, W_2 and W_3 are independently CR4R4, S, SO, SO2, O, or N-R_1.
85. In formula **1-C** Z1, Z2, Z3 are N and Z4 is carbon and is bonded to the remainder of the molecule; Y_1 is C; Y_2 is N; t = 1 to 3; and W_1, W_2 and W_3 are independently CR4R4, S, SO, SO2, O, or N-R_1.
86. In formula **1-C** Z1, Z3 and Z4 are N and Z2 is carbon and is bonded to the remainder of the molecule; Y_1 and Y_2 are C; t = 1 to 3; and W_1, W_2 and W_3 are independently CR4R4, S, SO, SO2, O, or N-R_1.

87. In formula **1-C** Z1, Z2 and Z4 are N and Z3 is carbon and is bonded to the remainder of the molecule; Y_1 and Y_2 are C and t = 1 to 3; and W_1, W_2 and W_3 are independently CR4R4, S, SO, SO2, O, or N-R_1.

88. In formula **1-C** Z2, Z3, Z4 are N and Z1 is carbon and is bonded to the remainder of the molecule; Y_1 and Y_2 are C and t = 1 to 3; and W_1, W_2 and W_3 are independently CR4R4, S, SO, SO2, O, or N-R_1.

The compounds according to the present invention have β-lactamase inhibitory and antibacterial properties and are useful for the treatment of infections in humans and animals. It should be noted that the compounds of the present invention, when used in combination with β-lactam antibiotics will result in the increased antibacterial activity (synergistic effect) against class-D producing organisms. β-Lactam antibiotics include penicillin antibiotics such as piperacillin, amoxycillin, ticarcillin, benzylpenicillins, ampicillin, sulbenicillin, other known penicillins, cephalosporins such as cefatrizine, cephaloridine, cephalothin, cefazolin, cephalexin, cephradine, other known cephalosporins, aztreonam and latamoxef (Moxalactam), and carbapenems such as meropenem and imipenem. Most preferably compounds of this present invention are used with piperacillin or amoxicillin which has a broad spectrum of activity against Gram positive and Gram negative pathogens.

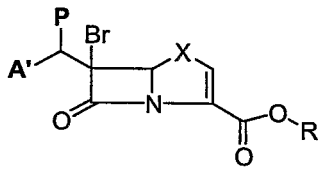
The compounds of the present invention may be provided prior to, simultaneously with, or subsequent to a β-lactam antibiotic ("co-administration"). By "provided", it is intended to include administering the compound directly or in vivo, e.g. pro-drugs. When the compounds of the present invention are co-administered with a β-lactam antibiotic, the ratio of the amount of the compound to the amount of the β-lactam antibiotic may vary in a wide range. The ratio of β-lactam antibiotic to β-lactamase inhibitor may vary from 1:1 to 100:1. Preferably the ratio of the β-lactam antibiotic to β-lactamase inhibitor is less than 10:1. The composition of the present invention may be in a form suitable for oral (PO), intravenous (IV) or topical administration. The compositions of the invention may be in a form of tablets, capsules, creams, syrups, suspension, sterile solutions suitable for injection or

infusion. Preferably, the compounds of the present invention are co-administered with piperacillin intravenously or amoxicillin intravenously or orally.

A compound's structural formula includes any tautomers, any stereoisomers (except where stereochemistry is clearly noted) and any crystalline forms.

General Synthetic Scheme(s) for Preparation of Compounds

This invention also provides a process for preparing a compound of formula **I** which comprises subjecting to reductive elimination a compound of fomula **II:**



II

wherein A' is A or B as defined above, X is O or S, P is an ester leaving group, e.g., acetate, mesylate, triflate or tosylate, and R is a protecting group, followed if necessary by removal of the protecting group, to give a compound of formula I wherein R_5 is hydrogen; and if desired converting to a pharmaceutically acceptable salt or to an ester wherein R_5 is C1 –C6 alkyl, C5 – C6 cycloalkyl, or CHR_3OCOC1-C6alkyl.

Conveniently compounds of the general formula **I** can be prepared by a novel, mild and a facile way, by condensing an appropriately substituted aldehyde **4** with a 6-bromo-penem derivative of structure **1**. (Scheme 1) in the presence of anhydrous $MgBr_2$ or $MgBr_2$: etherate and a base such as triethylamine or DBU or DMAP, preferably at –20°C to –40°C. The intermediate aldol product **5** can be functionalized with acid chlorides or anhydrides to an acetate, triflate or a tosylate **6**. Compound **6** can be smoothly converted to the desired product by a reductive elimination process using a metal such as activated zinc and phosphate buffer at 20°C to 35°C at a pH of 6.5 to 8.0. If the protecting group on the carboxylate oxygen is a *para*-nitrobenzyl substitiuent then the reductive elimination and deprotection can be achieved by a

single step. However, if the protecting group is other than a *para*-nitrobenzyl substituent, a two step procedure can be followed depending up on the nature of the protecting group. The product can be isolated as a free acid or as an alkali metal salt. The above mentioned two step procedure can be carried out in one step by carrying out the entire process without isolating the intermediate **6**. This is a very general, relatively simple and efficient procedure in terms of yield and economic feasibility. This procedure can be adopted to large scale synthesis and is amenable to a variety of aldehydes. Alternatively, compound **6** can be hydrogenated at 40 psi pressure in the presence of Pd/C (10%) in THF and 6.5 phosphate buffer to yield the final product.

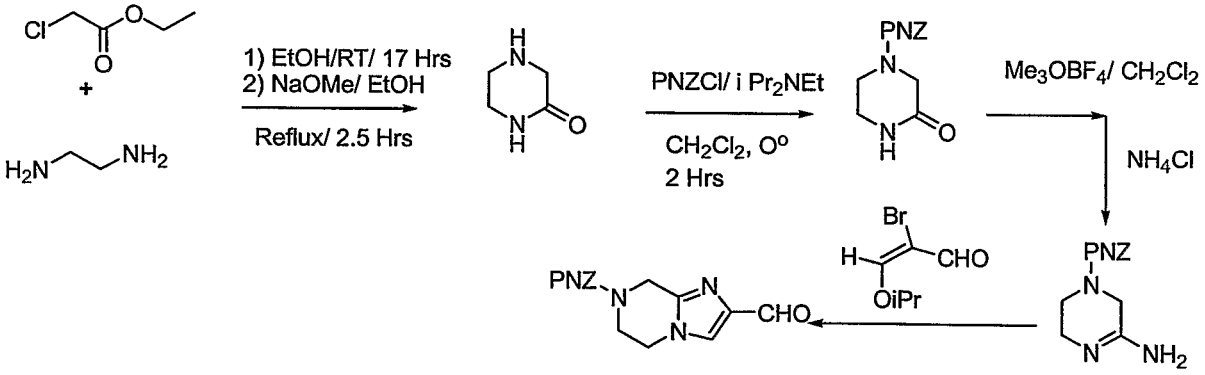
The above mentioned aldol condensation reaction is very versatile and it can be applied to any bromopenem derivative, where the carboxy group is protected other than 4-nitrobenzyl moiety. Example of other protecting group include benzyl, para-methoxy benzyl derivative, benzyhydrol , trityl, alkyl and allyl derivatives. However, when the protecting group is other than 4-nitrobenzyl group, a separate deprotection step need to be carried out after the reductive elimination procerdure. The chemistry involved in the deprotection step is well known to people who are skilled in that art.

The required aldehydes **4** for the above mentioned transformations can be prepared from their respective alcohol derivatives by MnO_2 oxidation or by Swern oxidation. In some cases the required aldehyde functionality can be introduced directly in the heterocyclic moiety by a Vilsmier Haack reaction using DMF/$POCl_3$. The aldehydes required for the present investigation may be prepared as depicted in Schemes 2 to 5. The N-(tert-butoxycarbonyl)- (ie) t-Boc protected –4-piperidone is treated with DMF/$POCl_3$ to yield 4-chloro –3 –formyl derivative. (Scheme 2). This reaction can be conducted on tetrahydro-4H-pyran-4-one and the corresponding tetrahydro-4H-thiopyran-4-one derivative to give the corresponding oxygen and the sulfur derivatives. This reaction can also be conducted on five to eight membered cyclic ketones derivatives. The chloro formyl intermediate can be reacted with 2-mercapto ethyl acetate to give the thieno derivative. The ester can be converted to alcohol, which can be converted to the starting aldehyde functionality. Scheme 3 illustrates the preparation of the imidazolo- tetrahydro pyridine derivative and imidazolo pyrazine derivative. 2- aminopyridine or 2-aminopyrazine can be reacted

with ethyl bromopyruvate in boiling ethanol to give the cyclized derivative (Scheme 3). Reduction of one ring can be achieved.

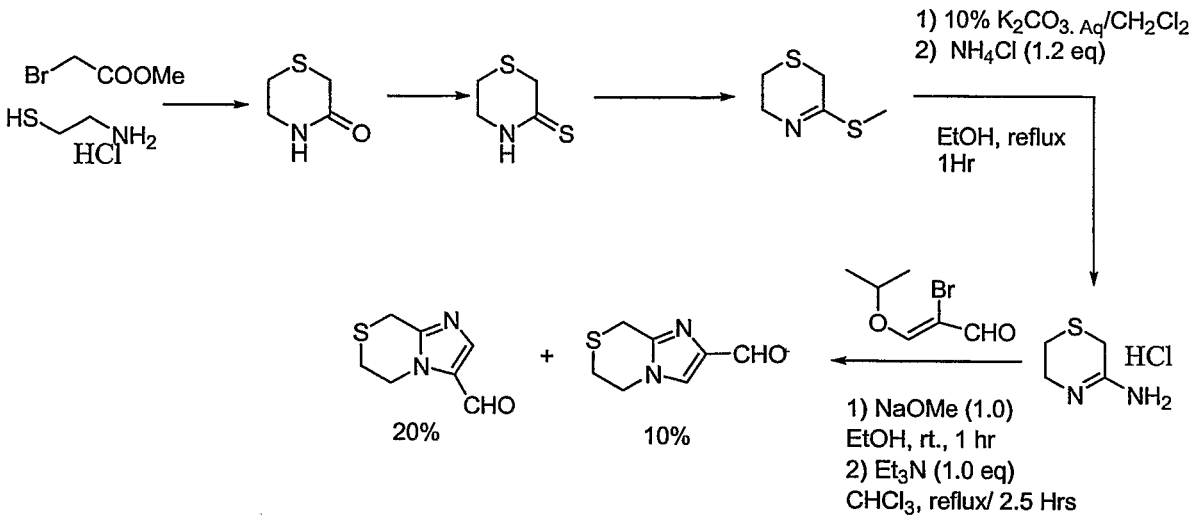
by hydrogenating it over Pd/C under 40 psi pressure in a par apparatus. Subsequently the ester group can be reduced to alcohol and converted to the aldehyde. In the case of X = N the intermediate amino ester can be derivatized using an appropriate R_1Q (where Q is a leaving group or a condensing group). In the case of Scheme 3, where R_1 = H can be synthesized by the procedure outlined in Scheme 4.

Scheme 4



Additional aldehydes may be synthesized as outlined in Schemes 5-7.

Scheme 5



Aldehydes required for examples 24-32 and 34, 35 were prepared by the route indicated schemes 8 to 18.

The following examples further illustrate the invention; they are not to be construed as limiting the invention. It will be readily apparent to one of ordinary skill in the art that additional embodiments can be made that are still within the spirit and scope of the invention.

Example 1

Preparation of (5R,6Z)-6-[(5-benzyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methylene]-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step1: Ethyl 5-benzoyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridine-2-carboxylate:

To a stirred dry DMF (7.3 g, 100 mmol), $POCl_3$ (12.25 g, 80 mmol) was slowly added between 0°C to 5° C. After the addition the solidified mass was dissolved in CH_2Cl_2 (20 ml) and stirred at room temperature for 2 hrs. Again the temperature was cooled to 0°C and 1-benzoyl-4-piperidone in CH_2Cl_2 was added slowly. After the addition the reaction mixture was stirred at room temperature for 2 hrs and poured over crushed ice and sodium acetate. It was stirred for 30 minutes at room temperature. Extracted with CH_2Cl_2; washed well with water; dired over anhydrous

$MgSO_4$ and concentrated. The crude product was dissolved in CH_2Cl_2 and ethylmercaptoacetae (9.6 g, 80 mmol) / Et_3N (10.1 g, 100 mmol) was added slowly at room temperature. The reaction mixture was refluxed for 2 hrs and quenched with water. CH_2Cl_2 layer was washed well with water; dried over anhydrous $MgSO_4$; filtered and concentrated. The product was purified by SiO_2 column chromatography by eluting it with 50% ethylacetae; hexane. Yellow oil; Yield: 6.4 gms (25%); M+H 316.

Step: 2 (5-benzyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methanol:

To stirred suspension of LAH (2.0 gms) a solution of ethyl 5-benzoyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridine-2-carboxylate (6.0 g, 19 mmol) in THF was added slowly at 0^0 C. After addition reaction mixture was stirred for 30 minutes and quenched with saturated NH_4Cl. It was diluted with $CHCl_3$ and filtered. The fitrate was washed with saturated brine solution and dried over anhydrous $MgSO_4$. It was filtered and taken to next step with out purifications. Yield: 4.5 g 91%. Yellow liquid.

Step 3: 2-Formyl (5-benzyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridine:

To a stirred solution of (5-benzyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methanol (4.0 g, 15.4 mmol) in CH_2Cl_2 (300 ml) active MnO_2 (20 g, excess) was added and stirred at room temperature for 18 hrs. At the end, the reaction mixture was filtered through celite and washed with $CHCl_3$. Reaction mixture was washed well with water; dried and concentrated. The product was found to be pure and taken to next step without purifications. Yield: 3.0 g (76%); (M+H: 257).

Step 4: 4-Nitrobenzy-6-[(acetyloxy)(5-benzyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

2-Formyl (5-benzyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (565 mg, 2.2 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (772 mg, 2.0 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (390 mg, 1.5 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl

acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 550 mg, 40%; M+H 687.

Step-5: (5R,6Z)-6-[(5-benzyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methylene]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid:

4-Nitrobenzy-6-[(acetyloxy)(5-benzyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate(450 mg, 0.65 mmol) was dissolved in THF (20 mL) and acetonitrile (10 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 hours at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 0.1 *M* NaOH was added to adjust pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 lits) and latter with 10% CAN: Water. The fractions containing the product were collected and concentrated at reduced pressure at room temperature. The yellow solid was washed with acetone and filtered. Dried. Yield: 50 mg, 18%; as yellow crystals; mp. 198^{0}C; (M+H) 411 .

^{1}H NMR (DMSO-d_6)δ d 2.7 (m, 2H), 2.8 (bm, 2H), 3.4 (m, 2H), 3.8 (s, 2H), 6.3 (s, 1H), 6.5 (s, 1H), 7.1(s, 1H), 7.28(s, 1H), 7.4 (s, 5H).

EXAMPLE 2

Preparation of (5*R*),(6*Z*)-6-(7-Methyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Imidazo[1,2-*a*]pyrazine-2-carboxylic acid ethyl ester:

Ethyl bromopyruvate (62.9 g) was added to the DME (258 mL) solution of 2-aminopyrazine (24.8 g) at room temperature and stirred for 2.5 h. The reaction mixture was cooled to 0 °C and stirred for 30 min to afford a pale brown precipitate. The precipitate was filtered and washed with Et_2O to give pale brown crystals. The suspension of the precipitate (66.1 g) in EtOH (1.29 L) was heated at reflux temperature to turn to clear solution. After refluxing for 2h, the reaction mixture was concentrated under reduced pressure, then mixed with $CHCl_3$ and saturated $NaHCO_3$aq. The mixture was filtered through a pad of Celite and the separated organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with $CHCl_3$ - MeOH (99/1 ~ 97/3), and collected fractions were concentrated under reduced pressure followed by recrystallization from $CHCl_3$ - Et_2O. The titled compound was obtained as pale pink crystals. Yield: 10.9 g, 22%).

^{1}H NMR($CDCl_3$)δ d 1.46(t, 3H, *J* = 7.2 Hz), 4.49(q, 2H, *J* = 7.2 Hz), 7.96(d, 1H, *J* = 4.7 Hz), 8.08(dd, 1H, *J* = 1.2, 4.7 Hz), 8.26(s, 1H), 9.21(d, 1H, *J* = 1.2 Hz).

Step 2: 5,6,7,8-Tetrahydroimidazo[1,2-*a*]pyrazine-2-carboxylic acid ethyl ester,

Hydrochloride:

0.46 *M* HCl - EtOH (169 mL) and 10% Pd-C (50% wet) (1.37 g) were added to the EtOH (546 mL) solution of imidazo[1,2-*a*]pyrazine-2-carboxylic acid ethyl ester (13.7 g). The mixture was hydrogenated under H_2 at 40 psi at room temperature for 15 h. The reaction mixture was filtered and Pd-C was washed with EtOH. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with $CHCl_3$ - MeOH (9/1 ~ 2/1). The titled compound was obtained as brown crystals Yield: 10.4 g, 63%.

^{1}H NMR($CDCl_3$)δ d 1.38(t, 3H, *J* = 7.1 Hz), 3.90(t, 2H, *J* = 5.7 Hz), 4.40(q, 2H, *J* = 7.1 Hz), 4.59(t, 2H, *J* = 5.7 Hz), 4.80(s, 2H), 8.20(s, 1H).

Step 3: 7-Methyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-2-carboxylic acid ethyl ester:

Et_3N (3.44 mL), 37% HCHO aq. (2.02 mL) and $NaBH_3CN$ (1.78 g) were added successively to the MeOH (75 mL) solution of 5,6,7,8-tetrahydroimidazo[1,2-

a]pyrazine-2-carboxylic acid ethyl ester, hydrochloride (5.2 g) at room temperature and stirred for 3.5 h under a nitrogen atmosphere. The mixture was diluted with CH_2Cl_2 and washed with 50% K_2CO_3 aq. The organic layer was dried (K_2CO_3) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with $CHCl_3$ - acetone (1/1 ~ 1/2). The titled compound was obtained as orange oil. Yield: 2.68 g, 57%).

^{1}H NMR($CDCl_3$)δ d 1.37(t, 3H, *J* = 7.1 Hz), 2.50(s, 3H), 2.85(t, 2H, *J* = 5.5 Hz), 3.69(s, 2H), 4.06(t, 2H, *J* = 5.5 Hz), 4.36(t, 2H, *J* = 7.1 Hz), 7.52(s, 1H).

Step 4: 7-Methyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-2-carbaldehyde:

1.01 *M* solution of DIBAL in toluene (13.6 mL) was added to the dry CH_2Cl_2 (86 mL) solution of 7-methyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-2-carboxylic acid ethyl ester (1.8 g) under a nitrogen atmosphere at –78 °C and stirred for 2 h. The mixture was quenched with 1*M* HCl. The reaction mixture was filtered through a pad of Celite. The filtrate was washed with 50% K_2CO_3 aq. and the aqueous layer was extracted with CH_2Cl_2 three times. The combined organic layer was dried (K_2CO_3) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with $CHCl_3$ - MeOH (19/1 ~ 9/1). The titled compound **5** was obtained as colorless crystals. Yield: 591 mg, 42%).

^{1}H NMR($CDCl_3$)δ d 2.51(s, 3H), 2.87(t, 2H, *J* = 5.5 Hz), 3.70(s, 2H), 4.10(t, 2H, *J* = 5.5 Hz), 7.53(s, 1H), 9.82(d, 1H, *J* = 1.4 Hz).

Step 5: (5*R*, 6*RS*)-6-[(*RS*)-Acetoxy(7-methyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (diastereo mixture):

7-Methyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-2-carbaldehyde (1.19 g) was added to the dry acetonitrile (97 mL) solution of anhydrous $MgBr_2$ (4.05 g) under a nitrogen atmosphere at room temperature. The dry THF solution (97 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (3.32 g) was added to the mixture, cooled to –20 °C, and Et_3N (3.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light.

The reaction mixture was stirred for 4.5 h at –20 °C and treated with acetic anhydride (1.36 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 17 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with $CHCl_3$ - acetone (9/1 ~ 2/1). The titled compound was obtained as two diastereo mixture. Red oil, Yield: 1.13 g.

^{1}H NMR($CDCl_3$)δ d 1.20(s, 0.81x3H), 2.24(s, 0.19x3H), 2.48(s, 3H), 2.80 ~ 2.84(m, 2H), 3.57 ~ 3.67(m, 2H), 3.97 ~ 4.02(m, 2H), 5.27(d, 1H, *J* = 13.6 Hz), 5.42(d, 0.19x1H, *J* = 13.6 Hz), 5.45(d, 0.81x1H, *J* = 13.6 Hz), 6.07(s, 0.19x1H), 6.30(s. 0.81x2H), 6.79(s, 0.19x1H), 6.80(s, 0.19x1H), 7.02(s, 0.81x1H), 7.44(s, 0.19x1H), 7.47(s, 0.81x1H), 7.60(d, 0.19x2H, *J* = 8.2 Hz), 7.62(d, 0.81x2H, *J* = 8.6 Hz), 8.22 ~ 8.26(m, 2H).

Step 6: (5*R*),(6*Z*)-6-(7-Methyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt:

(5*R*, 6*RS*)-6-[(*RS*)-Acetoxy(7-methyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazin-2-

yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (1.11 g) was dissolved in THF (32 mL) and acetonitrile (32 mL). Freshly activated Zn dust (4.46 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 48 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered through a pad of Celite, cooled to 3 °C, and 1 *M* NaOH was added to adjust pH to 7.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (20 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN(1/0 ~ 95/5). The combined fractions were concentrated under high vacuum at 35°C and lyophilized to give the title compound as a yellow amorphous solid. Yield: 417 mg, 65%: mp 200 °C (dec); ^{1}H NMR(D_2O)δ d 2.32(s,

3H), 2.79 ~ 2.81(m, 2H), 3.54(s, 2H), 3.95(t, 2H, *J* = 5.6 Hz), 6.39(s, 1H), 6.85(s, 1H), 6.87(s, 1H), 7.26(s, 1H).

EXAMPLE 3

Preparation of (5*R*), (6*Z*)-7-Oxo-6-(5,6,7,8-tetrahydroimidazo[1,2-a]pyrazin-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

2-Ketopiperazine

2-Ketopiperazine may be prepared according to procedures in US 2004-0077622A1

Step 1: 4-*p*-Nitrobenzyloxycarbonyl-2-ketopiperazine

The 48.7% solution of *p*-nitrobenzyloxycarbonyl chloride in 1,4-Dioxane (10.7 mL) was added to the dichloromethane (110 mL) solution of 2-Ketopiperazine (2.21 g) and diisopropylethylamine (4.6 mL) at 0°C and stirred for 0.5 h at 0 °C. Water (300 mL) was added to the reaction mixture, and extracted with dichloromethane (3 x 100 mL). The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduce pressure. The residue was applied to silica gel column chromatography, eluted with $CHCl_3$ – methanol (30 : 1), and the title compound was obtained as white solid (7.1 g, quant.).

^{1}H NMR (d, $CDCl_3$)δ 3.42-3.45 (m, 2H), 3.74 (t, 2H, *J* = 5.4 Hz), 4.19 (s, 2H), 5.26 (s, 2H), 6.39 (brs, 1H), 7.52 (d, 2H, *J* = 8.6 Hz), 8.24 (d, 2H, *J* = 8.6 Hz).

Step 2: 5-Methoxy-4-*p*-nitrobenzyloxycarbonyl-1,2,3,6-tetrahydropyrazine:

Trimethyloxonium tetrafluoroborate (97%, 3.7 g) was added to the dry dichloromethane (120 mL) solution of 4-*p*-nitrobenzyloxycarbonyl-2-ketopiperazine (6.7 g) at room temperature and stirred for 17 hours. The reaction mixture was treated with saturated sodium hydrogen carbonate aqueous solution, and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (3 x 100 mL), then the combined organic layer was washed with saturated sodium hydrogen carbonate aqueous solution and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduce pressure and the title compound was obtained as a pale brown solid. Yield; 5.7 g, 80.6.

^{1}H NMR (d, $CDCl_3$)δ 3.48 (m, 2H), 3.57 (m, 2H), 3.70 (s, 3H), 3.97 (s, 2H), 5.26 (s, 2H), 7.52 (d, 2H, *J* = 8.7 Hz), 8.23 (d, 2H, *J* = 8.7 Hz).

Step 3: 2-Imino-4-*p*-nitrobenzyloxycarbonyl piperazine:

The mixture of 5-methoxy-4-*p*-nitrobenzyloxycarbonyl-1,2,3,6-tetrahydropyrazine (5.7 g) and ammonium chloride (1.6 g) in dry ethanol (100 mL) was heated to reflux for 4 hours. The reaction mixture was then concentrated under reduced pressure. Dichloromethane (100 mL) was added to the residue and extracted with water (3 x 50 mL) then the combined aqueous layer was washed with dichloromethane. The aqueous layer was neutralized with 10% potassium carbonate aqueous solution and then extracted with dichloromethane (8 x 50 mL). The combined organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and the title compound was obtained as a white solid. Yield: 4.9 g, 91.2%.

^{1}H NMR (d, $CDCl_3$)δ 3.49 (brs, 4H), 3.98 (brs, 2H), 5.26 (s, 2H), 7,52 (d, 2H, *J* = 8.6 Hz), 8.23 (d, 2H, *J* = 8.6 Hz).

Step 4: 7-*p*-Nitrobenzyloxycarbonyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrazine-2-carbaldehyde (9) and 7-*p*-nitrobenzyloxycarbonyl-5,6,7,8 tetrahydroimidazo[1,2-a]pyrazine-3-carbaldehyde:

The mixture of 2-bromo-3-hydroxypropenal (2.8 g), p-toluenesulfonic acid monohydrate (33 mg) and 2-propanol (3.5 mL) in cyclohexane (28 mL) was azeotroped until the vaper temperature rose to 80°C. The reaction mixture was concentrated under reduce pressure. The residue was dissolved in dry acetonitrile (30 mL). The dry acetonitrile (310 mL) solution of 2-imino-4-*p*-nitrobenzyloxycarbonyl piperazine (4.7 g) was added at room temperature. The reaction mixture was stirred at room temperature for 3 h, and then the reaction solution was removed in vacuo. The residue was dissolved in ethyl acetate (170 mL) and triethylamin (2.4 mL) was added, then the reaction mixture was heated to reflux for 1.5 h. The reaction mixture was cooled to room temperature, and then water (170 mL) was added to the reaction mixture and separated. The aqueous layer was extracted with dichloromethane (2 x 100 mL). The combined organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduce pressure. The residue was applied to silica gel column chromatography, eluted with $CHCl_3$ – methanol (50 : 1), and the title

compound was obtained as a brown solid, (Yield: 2.9 g, 51.6%) and its regio isomer (orange amorphous solid, Yield; 0.8 g, 14.9%) were obtained.

^{1}H NMR (d, $CDCl_3$) 3.99 (t, 2H, J = 5.4 Hz), 4.14 (t, 2H, J = 5.4 Hz), 4.85 (s, 2H), 5.29 (s, 2H), 7.54 (d, 2H, J = 8.6 Hz), 7.57 (s, 1H), 8.24 (d, 2H, J = 8.6 Hz), 9.85 (s, 1H).

Regio isomer ^{1}H NMR (d, $CDCl_3$)δ 3.95 (t, 2H, J = 5.4 Hz), 4.44 (t, 2H, J = 5.4 Hz), 4.87 (s, 2H), 5.29 (s, 2H), 7.54 (d, 2H, J = 8.7 Hz), 7.78 (s, 1H), 8.24 (d, 2H, J = 8.7 Hz), 9.71 (s, 1H).

Step 5: (5R)-6-[Acetoxy-(7-p-nitrobenzyloxycarbonyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrazin-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid p-nitrobenzyl ester:

The dry acetonitrile (25 mL) solution of 7-*p*-nitrobenzyloxycarbonyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrazine-2-carbaldehyde (1.6 g) was added to the dry acetonitrile (55 mL) solution of $MgBr_2$ (2.2 g) under an nitrogen atmosphere at room temperature then the mixture was stirred for 10 min. The dry THF (80 mL) solution of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.8 g) was added, the mixture was cooled to –20 °C then triethylamine (1.6 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with 4,4-dimethylamino pyridine (58.3 mg) and acetic anhydride (0.89 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. 10% Citric acid aqueous solution (320 mL) was added to the reaction mixture and the aqueous layer was extracted with ethyl acetate (3 x 160 mL). The organic layer was washed with water, saturated sodium hydrogen carbonate and brine, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, eluted with CH_2Cl_2 – acetone (20 : 1), and the title compound was obtained as two diastereo mixture (81 : 19, brown foamy amorphous solid. Yield: 2.1 g, 59.9%.

^{1}H NMR (d, $CDCl_3$)δ 2.01 (s, 2.43H), 2.24 (s, 0.57H), 3.93-,3.96 (m, 2H), 4.02-4.05 (m, 2H), 4.74-4.76 (m, 2H), 5.28 (d, 1H, J = 13.5 Hz), 5.28 (s, 2H), 5.45 (d, 1H, J = 13.5 Hz), 6.07 (s, 0.19H), 6.29 (s, 0.81H), 6.31 (s, 0.81H), 6.80 (s, 0.19H), 6.83 (s, 0.19H), 7.08 (s, 0.81H), 7.43 (s, 0.19H), 7.46 (s, 0.81H), 7.54 (d, 2H, J = 8.6 Hz), 7.61 (d, 2H, J = 8.8 Hz), 8.24 (d, 4H, J = 8.3 Hz).

Step 6: (5*R*), (6*Z*)-7-Oxo-6-(5,6,7,8-tetrahydroimidazo[1,2-a]pyrazin-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt:

(5R)-6-[Acetoxy-(7-p-nitrobenzyloxycarbonyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrazin-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid p-nitrobenzyl ester (2.0 g) was dissolved in THF (63 mL). Freshly activated Zn dust (7.9 g) was added rapidly with 0.5 mol/L phosphate buffer (pH 6.5, 63 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction solution was filterd through a pad of Celite and the pad was washed with water (150 mL) and *n*-butanol (150 mL). The aqueous layer was separated and then the organic layer was extracted with water (2 x 50 mL). The combined aqueous layer was concentrated to 61 g and applied to Diaion HP-21 resin (80 mL, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column was eluted with water and then 5% acetonitrile aqueous solution. The combined fractions were concentrated under high vacuum at 35°C and lyophilized to give the title compound as a yellow amorphous solid. Yield: 172 mg, 20.1%: mp 150 °C (dec); ^{1}H NMR (d, D_2O) δ3.02 (t, 2H, J = 5.6 Hz), 3.82 (s, 2H), 3.89 (d, 2H, J = 5.6 Hz), 6.38 (s, 1H), 6.84 (s, 1H), 6.87 (s, 1H), 7.24 (s, 1H); IR (KBr)

Example 4

Preparation of (5R,6Z)-6-{[5-(4-methoxybenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)]methylene}-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step 1: 5-tert-butyl 2-ethyl 6,7-dihydrothieno[3,2-c]pyridine-2,5(4H)-dicarboxylate:

5-tert-butyl 2-ethyl 6,7-dihydrothieno[3,2-c]pyridine-2,5(4H)-dicarboxylate was prepared according to the procedure as outlined in Example 1, (Step 1). Starting from tert-butyl-1-piperidinecarboxylate (9.9 g, 50 mmol), $POCl_3$ (6.3 g, 40 mmol) and DMF (3.8 g, 50 mmol). The chloroformyl intermediate was reacted with ethyl mercaptoacetate (6.0 g, 50 mmol) and Et_3N. The product was purified by SiO_2 column chromatography by eluting it with 3:1 hexane; ethylacetae. Yield: 8.7 g, 56%; White liquid. (M+H) 312.

Step 2: tert-butyl 2-(hydroxymethyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-carboxylate:

tert-butyl 2-(hydroxymethyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-carboxylate was prepared according to the procedure outlined in Example 1, (Step 2). Starting from 5-tert-butyl 2-ethyl 6,7-dihydrothieno[3,2-c]pyridine-2,5(4H)-dicarboxylate (1.0 g, 3.21 mmol) and $LiAlH_4$ (500 mg , excess), 807 mg (92% yield) of the alcohol derivative was isolated as white liquid. (M+H) 270.

Step 3: tert-butyl 2-(formyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-carboxylate:

tert-butyl 2-(formyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-carboxylate was prepared according to the procedure outlined in Example 1, (Step 3). Starting from tert-butyl 2-(hydroxymethyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-carboxylate (1.0 g 3.7 mmol) in methylene chloride (100 ml) and active MnO_2 (5 g, excess), 800 g (81% Yield) of the aldehyde derivative was isolated as brown solid. (M+H) 268.

Step 4: 2-(formyl)-6,7-dihydrothieno[3,2-c]-5(4H)-pyridine:

2-(formyl)-6,7-dihydrothieno[3,2-c]-5(4H)-pyridine was prepared starting from tert-butyl 2-(formyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-carboxylate (1.0 g 3.7 mmol) was dissolved in CH_2Cl_2 (20 ml), MeOH (90% 20 ml) and 1N. HCl in dioxane (10 ml). The reaction mixture was stirred at room temperature for 48 hrs. At

the end reaction mixture was concentrated to dryness and taken to next step without purification. Yield: 750 mg (HCl salt, Quantitative); M+H 168.

Step 5: 2-Formyl [5-(4-methoxybenzyl)-4,5,6,7-tetrahydrothieno][3,2-c]pyridine:

To a stirred solution of 2-(formyl)-6,7-dihydrothieno[3,2-c]-5(4H)-pyridine (1.4 g, 5.2 mmol) in DMF (20 ml) , 4-methoxybenzyl chloride (0.94 g, 6.2 mmol) and N,N-diisopropylethylamine (10 ml, excess) was added at room temperature. The reaction mixture was stirred for 24 hrs and quenched with water. The reaction mixture was extracted with chloroform; washed well with water and dried over anhydrous $MgSO_4$. It was filtered and concentrated. The product was purified by SiO_2 column chromatography by eluting it with ethylacetate. Pale yellow oil. Yield: 470 mg , 35%; M+H 288.

Step 6: 4-Nitrobenzy-6-[(acetyloxy)[5(4-methoxybenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

2-Formyl [5-(4-methoxybenzyl)-4,5,6,7-tetrahydrothieno][3,2-c]pyridine (574 mg, 2.0 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (772 mg, 2.0 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (390 mg, 1.5 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 550 mg, 40%; M+H 714 and 716.

Step-7: (5R,6Z)-6-{[5-(4-methoxybenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)]methylene}-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid:

4-Nitrobenzy-6-[(acetyloxy)[5(4-methoxybenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (300 mg, 0.42 mmol) was dissolved in THF (20 mL) and acetonitrile (10 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 0.1 *M* NaOH was added to adjust pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 lits) and latter with 10% CAN: Water. The fractions containing the product were collected and concentrated at reduced pressure at room temperature. The yellow solid was washed with acetone and filtered. Dried. Yield: 50 mg, 18%; as yellow crystals; mp. 127^{0}C; (M+H) 441 .

^{1}H NMR (DMSO-d_6)δ d 2.7 (m, 2H), 2.8 (bm, 2H), 3.4 (m, 2H), 3.74 (s, 3H) 3.8 (s, 2H), 6.6 (s, 1H), 6.88 (dd, 2H), 7.14(s, 1H), 7.24(dd, 2H), 7.4 (s, 1H), 7.59 (s, 1H).

Example 5

Preparation of (5*R*), (6*Z*)-6-(5,6-dihydro-8*H*-imidazo[2,1-c][1,4]thiazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: 5-Methylthio-3,6-dihydro-2*H*-[1,4]thiazine hydroiodide

5-Methylthio-3,6-dihydro-2*H*-[1,4]thiazine hydroiodide may be prepared by the method as outlined in US 2004-0077622A1.

Step 2: 3-Iminothiomorpholin hydrochloride

5-Methylthio-3,6-dihydro-2*H*-[1,4]thiazine hydroiodide (7.1 g) is dissolved with 10% K_2CO_3 aqueous solution (150 mL) and the aqueous layer is extracted with CH_2Cl_2 (5 x 70 mL). The combined organic layer is dried ($MgSO_4$), filtered and concentrated under reduce pressure. Ammonium chloride (1.7 g) is added to the obtained residue in dry ethanol (128 mL) and heated to reflux for 1 h. The reaction mixture was cooled to room temperature. The reaction solution is removed in vacuo and the Iminothiomorpholin hydrochloride is obtained as brown solid (4.3 g, quant.).

^{1}H NMR (d, DMSO-d^6) δ3.15 (t, 2H, *J* = 5.9 Hz), 3.74 (t, 2H, *J* = 5.9 Hz), 3.83 (s, 2H), 8.97 (brs, 1H), 9.38 (brs, 1H), 9.99 (brs, 1H).

Step 3: 5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]thiazine-2-carbardehyde and 5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]thiazine-3-carbardehyde

The mixture of 2-bromo-3-hydroxypropenal (**7**, 4.3 g), *p*-toluenesulfonic acid monohydrate (52 mg) and 2-propanol (5.3 mL) in cyclohexane (43 mL) is azeotroped until the vaper temperature rose to 80°C. The reaction mixture is concentrated under reduce pressure. The residue is dissolved in dry ethanol (28 mL). The mixture of the dry ethanol (143 mL) solution of 3-iminothiomorpholin hydrochloride (4.3 g) and 28% methanol solution of sodium methylate (5.0 mL) are added at room temperature. The reaction mixture is stirred at room temperature for 1 h, and then the reaction solution is removed in vacuo. The residue is dissolved in chloroform (128 mL) and triethylamine (3.6 mL) is added, then the reaction mixture is heated to reflux for 2.5 h. The reaction mixture was cooled to room temperature and then concentrated under reduce pressure. The residue is dissolved with

dichloromethane (300 mL) and washed with 50% K_2CO_3 aqueous solution (2 x 100 mL). The organic layer is dried ($MgSO_4$) and filtered. The filtrate is concentrated under reduce pressure. The residue is applied to silica gel column chromatography, eluted with $CHCl_3$ – acetone (10 : 1), and 5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]thiazine-2-carbardehyde (brown solid, 445 mg, 10.3%) and 5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]thiazine-3-carbardehyde (brown solid, 872 mg, 20.2%) are obtained.

5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]thiazine-2-carbardehyde: ^{1}H NMR (d, $CDCl_3$)δ 3.07 (t, 2H, *J* = 5.7 Hz), 3.95 (s, 2H), 4.33 (t, 2H, *J* = 5.7 Hz), 7.55 (s, 1H), 9.83 (s, 1H).

5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]thiazine-3-carbardehyde: ^{1}H NMR (d, $CDCl_3$) δ3.05 (t, 2H, *J* = 5.7 Hz), 3.98 (s, 2H), 4.61 (t, 2H, *J* = 5.7 Hz), 7.73 (s, 1H), 9.69 (s, 1H).

Step 4: (5*R*), (6*Z*)-6-(5,6-dihydro-8*H*-imidazo[2,1-c][1,4]thiazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt:

The dry acetonitrile (20 mL) solution of 5,6-dihydro-8*H*-imidazo[2,1-c][1,4]thiazine-2-carbardehyde (392 mg) is added to the dry acetinitrile (20 mL) solution of $MgBr_2$ (1.1 g) under a nitrogen atmosphere at room temperature then the mixture is stirred for 10 min. The dry THF (40 mL) solution of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.0 g) is added and the mixture is cooled to –20 °C then triethylamine (0.8 mL) is added in one portion. The reaction vessel is covered with foil to exclude light. The reaction mixture is stirred for 3.5 h at –20 °C and treated with 4-dimethylamino pyridine (30 mg) and acetic anhydride (0.44 mL) in one portion. The reaction mixture is warmed to 0 °C and stirred for 14 h at 0 °C. 10% Citric acid aqueous solution (240 mL) is added to the reaction mixture and the aqueous layer is extracted with ethyl acetate (3 x 100 mL). The combined organic layer is washed with water, saturated sodium hydrogen carbonate and brine, dried ($MgSO_4$) and filtered. The filtrate is concentrated under reduced pressure. The residue is roughly purified by silica gel column chromatography, eluted with CH_2Cl_2 – acetone (50 : 1), and crude (5*R*)-6-[acetoxy-(5,6-dihydro-8*H*-imidazo[2,1-c][1,4]thiazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester is obtained as solid.

The solid obtained above is purified by SiO_2 column chromatography by eluting it with 505 ethylacetae:hexane. The pale yellow solid obtained is dissolved in THF (17 mL). Freshly activated Zn dust (2.2 g) is added rapidly with 0.5 mol/L phosphate buffer (pH 6.5, 17 mL). The reaction vessel is covered with foil to exclude light. The reaction mixture is vigorously stirred for 2 h at room temperature. The reaction solution is filterd through a pad of Celite and the pad is washed with water (40 mL) and *n*-butanol (30 mL). The aqueous layer is separated and then the organic layer is extracted with 0.5 mol/L phosphate buffer (pH 6.5, 2 x 10 mL). The combined aqueous layer is concentrated to 23 g, 1mol/L NaOH is added to adjust pH to 7.25 and applied to Diaion HP-21 resin (30 mL, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column is eluted with water and then 10% acetonitrile aqueous solution. The combined active fractions are concentrated under high vacuum at 35°C and lyophilized to give (5*R*), (6*Z*)-6-(5,6-dihydro-8*H*-imidazo[2,1-c][1,4]thiazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt as a yellow amorphous solid (168 mg, 20.9%).

mp 135 °C (dec); ^{1}H NMR (d, D_2O) δ3.00 (t, 2H, *J* = 5.7 Hz), 3.80 (AB, 2H, *J* = 16.7, 18.1 Hz), 4.19 (t, 2H, *J* = 5.7 Hz), 6.44 (d, 1H, *J* = 0.8 Hz), 6.89 (s, 1H), 6.93 (s, 1H), 7.29 (s, 1H); M+H = 322.

Example 6

Preparation of (5*R*), (6*Z*)-6-(6,7-Dihydro-5*H*-pyrrolo[1,2-a]imidazol-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: 6,7-Dihydro-5H-pyrrolo[1,2-a]imidazole-2-carbaldehyde

28% Sodium methoxide (5.26g) was added to the EtOH (250 mL) solution of 4,5-dihydro-3H-pyrrol-2-ylamine hydrochloride (3.27g) at room temperature. After stirring for 5 min at room temperature, 2-bromo-3-propoxy-propenal (5.79g) was added to the mixture at room temperature, then the reaction mixture was stirred for 1 h at room temperature. The reaction mixture was taken to dryness in vacuo. The residue was dissolved in $CHCl_3$ (300 mL) and triethylamine (3.8 mL) was added. The mixture was heated to reflux for 3 hours. The reaction mixture was cooled to room temperature, washed with 50% K2CO3, dried over anhydrous K2CO3, filtered, and evaporated under reduced pressure. The residue was applied with silicagel column

chromatography, eluted with CHCl3-acetone (2:1), and 6,7-Dihydro-5H-pyrrolo[1,2-a]imidazole-2-carbaldehyde (41%, 1.51g) was obtained as a pale yellow solid.

^{1}H NMR (d, $CDCl_3$):δ 2.62-2.7 (m, 2H), 2.90-2.94 (m, 2H), 4.07 (t, 2H, *J* = 7.2 Hz), 7.59 (s, 1H), 9.80 (s, 1H).

Step 2: (5*R*), (6*Z*)-6-(6,7-Dihydro-5*H*-pyrrolo[1,2-a]imidazol-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

6,7-Dihydro-5H-pyrrolo[1,2-a]imidazole-2-carbaldehyde (1.36 g) was added to the dry acetonitrile (155 mL) solution of anhydrous $MgBr_2$ (5.64 g) under an argon atmosphere at room temperature. The dry THF solution (155 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (3.86 g) was added to the mixture, cooled to –20 °C, and Et_3N (4.18 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with acetic anhydride (1.89 mL) and DMAP (370 mg) in one portion. The reaction mixture was warmed to 0 °C and stirred for 14.5 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 1 *M* citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was dissolved in THF (166 mL) and acetonitrile (77 mL). Freshly activated Zn dust (23.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 243 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 1 *M* NaOH was added to adjust pH to 8. The filtrate was washed with ethyl acetate and the aqueous layer was separated. 1 *M* NaOH was added to the aqueous layer again to adjust pH to 8. The resultant mixture was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (20 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN(1/0 ~ 9/1) to give the purified active fractions of (5*R*), (6Z)-6-(6,7-Dihydro-5*H*-pyrrolo[1,2-a]imidazol-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt . The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the titled as a yellow amorphous solid (681 mg, 24%, pH 7.8).

mp 190 °C (dec); ^{1}H NMR(d, D_2O): δ: 2.48-2.56 (m, 2H), 2.74-2.79 (m, 2H), 3.94-3.99 (m, 2H), 6.47 (d, 1H, *J* = 0.7 Hz), 6.94 (s, 1H), 6.95 (s, 1H), 7.36 (s, 1H); (M+H) 291.

Example 7

Preparation of (5*R*), (6*Z*)-6-(5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]oxazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

STEP 1: MORPHOLIN-3-ONE

Morpholin-3-one was prepared in the method of USP 5,349,045.

STEP 2: MORPHOLIN-3-THIONE

A mixture of morpholin-3-one (4.7 g) and Lawesson's reagent (10.3 g) in dry THF (94 mL) was heated to reflux for 1.5 h. The reaction mixture was cooled to room temperature and the reaction solvent was removed in vacuo. The residue was applied to silica gel column chromatography and eluted with $CHCl_3$ – methanol (50 : 1) to obtain a yellow solid. Recrystallization of the crude product from hexane-ethyl acetate gave the title (4.0 g, 72.2%) as yellow powder.

^{1}H NMR ($CDCl_3$) δ 3.45 (t, 2H, *J* = 5.1 Hz), 3.91 (t, 2H, *J* = 5.1 Hz), 4.55 (s, 2H).

Step 3: 5-Methylthio-3,6-dihydro-2*H*-[1,4]oxazine

A mixture of morpholin-3-thione (4.7 g) and methyl iodide (13 mL) in dry CH_2Cl_2 (140 mL) was stirred at room temperature for 15 h. The reaction mixture was filtered and the solid was washed with CH_2Cl_2. The obtained solid was dissolved with 50% K_2CO_3 aqueous solution (150 mL) and the aqueous layer was extracted with CH_2Cl_2 (8 x 100 mL). The combined CH_2Cl_2 layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduce pressure and the title was obtained as pale yellow oil (3.6 g, 67.8%).

^{1}H NMR ($CDCl_3$) δ 2.32 (s, 3H), 3.71-3.74 (m, 4H), 4.14-4.15 (m, 2H).

STEP 4: 3-IMINOMORPHOLIN HYDROCHLORIDE

A mixture of 5-methylthio-3,6-dihydro-2*H*-[1,4]oxazine (3.6 g) and ammonium chloride (1.5 g) in dry ethanol (136 mL) was heated to reflux for 1 h. The

reaction mixture was cooled to room temperature. The reaction solvent was removed in vacuo and the title was obtained as a pale brown solid (3.6 g, 97.7%).

^{1}H NMR (DMSO-d^6) δ 3.34 (m, 2H), 3.86 (t, 2H, *J* = 5.2 Hz), 4.47 (s, 2H).

Step 5: 5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]oxazine-2-carbaldehyde (9) and 5,6-dihydro-8*H*-imidazo[2,1-c][1,4]oxazine-3-carbaldehyde

The mixture of 2-bromo-3-hydroxypropenal (4.1 g), *p*-toluenesulfonic acid monohydrate (52 mg) and 2-propanol (5.2 mL) in cyclohexane (42 mL) was azeotroped until the vapor temperature rose to 80°C. The reaction mixture was concentrated under reduce pressure. The residue was dissolved in dry ethanol (50 mL). A mixture of the dry ethanol (200 mL) solution of 3-iminomorpholin hydrochloride (3.4 g) and 28% methanol solution of sodium methylate (4.8 g) was added at room temperature. The reaction mixture was stirred at room temperature for 2 h, and then the reaction solvent was removed in vacuo. The residue was dissolved in chloroform (125 mL) and triethylamine (3.5 mL) was added, then the reaction mixture was heated to reflux for 2 h. The reaction mixture was cooled to room temperature and then concentrated under reduce pressure. The residue was dissolved in dichloromethane (300 mL) and washed with 50% K_2CO_3 aqueous solution (2 x 100 mL). The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduce pressure. The residue was applied to silica gel column chromatography and eluted with $CHCl_3$ – acetone (4 : 1) to obtain the title (pale orange solid, 1.4 g, 36.3%) and the other regio isomer. (pale orange solid, 609 mg, 16.1%).

Desired product: ^{1}H NMR ($CDCl_3$) δ 4.08-4.15 (m, 4H), 4.88 (s, 2H), 7.58 (s, 1H), 9.85 (s, 1H).

The unwanted regio isomer: ^{1}H NMR ($CDCl_3$) δ 4.06 (t, 2H, *J* = 5.2 Hz), 4.40 (t, 2H, *J* = 5.2 Hz), 4.90 (s, 2H), 7.75 (s, 1H), 9.72 (s, 1H).

Step 6: 5*R*), (6*Z*)-6-(5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]oxazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

The dry acetonitrile (66 mL) solution of 5,6-dihydro-8*H*-imidazo[2,1-c][1,4]oxazine-2-carbaldehyde (1.2 g) was added to the dry acetonitrile (66 mL) solution of $MgBr_2$ (3.6 g) under a nitrogen atmosphere at room temperature then the

mixture was stirred for 10 min. The dry THF (132 mL) solution of *p*-nitrobenzyl (5R, 6S)-6-bromopenem-3-carboxylate (3.4 g) was added and the mixture was cooled to –20 °C then triethylamine (2.8 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 4 h at –20 °C and treated with 4-dimethylamino pyridine (100 mg) and acetic anhydride (1.5 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 18 h at 0 °C. 10% Citric acid aqueous solution (1 L) was added to the reaction mixture and the aqueous layer was extracted with ethyl acetate (3 x 500 mL). The combined organic layer was washed with water, saturated sodium hydrogen carbonate and brine, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and crude (5*R*)-6-[acetoxy-(5,6-dihydro-8*H*-imidazo[2,1-c][1,4]oxazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester was obtained as brown amorphous solid.

Freshly activated Zn dust (14 g) was added rapidly with 0.5 mol/L phosphate buffer (pH 6.5, 72 mL) to the THF (72 mL) solution of (5*R*)-6-[acetoxy-(5,6-dihydro-8*H*-imidazo[2,1-c][1,4]oxazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2.5 h at room temperature. The reaction solution was filtered through a pad of Celite and the pad was washed with water (170 mL) and *n*-butanol (170 mL). The aqueous layer was separated and then the organic layer was extracted with 0.5 mol/L phosphate buffer (pH 6.5, 2 x 50 mL). The combined aqueous layer was concentrated to 90 g, 1mol/L NaOH was added to adjust pH to 7.5 and applied to Diaion HP-21 resin (120 mL, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column was eluted with water and then 5% acetonitrile aqueous solution. The combined active fractions was concentrated under high vacuum at 35°C and lyophilized to give the title as a yellow amorphous solid (756 mg, 29.1%).

Mp 130 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.98-4.01 (m, 2H), 4.04-4.07 (m, 2H), 4.74 (AB, 2H, *J* = 15.3, 22.9 Hz), 6.40 (d, 1H, *J* = 0.8 Hz), 6.55 (s, 1H), 6.95 (d, 1H, *J* = 0.6 Hz), 7.54 (s, 1H); IR (KBr) 3412, 1741, 1672, 1592, 1549 cm^{-1}; λ^{max} (H_2O) 304 nm.

Example 8

Preparation of (5*R*),(6*Z*)-6-(5,6-Dihydro-4*H*-pyrrolo[1,2-*b*]pyrazol-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: 5,6-Dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-2-carboxylic acid ethyl ester

The titled compound was prepared in the same way of Ranganathan and co-workers (*Indian J. Chem.* **1991**, *30 B*, 169-175).

Step 2: (5,6-Dihydro-4*H*-pyrrolo[1,2-*b*]pyrazol-2-yl)methanol

MeOH (2.73 mL) was added to the THF (180 mL) solution of $LiBH_4$ (1.63 g) under a nitrogen atmosphere at room temperature, and then 5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-2-carboxylic acid ethyl ester (8.11 g) was added to the suspension and stirred for 2 h at 40 °C. The mixture was quenched with 1 mol/L HCl at pH 1 and stirred for 1 h at room temperature. Solid K_2CO_3 was added to the solution to adjust pH to 8 and the mixture was extracted with AcOEt. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure to afford the title compound as brown crystals (4.87 g, 78%).

^{1}H NMR ($CDCl_3$) δ 2.44 (t, 1H, *J* = 5.8 Hz), 2.54 – 2.62 (m, 2H), 2.87 (t, 2H, *J* = 7.4 Hz), 4.10 (t, 2H, *J* = 7.2 Hz), 4.63 (d, 2H, *J* = 5.8 Hz), 5.96 (s, 1H).

Step 3: 5,6-Dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-2-carbaldehyde

MnO_2 (activated) (24.4 g) was added to the $CHCl_3$ (350 mL) solution of (5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazol-2-yl)methanol (4.87 g) and refluxed for 1 h under a nitrogen atmosphere. The reaction mixture was filtered through a pad of Celite. The filtrate was reduced under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane – AcOEt (1/1 – 1/2). The title compound was obtained as yellow oil (4.35 g, 91%).

^{1}H NMR ($CDCl_3$) δ 2.63 – 2.71 (m, 2H), 2.95 (t, 2H, *J* = 7.4 Hz), 4.22 (t, 2H, *J* = 7.4 Hz), 6.52 (s, 1H), 9.89 (s, 1H).

Step 4: (5*R*),(6*Z*)-6-(5,6-Dihydro-4*H*-pyrrolo[1,2-*b*]pyrazol-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

5,6-Dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-2-carbaldehyde (1.36 g) was added to the dry acetonitrile (148 mL) solution of anhydrous $MgBr_2$ (5.52 g) under a nitrogen atmosphere at room temperature. The dry THF solution (148 mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-

nitrobenzyl ester (cont. 97%) (3.97 g) was added to the mixture, cooled to –20 °C, and Et_3N (4.18 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 4 h at –20 °C and treated with acetic anhydride (1.89 mL) and DMAP (123 mg) in one portion. The reaction mixture was warmed to 0 °C and stirred for 14 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, water and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure.

The residue was dissolved in THF (106 mL) and acetonitrile (49 mL). Freshly activated Zn dust (22.5 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 155 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 h at room temperature. The reaction mixture was filtered through a pad of Celite. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was cooled to 3 °C and 1 *M* NaOH was added to adjust pH to 8.0. The mixture was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (79 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN (1/0 – 9/1). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (848 mg, 29%, pH 7.1).

Mp 190 °C (dec); ^1H NMR (D_2O) δ 2.49 (m, 2H), 2.78 (t, 2H, J = 7.4 Hz), 4.02 (t, 2H, J = 7.4 Hz), 6.01 (s, 1H), 6.29 (s, 1H), 6.90 (s, 2H).

Example 9

Preparation of (5*R*)(6*Z*)-7-Oxo-6-(4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridin-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Tetrahydropyridino[1,2-c][1,2,3]oxadiazolone

Conc. HCl (1.96 mL) and $NaNO_2$ (2.2 g) were added to the H_2O (21 mL) solution of *DL*-pipecolic acid (3.04 g) under a nitrogen atmosphere at 0 °C and stirred for 1 h. The solution was extracted with CH_2Cl_2 and organic layer was washed with brine. The mixture was dried over Na_2SO_4 and concentrated under reduced

pressure to afford crude (2*RS*)-1-nitrosopiperidine-2-carboxylic acid as pale yellow crystals.

Trifluoroacetic anhydride (1.93 g) was added to the THF (92 mL) solution of crude (2*RS*)-1-nitrosopiperidine-2-carboxylic acid under a nitrogen atmosphere at 0 °C and stirred for 5 h at 0 °C and for 2 h at room temperature. The solution was concentrated under a reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (1/1 – 0/1). The titled compound was obtained as colorless crystals (1.10 g, 33%).

^{1}H NMR ($CDCl_3$) δ 1.93 – 1.99 (m, 2H), 2.08 – 2.15 (m, 2H), 2.65 (t, 2H, *J* = 6.5 Hz), 4.26 (t, 2H, *J* = 6.1 Hz).

Step 2: 4,5,6,7-Tetrahydropyrazolo[1,5-*a*]pyridine-2-carboxylic acid ethylester:

Ethyl propiolate (804 mg) was added to the *o*-xylene (15 mL) solution of tetrahydropyridino[1,2-*c*][1,2,3]oxadiazolone (1.04 g) under a nitrogen atmosphere and refluxed for 16 h. The solution was concentrated under a reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (2/1 – 1/1). The titled compound was obtained as yellow oil (871 mg, 65%), and 4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridine-3-carboxylic acid ethyl ester was obtained as yellow oil (345 mg, 26%).

^{1}H NMR ($CDCl_3$) δ 1.39 (t, 3H, *J* = 7.1 Hz), 1.84 – 1.91 (m, 2H), 2.02 – 2.09 (m, 2H), 2.82 (t, 2H, *J* = 6.4 Hz), 4.22 (t, 2H, *J* = 6.2 Hz), 4.39 (q, 2H, *J* = 7.1 Hz), 6.53 (s, 1H).

Step 3: (4,5,6,7-Tetrahydropyrazolo[1,5-*a*]pyridin-2-yl)methanol

MeOH (0.29 mL) was added to the THF (19 mL) solution of $LiBH_4$ (cont. 90%) (174 mg) under a nitrogen atmosphere at room temperature, then 4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridine-2-carboxylic acid ethyl ester (862 mg) was added to the suspension and stirred for 1 h at room temperature and 1.5 h at 40 °C. The mixture was quenched with 1 mol/L HCl at pH 1 and stirred for 1 h at room temperature. Solid K_2CO_3 was added to the solution to adjust pH to 8 and the mixture was extracted with AcOEt. The organic layer was dried ($MgSO_4$) and

filtered. The filtrate was concentrated under reduced pressure to afford titled compound as pale yellow oil (691 mg, 95%).

^{1}H NMR ($CDCl_3$) δ 1.80 – 1.87 (m, 2H), 1.98 – 2.05 (m, 2H), 2.77 (t, 2H, *J* = 6.4 Hz), 2.81 – 2.84 (br, 1H), 4.09 (t, 2H, *J* = 6.1 Hz), 4.62 (d, 2H, *J* = 5.3 Hz), 5.96 (s, 1H).

Step 4: 4,5,6,7-Tetrahydropyrazolo[1,5-*a*]pyridine-2-carbaldehyde

MnO_2 (activated) (3.36 g) was added to the $CHCl_3$ (44 mL) solution of (4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridin-2-yl)methanol (673 mg) and refluxed for 1 h under a nitrogen atmosphere. The reaction mixture was filtered through a pad of Celite. The filtrate was reduced under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (2/1 - 1/2). The titled compound was obtained as pale yellow oil (510 mg, 77%).

^{1}H NMR ($CDCl_3$) δ 1.90 (m, 2H), 2.10 (m, 2H), 2.84 (t, 2H, *J* = 6.4 Hz), 4.23 (t, 2H, *J* = 6.2 Hz), 6.52 (s, 1H), 9.92 (s, 1H).

Step 5: (5*R*)(6*Z*)-7-Oxo-6-(4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridin-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

4,5,6,7-Tetrahydropyrazolo[1,5-*a*]pyridine-2-carbaldehyde (483 mg) was added to the dry acetonitrile (48 mL) solution of anhydrous $MgBr_2$ (1.81 g) under a nitrogen atmosphere at room temperature. The dry THF solution (48 mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (cont. 97%) (1.28 g) was added to the mixture, cooled to –20 °C, and Et_3N (1.35 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (0.61 mL) and DMAP (40 mg) in one portion. The reaction mixture was warmed to 0 °C and stirred for 16 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, water and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure.

The residue was dissolved in THF (35 mL) and acetonitrile (16 mL). Freshly activated Zn dust (7.43 g) was added rapidly with 0.5 *M* phosphate buffer (pH

6.5, 51 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 h at room temperature. The reaction mixture was filtered through a pad of Celite. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was cooled to 3 °C and 1 *M* NaOH was added to adjust pH to 8.0. The mixture was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (105 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN (1/0 - 85/15). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (427 mg, 41%, pH 7.7).

Mp 190 °C (dec); ^{1}H NMR (D_2O) δ 1.67 – 1.71 (m, 2H), 1.85 – 1.89 (m, 2H), 2.64 (t, 2H, J = 6.3 Hz), 3.97 (t, 2H, J = 6.1 Hz), 5.97 (s, 1H), 6.25 (s, 1H), 6.85 (s, 1H), 6.88 (s, 1H).

Example 10

Preparation of (5*R*),(6*Z*)-6-(7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt

Step 1: 5-Methoxy-1-methyl-3,6-dihydro-1H-pyrazin-2-one

The titled compound was prepared in the same way of S.Rajappa and B.G.Advani (*Tetrahedron.* **1973**, *29*, 1299-1302).

Step 2: 5-Amino-1-methyl-3,6-dihydro-1H-pyrazin-2-one

A mixture of 5-methoxy-1-methyl-3,6-dihydro-1H-pyrazin-2-one (2.3 g) and ammonium chloride (936 mg) in dry ethanol (32 mL) was stirred at room temperature for 1 h and then refluxed for 2 h. The reaction mixture was cooled to room temperature and evaporated under reduced pressure. The residue was triturated with chloroform at room temperature for 30 min. The precipitate was filtered off and dried in vacuo. The 5-amino-1-methyl-3,6-dihydro-1H-pyrazin-2-one hydrochloride was obtained as a pale brown powder (1.7 g, 66%).

A solution of 5-amino-1-methyl-3,6-dihydro-1H-pyrazin-2-one hydrochloride (662mg) in methanol (10 mL) was added 10% potassium carbonate aqueous solution at 0□ and then stirred for 40 min at 0□. The mixture was concentrated under reduced pressure. The residue was triturated with chloroform (18mL) and methanol (2 mL) at room temperature for 30 min. The precipitate was filtered off and dried in vacuo. The compound was obtained as a pale brown powder (515 mg, quantitative).

^{1}H NMR (DMSO-d^6) δ 2.88 (s, 3H), 3.94 (s, 2H), 4.42 (s, 2H).

Step 3: 7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo [1,2-a]pyrazine-2-carbaldehyde and 7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazine-3-carbaldehyde

The solution of 2-bromo-3-isopropoxy-propenal (1.3 g) in dry acetonitrile (60 mL) was added to the solution of 5-amino-1-methyl-3,6-dihydro-1H-pyrazin-2-one (782 mg) in dry acetonitrile (60 mL) at room temperature. The reaction mixture was stirred at room temperature for 20 h, added triethylamine (0.95 mL) and then refluxed for 2 h. The reaction mixture was cooled to room temperature and then evaporated under reduce pressure. The residue was dissolved in chloroform (10mL) and washed with 50% K_2CO_3 aqueous solution (10mL). The aqueous layer was

extracted with chloroform. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was evaporated under reduce pressure. The residue was applied to silica gel column chromatography and eluted with $CHCl_3$ – MeOH (95 : 5) to obtain the title compound 7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo [1,2-a]pyrazine-2-carbaldehyde as a pale yellow solid (541 mg, 49.1%) and its regio isomer 7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazine-3-carbaldehyde as a pale yellow solid (128 mg, 11.6%).

7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo [1,2-a]pyrazine-2-carbaldehyde: ^1H NMR ($CDCl_3$) δ 3.17 (s, 3H), 4.68 (s, 2H), 4.78 (s, 2H) , 7.66 (s, 1H) , 9.83 (s, 1H).

7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazine-3-carbaldehyde: ^1H NMR ($CDCl_3$) δ 3.16 (s, 3H), 4.70 (s, 2H), 5.03 (s, 2H) , 7.82 (s, 1H) , 9.73 (s, 1H).

Step 4: (5*R*, 6*RS*)- 6- [Acetoxy-(7-methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester

7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo [1,2-a]pyrazine-2-carbaldehyde (319 mg) was added to the dry acetonitrile (32 mL) solution of anhydrous $MgBr_2$ (786 mg) under a nitrogen atmosphere at room temperature. The dry THF solution (32 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (687 mg) was added to the mixture, cooled to –20 °C, and triethylamine (0.60 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with 4-dimethylaminopyridine (44 mg) and acetic anhydride (0.35 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 20 h at 0°C. The mixture was diluted with ethyl acetate and H_2O. After separating organic layer, the aqueous layer was extracted with ethyl acetate. The organic layers were combined and washed with 5% citric acid aqueous solution and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then eluted with chloroform. The title compound was obtained as diastereo mixture (yellow amorphous solid ; 410 mg, 38%).

^1H NMR (δ, $CDCl_3$) 2.03 (s, 0.7 x 3 H), 2.09 (s, 0.3 x 3H), 3.15 (s, 3H), 4.59-4.62 (m, 2H), 4.66 (s, 0.3 x 2H), 4.67 (s, 0.7 x 2H), 5.28 (d, 1H, *J* = 13.5 Hz), 5.43 (d, 0.3 x 1H, *J* = 13.5 Hz), 5.45 (d, 0.7 x 1H, *J* = 13.5 Hz), 6.07 (s, 0.3 x 1H),

6.28 (s, 0.7 x 1H), 6.32 (s, 0.7 x 1H), 6.83 (s, 0.3 x 1H), 6.86 (s, 0.3 x 1H), 7.10 (s, 0.7 x 1H), 7.44 (s, 0.3 x 1H), 7.47 (s, 0.7 x 1H), 7.60 (d, 0.7 x 2H, *J* = 8.6 Hz), 7.61 (d, 0.3 x 2H, *J* = 8.6 Hz), 8.24 (d, 2H, *J* = 8.6 Hz),

Step 5: (5*R*),(6*Z*)-6-(7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt and (5*R*),(6*E*)-6-(7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt

(5*R*, 6*RS*)- 6- [Acetoxy-(7-methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (481 mg) was dissolved in THF (6.7 mL) and acetonitrile (3.1 mL). Freshly activated Zn dust (1.92 g) and 0.5 *M* phosphate buffer (pH 6.5, 9.9 mL) were added to the mixture. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction solution was mixed with ethyl acetate and filtered through a pad of Celite. The pad was washed with water and the aqueous layer was separated. The aqueous layer was cooled to 3 °C and 1 *M* NaOH was added to adjust pH to 8.0. The mixture was concentrated under high vacuum at 35 °C and lyophilized. The residue was separated by the preparative HPLC (Inertsil ODS-2, GL Science Inc., 10 x 250 mm, 0.05 mol/L phosphate buffer (pH 7.1) : CH_3CN = 93 : 7, 4.0 mL/min.). The separated fractions of (5*R*),(6*Z*)-6-(7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt and (5*R*),(6*E*)-6-(7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt were cooled to 3 °C and 1 *M* NaOH was added to adjust pH to 8.0 respectively. Each solution was concentrated under high vacuum at 35 °C. Each concentrate was applied to Diaion HP-21 (60 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with water and then with 5% acetonitrile-water. The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the title compound(5*R*),(6*Z*)-6-(7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt as a yellow amorphous solid (125 mg, 44.4 %, Mp 115-117 °C (dec)) and compound (5*R*),(6*E*)-6-(7-Methyl-6-oxo-5,6,7,8-tetrahydro-

imidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt as yellow amorphous solid (19 mg, 6.7 %) respectively.

Compound (5*R*),(6*Z*)-6-(7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt ^{1}H NMR (δ, D_2O) 2.99 (s, 3H), 4.54 (s, 2H), 4.66 (s, 2H), 6.38 (s, 1H), 6.85 (s, 1H), 6.90 (s 1H) , 7.30 (s, 1H).

Compound (5*R*),(6*E*)-6-(7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt ^{1}H NMR (δ, D_2O) 2.94 (s, 3H), 4.45 (s, 2H), 4.56 (s, 2H), 6.22 (s, 1H), 6.48 (s, 1H), 6.94 (s, 1H), 7.69 (s 1H).

Example 11

Preparation of (5*R*)(6*Z*)-6-(6,7-Dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt:

Step 1: (3*R*)-Thiomorpholine-3-carboxylic acid

The titled compound was prepared in the same way of Shiraiwa and co-workers (*Biosci. Biotechnol. Biochem.* **1998**, *62*, 2382-2387).

Step 2: 3-Oxo-3a,4,6,7-tetrahydro-3*H*-2-oxa-5-thia-1-aza-7a-azonioindenide

$NaNO_2$ (3.14 g) was added to the 1 mol/L HCl (33.7 mL) solution of (3*R*)-thiomorpholine-3-carboxylic acid (4.96 g) under a nitrogen atmosphere at 0 °C and stirred for 0.5 h. The solution was extracted with $CHCl_3$ (5 times) and the organic layer was washed with brine. The mixture was dried over $MgSO_4$ and concentrated under reduced pressure to afford crude (3*R*)-4-nitrosothiomorpholine-3-carboxylic acid as pale yellow crystals.

Trifluoroacetic anhydride (7.07 g) was added to the THF (169 mL) solution of crude (3*R*)-4-nitrosothiomorpholine-3-carboxylic acid under a nitrogen atmosphere at 0 °C and stirred for 3 h at 0 °C and for 17 h at room temperature. The solution was concentrated under a reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane -

AcOEt (1/1 – 0/1). The titled compound was obtained as pale brown crystals (3.41 g, 64%).

^{1}H NMR ($CDCl_3$) δ 3.15 (t, 2H, *J* = 5.5 Hz), 3.71 (s, 2H), 4.54 (t, 2H, *J* = 5.5 Hz).

Step 3: 6,7-Dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazine-2-carboxylic acid ethyl ester

Ethyl propiolate (2.33 g) was added to the *o*-xylene (72 mL) solution of 3-oxo-3a,4,6,7-tetrahydro-3*H*-2-oxa-5-thia-1-aza-7a-azonioindenide (3.41 g) under a nitrogen atmosphere and refluxed for 15 h. The solution was concentrated under a reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (2/1 – 1/1). The titled compound was obtained as yellow oil (3.13 g, 68%), and the other unwanted regio isomer 6,7-dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazine-3-carboxylic acid ethyl ester was obtained as yellow oil (556 mg, 12%).

^{1}H NMR ($CDCl_3$) δ 1.31 (t, 3H, *J* = 7.1 Hz), 3.04 (t, 2H *J* = 5.7 Hz), 3.81 (s, 2H), 4.32 (q, 2H, *J* = 7.1 Hz), 4.40 (t, 2H, *J* = 5.7 Hz), 6.54 (s, 1H).

Step 4: (6,7-Dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazin-2-yl)methanol

$LiBH_4$ (cont. 90%) (536 mg) and MeOH (0.9 mL) was added to the THF (59 mL) solution of 6,7-dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazine-2-carboxylic acid ethyl ester (3.13 g) under a nitrogen atmosphere at room temperature and stirred for 3 h at 40 °C. The mixture was quenched with 1 mol/L HCl at pH 1 and stirred for 1 h at room temperature. Solid K_2CO_3 was added to the solution to adjust pH to 8 and the mixture was extracted with AcOEt. The organic layer was dried (K_2CO_3) and filtered. The filtrate was concentrated under reduced pressure to afford titled compound as pale yellow oil (2.51 g, quant.).

^{1}H NMR ($CDCl_3$) δ 2.58 (br, 1H), 3.07 (t, 2H, *J* = 5.7 Hz), 3.84 (s, 2H), 4.33 (t, 2H, *J* = 5.7 Hz), 4.63 (d, 2H, *J* = 3.9 Hz), 6.05 (s, 1H).

Step 5: 6,7-Dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazine-2-carbaldehyde

MnO_2 (activated) (11.46 g) was added to the $CHCl_3$ (135 mL) solution of (6,7-dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazin-2-yl)methanol (2.31 g) and refluxed for 1 h under a nitrogen atmosphere. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under a reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (1/1). The titled compound was obtained as pale yellow crystals (1.78 g, 78%).

^{1}H NMR ($CDCl_3$) δ 3.15 (t, 2H, *J* = 5.8 Hz), 3.90 (s, 2H), 4.48 (t, 2H, *J* = 5.8 Hz), 6.58 (s, 1H), 9.92 (s, 1H).

Step 6: (5*R*)(6*Z*)-6-(6,7-Dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

6,7-Dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazine-2-carbaldehyde (841 mg) was added to the dry acetonitrile (39 mL) solution of anhydrous $MgBr_2$ (1.88 g) under a nitrogen atmosphere at room temperature. The dry THF solution (39 mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (cont. 99.7%) (1.93 g) was added to the mixture, cooled to –20 °C, and Et_3N (2.79 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with acetic anhydride (0.94 mL) and DMAP (61 mg) in one portion. The reaction mixture was warmed to 0 °C and stirred for 17 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, water and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure.

The residue was dissolved in THF (83 mL) and acetonitrile (39 mL). Freshly activated Zn dust (7.72 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 122 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 h at room temperature. The reaction mixture was filtered through a pad of Celite. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was cooled to 3 °C and 1 *M* NaOH was added to adjust pH to 8.0. The mixture was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (150

mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN (1/0 - 85/15). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (371 mg, 22%, pH 8.0).

Mp 190 °C (dec); ^{1}H NMR (D_2O) δ 3.03 (t, 2H, *J* = 5.7 Hz), 3.75 (s, 2H), 4.22 (t, 2H, *J* = 5.7 Hz), 6.07 (s, 1H), 6.27 (s, 1H), 6.86 (s, 1H), 6.89 (s, 1H).

Example 12

Preparation of (5*R*)(6*Z*)-7-Oxo-6-(4*H*-5-thia-1,6a-diazapentalen-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: 3-Oxo-3a, 4-dihydro-3*H*, 6*H*-2-oxa-5-thia-1-aza-6a-azonio-3a-pentalenide

Conc. HCl (15 mL) and $NaNO_2$ (16.6 g) were added to the H_2O (166 mL) solution of *L*-thioproline (24.3 g) under a nitrogen atmosphere at 0 °C and stirred for 2 h. The solution was extracted with CH_2Cl_2, organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to afford the crude N-nitroso derivative as a yellow solid.

Trifluoroacetic anhydride (5.0 mL) was added to the THF (350 mL) solution of crude N-nitrosothioproline under a nitrogen atmosphere at 0 °C and stirred for 5 h at 0 °C. The solution was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (1 : 1). The titled compound was obtained as a pale brown solid (4.0 g, 15.1%).

^{1}H NMR ($CDCl_3$): δ4.04 (t, 2H, *J* = 1.7 Hz), 5.40 (t, 2H, *J* = 1.7 Hz).

Step 2: 4*H*-5-Thia-1,6a-diazapentalen-2-carboxylic acid ethylester

Ethyl propiolate (3.1 mL) was added to the *o*-xylene (130 mL) solution of 3-oxo-3a, 4-dihydro-3*H*, 6*H*-2-oxa-5-thia-1-aza-6a-azonio-3a-pentalenide (4.0 g) under a nitrogen atmosphere and refluxed for 19 h. The solution was cooled to room temperature and concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (4 : 1). The titled compound was obtained as a yellow solid (2.7 g, 49.3%),

and 4*H*-5-thia-1,6a-diazapentalen-3-carboxylic acid ethylester was obtained as pale yellow crystals (1.2 g, 21.7%).

^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3H, *J* = 7.1 Hz), 4.11 (d, 2H, *J* = 2.1 Hz), 4.40 (q, 2H, *J* = 7.1 Hz), 5.24 (t, 2H, *J* = 1.6 Hz), 6.61 (s, 1H).

Step 3: (4*H*-5-Thia-1,6a-diazapentalen-2-yl)methanol

$LiBH_4$ (cont. 90%) (459 mg) was added to the ether (126 mL) solution of 4*H*-5-thia-1,6a-diazapentalen-2-carboxylic acid ethylester (2.5 g) and MeOH (0.77 mL) under a nitrogen atmosphere at room temperature, then refluxed for 1.5 h. The mixture was quenched with 1 mol/L HCl (25 mL) and stirred for 1 h at room temperature. The mixture was neutralized by saturated sodium hydrogen carbonate solution and separated. The aqueous layer was extracted with dichloromethane (10 x 25 mL). The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with AcOEt. The titled compound was obtained as a pale yellow solid (1.7 g, 87.9%).

^{1}H NMR ($CDCl_3$) δ 2.95 (t, 1H, *J* = 5.6 Hz), 4.07 (s, 2H), 4.62 (d, 2H, *J* = 5.1 Hz), 5.13 (t, 1H, *J* = 1.6 Hz), 6.04 (s, 1H).

Step 4: 4*H*-5-Thia-1,6a-diazapentalen-2-carbaldehyde

The dry dichloromethane (8 mL) solution of dimethylsulfoxide (2.2 mL) was added dropwise to the dry dichloromethane (110 mL) solution of oxalyl chloride (2.0 mL) at -78°C. The reaction mixture was stirred for 15 min at the same temperature. The dry dichloromethane (40 mL) solution of (4*H*-5-thia-1,6a-diazapentalen-2-yl)methanol, (1.7 g) was added dropwise to the reaction mixture at -78°C, and stirring was continued for an additional 15 min. The reaction mixture was allowed to warm to -45°C and stirred for 1 h. Triethylamine (11.3 mL) was added dropwise and the reaction mixture was allowed to warm to 0°C. After 20min, saturated ammonium chloride solution (50 mL) and water (100 mL) were added and separated. The aqueous layer was extracted with AcOEt (3 x 150 mL). The combined organic layers were washed with water (200 mL) and brine (200 mL), dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with

hexane – AcOEt (1 : 1). The titled compound was obtained as a yellow solid (1.7 g, quant.).

^{1}H NMR ($CDCl_3$) δ 4.13 (s, 2H), 5.26 (d, 2H, J = 1.4 Hz), 6.59 (s, 1H), 9.90 (s, 1H).

Step 5; (5*R*)(6*Z*)-7-Oxo-6-(4*H*-5-thia-1,6a-diazapentalen-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

The dry acetonitrile (92 mL) solution of 4*H*-5-thia-1,6a-diazapentalen-2-carbaldehyde (1.7 g) was added to the dry acetinitrile (92 mL) solution of $MgBr_2$ (5.0 g) under a nitrogen atmosphere at room temperature then the mixture was stirred for 10 min. The dry THF (184 mL) solution of *p*-nitrobenzyl (5R, 6S)-6-bromopenem-3-carboxylate (4.3 g) was added and the mixture was cooled to –20 °C then triethylamine (7.4 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with 4-dimethylamino pyridine (138 mg) and acetic anhydride (2.1 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The 1mol/L Citric acid aqueous solution (1000 mL) was added to the reaction mixture and the aqueous layer was extracted with ethyl acetate (3 x 400 mL). The combined organic layers were washed with water, saturated sodium hydrogen carbonate and brine, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and crude (5*R*)-6-[acetoxy-(4*H*-5-thia-1,6a-diazapentalen-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester was obtained as a brown amorphous solid.

Freshly activated Zn dust (19.3 g) was added rapidly with 0.5 mol/L phosphate buffer (pH 6.5, 100 mL) to the THF (100 mL) solution of crude (5*R*)-6-[acetoxy-(4*H*-5-thia-1,6a-diazapentalen-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2.5 h at room temperature. The reaction solution was filtered through a pad of Celite and the pad was washed with water (200 mL) and *n*-butanol (200 mL). The aqueous layer was separated and then the organic layer was extracted with 0.5 mol/L phosphate buffer (pH 6.5, 2 x 50 mL). The combined aqueous layers were concentrated to 90 g, 1mol/L NaOH was added to adjust pH to 8.0 and applied to Diaion HP-21 resin (180 mL, Mitsubishi Kasei Co. Ltd.) column chromatography.

After adsorbing, the column was eluted with water and then 15% acetonitrile aqueous solution. The combined active fractions were concentrated under high vacuum at 35°C and lyophilized to give the title compound as a yellow amorphous solid (634 mg, 17.4%, pH 7.25).

Mp 150 °C (dec); ^{1}H NMR (D_2O) δ4.00 (s, 2H), 5.09 (s, 2H), 6.14 (s, 1H), 6.36 (s, 1H), 6.91 (s, 1H), 6.92 (s, 1H); IR (KBr) 3381, 1752, 1683, 1600, 1558 cm^{-1}; λ^{max} (H_2O) 292, 196 nm.

Example 13

Preparation of (5*R*)(6*Z*)-6-(7*H*-Imidazo[1,2-c]thiazol-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Thiazolidin-4-one

The titled compound was prepared in the same way of Marvin M. and Allen R. Harkness. (*Tetrahedron Letters.* **1994**, *35*, 6971-6974).

Step 2: Thiazolidine-4-thione

Lawesson's reagents (33.5 g) added to the solution of thiazolidin-4-one (14.2 g) in dry THF (690 mL) and the reaction mixture was refluxed for 2 h. The mixture was cooled to room temperature and evaporated under reduced pressure. The residue was triturated with $CHCl_3$:MeOH=7:3 solution (65 mL) at room temperature for 30 min. The precipitate was filtered off, washed with $CHCl_3$:*n*-hexane=7:3 solution (15 mL) and dried in vacuo. The thiazolidine-4-thione was obtained as a pale yellow powder (10.7 g, 65%).

^{1}H NMR ($CDCl_3$) δ 4.08 (s, 2H), 4.70 (s, 2H).

Step 3: 4-Methylthio-2,5-dihydro-thiazole

Methyl iodide (28.4 g) was added to the boiling solution of thiazolidine-4-thione (9.5 g) in chloroform (400 mL), and the reaction mixture was refluxed for 1.5 h. To the reaction mixture, an additional methyl iodide (56.8 g) was added in 5 portions at 30-60 min intervals. After refluxing for additional 1 h, the reaction mixture was cooled to room temperature. Then 10% potassium carbonate aqueous solution (200 mL) was added and stirred for 15min at room temperature. After separating organic layer, the aqueous layer was extracted with $CHCl_3$ (100 mL x 3). Organic layers were combined, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and dried in vacuo. After drying, the title compound was obtained as brown oil (11.0 g, quant.).

^{1}H NMR ($CDCl_3$) δ 2.51 (s, 3H), 3.91 (t, 2H, *J* = 3.5 Hz), 5.21 (t, 2H, *J* = 3.5 Hz).

Step 4: Thiazolidin-4-ylideneamine

A mixture of 4-methylthio-2,5-dihydrothiazole (10.7 g) and ammonium chloride (6.4 g) in dry ethanol (400 mL) was refluxed for 27.5 h. The reaction mixture was cooled to room temperature and evaporated under reduced pressure. The residue was dissolved in chloroform (300 mL) and 10% potassium carbonate aqueous solution (200 mL), then stirred for 20 min at room temperature. After separating organic layer, the aqueous layer was extracted with chloroform (100 mL x 5). Organic layers were combined, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and dried in vacuo to obtain crude thiazolidin-4-ylideneamine (5.5g) as a brown solid that included by product, which is an ethoxy derivative and 4-methylthio-2,5-dihydrothiazole, which is the starting material . The ratio of these three compounds was determined to be 61:34:5 respectively by ^{1}H-NMR.

^{1}H NMR ($CDCl_3$) δ 3.75 (t, 2H, *J* = 2.8 Hz), 4.97 (t, 2H, *J* = 2.9 Hz).

Step 5: 7*H*-Imidazo[1,2-c]thiazole-2-carbaldehyde

The solution of 2-bromo-3-isopropoxypropenal (6.9 g) in dry acetonitrile (326 mL) was added to the solution of crude thiazolidin-4-ylideneamine (3.3 g) in dry acetonitrile (326 mL) at room temperature. The reaction mixture was stirred at room temperature for 19.5 h, added triethylamine (4.9 mL) and then refluxed for 2 h. The reaction mixture was cooled to room temperature and then evaporated under reduce pressure. The residue was dissolved in dichloromethane (300 mL) and washed with 50% potassium carbonate aqueous solution (20 g). After filtration and separation, the aqueous layer was extracted with dichloromethane (50 mL x 4). The organic layers were combined, dried ($MgSO_4$) and filtered. The filtrate was evaporated under reduced pressure. The residue was applied to silica gel column chromatography and eluted with $CHCl_3$ – MeOH (100 : 3) to obtain crude 7*H*-Imidazo[1,2-c]thiazole-2-carbaldehyde as a brown solid. The crude product was re-crystallized twice from $CHCl_3$ – *n*-hexane (1st: 30:5, 2nd: 30:60) at 0 °C to give the required aldehyde as pale brown crystals (Yield: 1.84 g, 15 %).

^{1}H NMR ($CDCl_3$) δ 4.09 (t, 2H, *J* = 1.3 Hz), 5.08 (t, 2H, *J* = 1.2 Hz), 7.63 (s, 1H), 9.81 (s, 1H).

Step 6: (5*R*)(6*Z*)-6-(7*H*-Imidazo[1,2-c]thiazol-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo

[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

7*H*-Imidazo[1,2-c]thiazole-2-carbaldehyde (841 mg) was added to the dry acetonitrile (116 mL) solution of anhydrous $MgBr_2$ (2.93 g) under a nitrogen atmosphere at room temperature. The dry THF solution (116 mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (cont. 99.7%) (2.51 g) was added to the mixture, cooled to –20 °C, and Et_3N (2.20 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 4 h at –20 °C and treated with acetic anhydride (1.26 mL) and DMAP (160 mg) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, water and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure.

The residue was dissolved in THF (53 mL) and acetonitrile (25 mL). Freshly activated Zn dust (15.1 g) and 0.5 *M* phosphate buffer (pH 6.5, 78 mL) were added to the mixture. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 h at room temperature. The reaction mixture was filtered through a pad of Celite. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was cooled to 3 °C and 1 *M* NaOH was added to adjust pH to 8.0. The mixture was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (321 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN (1/0 - 9/1). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (1.1 g, 51%, pH 7.5).

Mp 145 °C (dec); ^1H NMR (D_2O) δ 3.85 (s, 2H), 4.88 (s, 2H), 6.32 (s, 1H), 6.78 (s, 1H), 6.85 (s, 1H), 7.27 (s, 1H).

Example 14

Preparation of (5R,6Z)-7-oxo-6-[(4-oxo-6,7-dihydro-4H-pyrazolo[5,1-c][1,4]oxazin-2-yl)methylene]-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid.

Step 1: Diethyl 1-(2-{[tert-butyl(dimethyl)silyl]oxy}ethyl)-1H-pyrazole-3,5-dicarboxylate

To a solution of diethyl 3,5-pyrazoledicarboxylate (2.17 g, 10 mmol) in acetonitrile (10 ml), under nitrogen, was added potassium carbonate (2.07 g, 15 mmol), and 2-bromoethoxy-t-butyldimethylsilane (2.90 g, 12 mmol). The mixture was stirred at reflux for 18 hr. It was then cooled to room temperature, diluted with ethyl acetate (20 ml), and filtered through Magnesol. The filter pad was eluted wtih 2 x 10 ml of ethyl acetate, and the combined filtrate was evaporated. The residue was dissolved in hexanes and passed through a column of silica gel (70 g). After eluting with hexanes (100 ml), the column was eluted with ethyl acetate. The ethyl acetate eluent was evaporated to give 3.71 g of a colorless oil; MS m/e 371 (MH^+).

Step 2: 1-(2-{[tert-butyl(dimethyl)silyl]oxy}ethyl)-1H-pyrazole-3,5-dimethanol

To a solution of diethyl 1-(2-{[tert-butyl(dimethyl)silyl]oxy}ethyl)-1H-pyrazole-3,5-dicarboxylate (0.74 g, 2 mmol) in methylene chloride (8 ml), under nitrogen, was added 12 ml of a 1.0 M solution of diisobutylaluminum hydride in methylene chloride at 0 °C. After stirring at 0 °C for 0.5 hr, the mixture waas warmed to room temperature for 0.5 hr. It was then quenched with 15 ml of saturated ammonium chloride solution and extracted with ethyl acetate. The organic extract was washed with brine, dried over anhydrous sodium sulfate, and evaporated to give 0.44 g of a white solid; mp 82-83 °C; MS m/e 287 (MH^+).

Step 3: 1-(2-{[tert-butyl(dimethyl)silyl]oxy}ethyl)-1H-pyrazole-3,5-dicarbaldehyde

To a stirred solution of 1-(2-{[tert-butyl(dimethyl)silyl]oxy}ethyl)-1H-pyrazole-3,5-dimethanol (1.18 g, 4 mmol) in methylene chloride (20 ml), was added 4-methylmorpholine-N-oxide (2.89 g, 24 mmol) and molecular sieve 4A (4 g). The reaction mixture was stirred at room temperature for 10 min. and then treated with tetrapropylammonium peruthenate (0.15 g, 0.4 mmol). Stirring was continued for 2 hr. The methylene chloride solution was concentrated and diluted with ether (40 ml). The mixture was filtered through a pad of silica gel (40 g) and the filter pad was eluted with 2 x 20 ml ether. The combined eluent was washed with 1N HCl and brine, dried over anhydrous sodium sulfate, and evaporated to give 0.79 g of a white solid; mp 63-64 °C; MS m/e 283 (MH^+).

Step 4: 4-oxo-6,7-dihydro-4H-pyrazolo[5,1-c][1,4]oxazine-2-carbaldehyde

To a solution of 1-(2-{[tert-butyl(dimethyl)silyl]oxy}ethyl)-1H-pyrazole-3,5-dicarbaldehyde (1.02 g, 6.07 mmol) in THF (30 ml) was added 6.68 ml of a 1.0 M solution of tetrabutylammonium fluoride in THF at 0 °C. After stirring for 1 hr, the mixture was treated with 10 ml of saturated ammonium chloride solution and extracted with ethyl acetate. The organic solution was washed with brine, dried over anhydrous sodium sulfate, filtered through Magnesol and evaporated. The crude gum was washed with hexanes, dried in vacuo, and then dissolved in methylene chloride (20 ml). To this solution was added 4-methylmorpholine-N-oxide (2.89 g, 24 mmol) and molecular sieve 4A (6 g). The mixture was stirred at room temperature for 10 min. and then treated with tetrapropylammonium peruthenate (0.11 g, 0.3 mmol). Stirring was continued for 2 hr. The methylene chloride solution was concentrated and diluted with ethyl acetate (40 ml). The mixture was filtered through a pad of silica gel (40 g) and the filter pad was eluted with 2 x 20 ml ethyl acetate. The combined eluent was washed with 1N HCl and brine, dried over anhydrous sodium sulfate, and evaporated to give 0.30 g of a white solid; mp 135-136 °C; MS m/e 167 (MH^+).

Step 5: 4-nitrobenzyl (5R)-6-[(acetyloxy)(4-oxo-6,7-dihydro-4H-pyrazolo[5,1-c][1,4]oxazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate

To a solution of $MgBr_2$ (0.46 g, 2.52 mmol) in acetonitrile (13 ml) under nitrogen was added 4-oxo-6,7-dihydro-4H-pyrazolo[5,1-c][1,4]oxazine-2-carbaldehyde (0.14 g, 0.84 mmol) at room temperature with stirring. A solution of (5R,6S)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (0.32 g, 0.84 mmole) in THF (13 ml) was then added, and the mixture was cooled to –20 °C. Triethylamine (0.35 ml, 2.52 mmol) was introduced, and the mixture was stirred at –20 °C in the dark for 4 hr. It was then treated with acetic anhydride (0.2 ml, 2.0 mmol), and 4-N,N-dimethylaminopyridine (12 mg, 0.1 mmol), and kept at 0 °C for 18 hr. The mixture was concentrated and the residue was dissolved in ethyl acetate. The ethyl acetate solution was washed with 5% citric acid, saturated sodium bicarbonate solution, and brine, dried over anhydrous sodium sulfate, and evaporated. The crude material was chromatographed with silica gel (EtOAc-CH2Cl2/1:5) to give 0.27 g of an off-white solid; mp 107-110 °C; MS m/e 595 (MH^+).

Step 6: (5R,6Z)-7-oxo-6-[(4-oxo-6,7-dihydro-4H-pyrazolo[5,1-c][1,4]oxazin-2-yl)methylene]-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid

To a solution of 4-nitrobenzyl (5R)-6-[(acetyloxy)(4-oxo-6,7-dihydro-4H-pyrazolo[5,1-c][1,4]oxazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (0.22 g, 0.37 mmol) in THF (15 ml), under nitrogen, was added 15 ml of a phosphate buffer solution (0.5M, pH 6.5), and 80 mg of 10% Pd/C. The mixture was hydrogenated at 40-50 psi for3 hr, and then filtered through Celite. The filter pad was washed with THF, and the filtrate was extracted with ethyl acetate. The organic extract was dried over anhydrous magnesium sulfate and evaporated. The residue was washed with ether to give 0.07 g of a yellow solid; MS m/e 320 (MH^+); ^1H NMR (DMSO-d_6) δ 4.55-4.57 (m, 2H), 4.76-4.80 (m, 2H), 6.50 (s, 1H), 6.63 (s, 1H), 7.58 (s, 1H), 7.76 (s, 1H).

EXAMPLE 15

Preparation of 6-(6,7-Dihydro-4H-thieno[3,2-c]pyran-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

STEP 1: PREPARATION OF 6,7-DIHYDRO-4H-THIENO[3,2-C]PYRAN-2-CARBALDEHYDE

POCl3 (3.83ml, 50mmol) was added dropwise to ice cooled DMF (3.85ml, 50mmol) within 3 minutes. DCM (20ml) was added and the bath was removed when the reaction media appeared to be pasty. The reaction was kept at 23oC for 2 hrs. Then it was cooled to 0oC again. 4H-pyran-4-one (5 gram, 50mmol) in 10ml DCM was then added dropwise within 3 minutes. The reaction was kept at 0oC for 2 hrs. Pour the mixture onto ice and sodium acetate solution and extract with DCM (2x200). The combined organic layers were dried over magnesium sulfate. Filter off the drying agent and concentrate gave 5.0 gram of product. The compound was dissolved in DCM (200ml) and was added 6.0 gram of ethyl 2-6,7-Dihydro-4H-thieno[3,2-c]thiopyran-2-carbaldehyde-acetate and 10 ml TEA. The mixture was refluxed for 18 hrs. Then it was washed with water and dried over magnesium sulfate. It was then filtered, concentrated and flash chromatographed with 20 ethyl acetate in hexane. The collected material was dissolved in 100ml THF and LAH (150ml, 0.5M in THF) was injected and left at 23oC for 10 minutes. Then it was refluxed for 18 hrs. Quenched at 23oC by adding water and eventually 1N HCl to clear up the mixture.

Extract with ethyl acetate (2x200ml) and combined organic layers dried over magnesium sulfate. Filter and concentrate gave 2.3gram product. The crude material was dissolved in DCM (300ml) and manganese dioxide (15 gram was added). The reaction was carried on at 23oC for 0.5 hr. Then 2x15 gram of oxidant was added each half an hour later. The material was then filtered through a pad of celite concentrated. Flash column chromatography gave 1.206gram (14% yield) oil product.

H-NMR:δ 9.84(s, 1H), 7.41(s, 1H), 4.74 (s, 2H), 4.00 (t, 2H, J=5.6 Hz), 2.96 (t, 2H, J=5.6Hz); MS: 169.1(M+H)

Step 2: Preparation of 6-(6,7-Dihydro-4H-thieno[3,2-c]pyran-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

6,7-Dihydro-4H-thieno[3,2-c]pyran-2-carbaldehyde (336mg, 2mmol) was dissolved in 20ml acetonitrile and magnesium bromide (516 mg, 2mmol) was then added under N2 atmosphere. The mixture was stirred at 23oC for half an hour. 6-Bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (770mg, 2mmol) in 20ml THF was then injected all at once and the mixture was immediately cooled to –20oC/ Triethylamine (1ml) was then injected and the mixture stirred at –20oC for three hrs. Then acetic anhydride (0.4ml) was injected and the mixture was stirred at 0oC for 18 hrs. The reaction media was then diluted with 400ml ethyl acetate and washed with 100 ml 5% citric acid, 100 ml saturated sodium bicarbonate, and 100ml brine. The organic layer was then dried over magnesium sulfate, filtered and concentrated. Flash column chromatography using 20% ethyl acetate in hexane gave 491mg (41%) product. This product was then dissolved in 15ml THF and 15ml aqueous phosphate buffer (pH=6.5). The mixture was then subjected to 45psi hydrogen for one hour with 0.5gram 10% palladium on carbon. Then it was filtered through a pad of celite and concentrated in vacuo to remove most of the THF. The solution was then cooled to zero degree and basified to pH=8 with 1 N sodium hydroxide. Then it was purified via reverse phase HPLC using 2 liter of water followed by 5% acetonitrile in water. Water was then removed through concentrate in vacuo and 100 mg (38%) of product was collected; MP: >250^0 C;

H-NMR: δ 7.36 (s, 1H), 7.15(s, 1H), 6.55(s, 1H), 6.44(s, 1H), 4.61 (s, 2H), 3.88(m, 2H), 2.86 (m, 2H), 2.27 (m, 2H), 1.43 (t, 3H)

MS: 320.3(M-H)

EXAMPLE 16

Preparation of 6-(6,7-Dihydro-4H-thieno[3,2-c]thiopyran-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

STEP 1: PREPARATION OF 6,7-DIHYDRO-4H-THIENO[3,2-C]THIOPYRAN-2-CARBALDEHYDE

POCl3 (4.02ml, 43mmol) was added dropwise to ice cooled DMF (3.34ml, 43mmol) within 3 minutes. DCM (20ml) was added and the bath was removed when the reaction media appeared to be pasty. The reaction was kept at 23oC for 2 hrs. Then it was cooled to 0oC again. Tetrahydro-thiopyran-4-one (5 gram, 43mmol) in 10ml DCM was then added dropwise within 3 minutes. The reaction was kept at 0oC for 2 hrs. Dilute with DCM (250 ml) and then wash with ice cold 200ml saturated sodium acetate aqueous solution. The organic layer was dried over sodium sulfate. Filter off the drying agent, concentrate and flash column chromatography using 10% ethyl acetate in hexane gave 1.3 gram (8mmol) of product. The compound was dissolved in DCM (100ml) and was added 1.2ml (11 mmol) of ethyl 2-mercapto-acetate and 1ml TEA. The mixture was refluxed for 18 hrs. Then it was washed with water and dried over magnesium sulfate. Filter, concentrate and flash chromatograph with 20 ethyl acetate in hexane produced 1.1gram (11% yield) of product

H-NMR:δ 6.68(s, 1H), 4.73 (s, 2H), 3.68(s, 2H), 3.04 (t, 2H, J=7.6 Hz), 2.91 (t, 2H, J=7.6Hz).; MS (EI): 185.99 (M+)

The 1.1 gram (4.8mmol) 6,7-Dihydro-4H-thieno[3,2-c]thiopyran-2-carboxylic acid ethyl ester was dissolved in 100ml THF and LAH (40ml, 0.5M in DMG) was injected and the reaction was left at 23oC for 10 minutes. Then it was refluxed for 18 hrs. Quenched at 23oC with water (10ml). The organic layer decanted and the remaining was washed with 20ml DCM. The combined organic layers dried over sodium sulfate. Filter, concentrate and flash column chromatograph with 10-20% ethyl acetate produced 940mg crude product. This crude material was dissolved in DCM (40ml) and manganese dioxide (2 gram was added). The reaction was carried on at 23oC for half an hour. The material was then filtered through a pad of celite concentrated. Flash column chromatography gave 320mg (36%) product.

H-NMR:δ 9.82(s, 1H), 7.46 (s, 1H), 3.56 (s, 2H), 3.15 (t, 2H, J=7.2 Hz), 2.95 (t, 2H, J=7.2 Hz).; MS (EI): 228.02 (M+)

Step 2: Preparation of 6-(6,7-Dihydro-4H-thieno[3,2-c]thiopyran-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

6,7-Dihydro-4H-thieno[3,2-c]thiopyran-2-carbaldehyde (320mg, 1.72mmol) was dissolved in 17ml acetonitrile and magnesium bromide etherate (450 mg, 1.74mmol) was then added under N2 atmosphere. The mixture was stirred at 23oC for half an hour. 6-Bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (660mg, 1.72mmol) in 17ml THF was then injected all at once and the mixture was immediately cooled to –20oC/ Triethylamine (1ml) was then injected and the mixture stirred at –20oC for three hrs. Then acetic anhydride (0.4ml) was injected and the mixture was stirred at 0oC for 18 hrs. The reaction media was then diluted with 400ml ethyl acetate and washed with 100 ml 5% citric acid, 100 ml saturated sodium bicarbonate, and 100ml brine. The organic layer was then dried over magnesium sulfate, filtered and concentrated. Flash column chromatography using 20% ethyl acetate in hexane gave 461mg (44%) product. This product was then dissolved in 20ml THF and 20ml aqueous phosphate buffer (pH=6.5). The mixture was then subjected to 40psi hydrogen for one hour and half with 0.5gram 10% palladium on carbon. Then it was filtered through a pad of celite and concentrated in vacuo to remove most of the THF. The solution was then cooled to zero degree and basified to pH=8 with 1 N sodium hydroxide. Then it was purified via reverse phase HPLC using 2 liter of water followed by 5% acetonitrile in water. Water was then removed through concentrate in vacuo and 21 mg (8.6%) of product was collected.

MP: >250^0 C

H-NMR: 7.34 (s, 1H), 7.18(s, 1H), 6.59(s, 1H), 6.44(s, 1H), 3.71 (s, 2H), 2.93(s, 2H), 2.50 (s, 2H).; MS: 338.0(M+H)

EXAMPLE 17

Preparation of 6-(5-Methyl-4,5,6,7-tetrahydro-thieno[3,2-c]pyridin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step 1: Preparation of (5-Methyl-4,5,6,7-tetrahydro-thieno[3,2-c]pyridin-2-yl)-methanol

6,7-Dihydro-4H-thieno[3,2-c]pyridine-2,5-dicarboxylic acid diethyl ester (46 gram, 163mmol) was dissolved in 200ml THF. The solution was injected LAH (1M, THF) 300ml at 23oC. Then it was stirred at 23oC for 18 hrs. The reaction was quenched with 10ml water and dried directly over sodium sulfate. Filter and concentrate yielded 29.3 gram (160mmol, 98%) crude product.

H-NMR: 6.55(s, 1H), 4.70 (s, 2H), 3.41 (s, 2H), 2.86 (t, 2H, J=5.6 Hz), 2.73 (t, 2H, J=5.6 Hz), 2.38 (s, 3H); MS: 184.0(M+H)

Step 2: Preparation of 5-Methyl-4,5,6,7-tetrahydro-thieno[3,2-c]pyridine-2-carbaldehyde

DMSO (1.7ml, 24mmol) in 5ml CH_2Cl_2 was cooled to –50-60oC. Oxalyl chloride (1ml, 11mmol) in 20ml DCM was then added within 5 minutes at 50oC. The mixture was kept at –50oC for 5 minutes and then 1.67 gram (9mmol) of (5-Methyl-4,5,6,7-tetrahydro-thieno[3,2-c]pyridin-2-yl)-methanol in 20ml DCM was added at 50oC and the mixture was stirred for another 15minutes at 50oC. Triethylamine (7ml) was then added at –50oC and after 5 minutes the bath was removed and the mixture is naturally warmed up to 23oC. It was washed with 100ml water and extracted with 100ml ethyl acetate. The combined organic layers were dried over magnesium sulfate. Filter. Concentrate and flash column chromatograph using 0-15% methanol in ethyl acetate yielded 736mg (45% yield) product.

H-NMR: 9.81(s, 1H), 7.42 (s, 1H), 3.56 (s, 2H), 3.00 (t, 2H, J=5.6 Hz), 2.91 (t, 2H, J=5.6 Hz), 2.51 (s, 3H); MS: 182.1(M+H)

Step 3: Preparation of 6-(5-Methyl-4,5,6,7-tetrahydro-thieno[3,2-c]pyridin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

2-formyl-6,7-dihydro-4H-thieno[3,2-c]pyridine-5-carboxylic acid ethyl ester (724mg, 4mmol) was dissolved in 40ml acetonitrile and magnesium bromide etherate (1.2 gram, 4.65mmol) was then added under N2 atmosphere. The mixture was stirred at 23oC for half an hour. 6-Bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.54gram, 4mmol) in 40ml THF was then injected all at once and the mixture was immediately cooled to –20oC. Triethylamine (2ml) was then injected and the mixture stirred at –20oC for 3 hrs. Then acetic anhydride (0.66ml) was injected and the mixture was stirred at 0oC for 48 hrs. The reaction media was then diluted with 500ml ethyl acetate and washed with 50 ml 5% citric acid, 50 ml saturated sodium bicarbonate, and 50ml brine. Another 300ml ethyl

acetate was used to wash each aqueous solution. The combined organic layers were then dried over sodium sulfate. Filter, concentrate, and flash column chromatograph using 20% ethyl acetate in hexane gave 1.56 gram (64% yield) product. This product was then dissolved in 20ml THF and 20ml aqueous phosphate buffer (pH=6.5). The mixture was then subjected to 40psi hydrogen for two hrs with 0.5gram 10% palladium on carbon. Then it was filtered through a pad of celite and concentrated in vacuo to remove most of the THF. The solution was then cooled to zero degree and basified to pH=8 with 1 N sodium hydroxide. Then it was purified via reverse phase HPLC using 2 liter of water followed by 5% acetonitrile in water. Water was then removed through concentrate in vacuo and 112 mg (13%) of product was collected.

MP: >250oC

H-NMR: δ 7.48 (s, 1H), 7.37(s, 1H), 7.21(s, 1H), 7.10(s, 1H), 3.41(s, 2H), 2.88 (s, 2H), 2.68(s, 2H), 2.37(s, 3H); MS: 335.0(M+H)

EXAMPLE 18

Preparation of 2-(2-Carboxy-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-en-6-ylidenemethyl)-6,7-dihydro-4H-thieno[3,2-c]pyridine-5-carboxylic acid ethyl ester

2-Formyl-6,7-dihydro-4H-thieno[3,2-c]pyridine-5-carboxylic acid ethyl ester (480mg, 2mmol) was dissolved in 20ml acetonitrile and magnesium bromide etherate (516mg, 2mmol) was then added under N2 atmosphere. The mixture was stirred at 23oC for half an hour. 6-Bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (770mg, 2mmol) in 20ml THF was then injected all at once and the mixture was immediately cooled to –20oC. Triethylamine (1ml) was then injected and the mixture stirred at –20oC for 3 hrs. Then acetic anhydride (0.4ml) was injected and the mixture was stirred at 0oC for 48 hrs. The reaction media was then diluted with 200ml ethyl acetate and washed sequentially with 50 ml 5% citric acid, 50 ml saturated sodium bicarbonate, and 50ml brine. The organic layer was then dried over sodium sulfate. Filter, concentrate, and flash column chromatograph using 20% ethyl acetate in hexane gave 690mg (50%, yield) product. A fraction of this product (456mg, 0.69mmol) was then dissolved in 15ml THF and 15ml aqueous phosphate buffer (pH=6.5). The mixture was then subjected to 40psi

hydrogen for two hrs with 0.5gram 10% palladium on carbon. Then it was filtered through a pad of celite and concentrated in vacuo to remove most of the THF. The solution was then cooled to zero degree and basified to pH=8 with 1 N sodium hydroxide. Then it was purified via reverse phase HPLC using 2 liter of water followed by 5% acetonitrile in water. Water was then removed through concentrate in vacuo and 18 mg (5%) of product was collected.

MP: >250oC

H-NMR: 7.35 (s, 1H), 7.24 (s, 1H), 6.61 (s, 1H), 6.45(s, 1H), 4.48 (s, 2H), 4.08 (quartet, 2H, J=7.2Hz), 3.68 (m, 2H), 2.87(m, 2H), 1.20 (t, 3H, J=7.2Hz); MS: 393.0(M+H)

EXAMPLE 19

Preparation of 7-Oxo-6-(6,7,8,9-tetrahydro-5H-imidazo[1,2-a]azepin-2-ylmethylene)-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

STEP 1: PREPARATION OF 6,7,8,9-TETRAHYDRO-5H-IMIDAZO[1,2-A]AZEPINE-2-CARBALDEHYDE

Thiocaprolactam (6.45 gram, 50mmol) was dissolved in 400ml CH_2Cl_2 and methyl iodide (16ml, 5eq) was next added. The mixture was stirred under nitrogen for 18 hrs. Then it was treated with 100ml potassium carbonate (50%, aq.). The organic layer was then dried over magnesium sulfate. After filtration and concentration 7.3 gram of material was obtained. This material was dissolved 300ml ethanol and 2.83 gram of ammonium chloride was added. The mixture was refluxed for 1 hr. Then the solvent was removed in vacuo. Half of the material was added 200ml ethanol and then followed by addition of 1.35gram (25mmol) sodium methoxide and 4.8gram (25mmol) 2-Bromo-3-isopropoxy-propenal and the mixture was stirred at 23oC for 2 hrs. Then the solvent was removed and 200ml chloroform was added along with 10ml triethyl amine. The mixture was refluxed for 2 hrs and then cooled to 23oC. The reaction media was partitioned between 300ml DCM and 2x150 potassium carbonate (50%). The organic layer was dried over magnesium sulfate. After filtration and concentration 2.1gram of oil product was obtained.

H-NMR: 9.62 (s, 1H), 7.60 (s, 1H), 6.61 (s, 1H), 6.45(s, 1H), 4.58 (s, 2H), 2.96 (2m, H), 1.90(m, 2H), 1.72 (m, 2H); MS: 164.9(M+H)

Step 2: Preparation of 7-Oxo-6-(6,7,8,9-tetrahydro-5H-imidazo[1,2-a]azepin-2-ylmethylene)-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

6,7,8,9-Tetrahydro-5H-imidazo[1,2-a]azepine-2-carbaldehyde (1.312gram, 8mmol) was dissolved in 80ml acetonitrile and magnesium bromide etherate (2.94gram, 8mmol) was then added under N2 atmosphere. The mixture was stirred at 23oC for half an hour. 6-Bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.155gram, 3mmol) in 60ml THF was then injected all at once and the mixture was immediately cooled to –20oC. Triethylamine (4ml) was then injected and the mixture stirred at –20oC for 4 hrs. Then acetic anhydride (1ml) was injected and the mixture was stirred at 0oC for 20 hrs. The reaction media was then diluted with 500ml ethyl acetate and washed with 100ml 5% citric acid, 100 ml saturated sodium bicarbonate, and 100ml brine. The organic layer was then dried over sodium sulfate. Filter, concentrate, and flash column chromatograph using 20% ethyl acetate in hexane gave 800mg product. This product was then dissolved in 20ml THF and 20ml aqueous phosphate buffer (pH=6.5). The mixture was then subjected to 40psi hydrogen for 1 hr with 0.5gram 10% palladium on carbon. Then it was filtered through a pad of celite and concentrated in vacuo to remove most of the THF. The solution was then cooled to zero degree and basified to pH=8 with 1 N sodium hydroxide. Then it was purified via reverse phase HPLC using 2 liter of water followed by 5% acetonitrile in water. Water was then removed through concentrate in vacuo and 131 mg (31%) of product was collected.

MP: >250^0 C

H-NMR: δ 7.78 (s, 1H), 7.02 (s, 1H), 6.94 (s, 1H), 6.36 (s, 1H), 3.92(m, 2H), 2.80 (m, 2H), 1.78 (m, 2H), 1.61(m, 2H), 1.54(m, 2H); MS: 318.2(M+H).

EXAMPLE 20

Preparation of (5*R*),(6*Z*)-6-(7-Benzyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: 7-Benzyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-2-carboxylic acid ethyl ester

Et_3N (6.27 mL), PhCHO (4.92 mL) were added successively to the EtOH (81 mL) solution of 5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-2-carboxylic acid ethyl ester, hydrochloride (9.47 g) at room temperature and stirred for 3 h under a nitrogen atmosphere. Then $NaBH_3CN$ (2.97 g) was added to the reaction mixture and stirred for 19 h. The mixture was filtered through a pad of Celite and diluted with CH_2Cl_2 and washed with 50% K_2CO_3 aq. The organic layer was dried (K_2CO_3) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with $CHCl_3$ - acetone (1/0 ~ 9/1) and $CHCl_3$ - MeOH (19/1 ~ 9/1). The titled compound was obtained as pale yellow crystals (4.16 g, 36%).

^1H NMR($CDCl_3$) δ 1.36(t, 3H, *J* = 7.1 Hz), 2.87(t, 2H, *J* = 5.2 Hz), 3.71(s, 2H), 3.75(s, 2H), 4.01(m, 2H), 4.34(q, 2H, *J* = 7.1 Hz), 7.25-7.34(m, 5H), 7.51(s, 1H).

Step 2: 7-Benzyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-2-carbaldehyde

1.01 *M* solution of DIBAL in toluene (1 mL + 0.2 mL + 0.3 mL) was added to the dry CH_2Cl_2 (5 mL) solution of 7-benzyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-2-carboxylic acid ethyl ester (283 mg) under a nitrogen atmosphere at – 78 °C and stirred for 1.5 h. The mixture was quenched with 1*M* HCl (5 mL). The reaction mixture was filtered through a pad of Celite. The filtrate was washed with 50% K_2CO_3 aq. and the aqueous layer was extracted with CH_2Cl_2. The combined organic layer was dried (K_2CO_3) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with $CHCl_3$ - acetone (9/1 ~ 4/1) and $CHCl_3$ - MeOH (19/1). The titled compound was obtained as colorless crystals (148 mg, 61%).

^1H NMR($CDCl_3$) δ 2.90(t, 2H, *J* = 5.5 Hz), 3.74(s, 2H), 3.76(s, 2H), 4.06(t, 2H, *J* = 5.5 Hz), 7.28 ~ 7.35(m, 5H), 7.53(s, 1H), 9.80(s, 1H).

Step 3: (5*R*, 6*RS*)-6-[(*RS*)-Acetoxy(7-benzyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (diastereo mixture)

7-Benzyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-2-carbaldehyde (139 mg) was added to the dry acetonitrile (8.7 mL) solution of anhydrous $MgBr_2$ (325

mg) under a nitrogen atmosphere at room temperature. The dry THF solution (8.7 mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (223 mg) was added to the mixture, cooled to –20 °C, and Et_3N (0.24 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 5 h at –20 °C and treated with acetic anhydride (0.11 mL) and DMAP (7 mg) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, water and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (3/1 ~ 1/1). The titled compound was obtained as two diastereo mixture (80/20, purple amorphous solid, 233 mg, 61%).

^1H NMR($CDCl_3$) δ 1.99(s, 0.8x3H), 2.23(s, 0.2x3H), 2.83 ~ 2.89(m, 2H), 3.68(d, 2H, *J* = 4.9 Hz), 3.71(s, 2H), 3.94 ~ 4.13(m, 2H), 5.27(d, 1H, *J* = 13.6 Hz), 5.41(d, 0.2x1H, *J* = 13.6 Hz), 5.45(d, 0.8x1H, *J* = 13.6 Hz), 6.05(s, 0.2x1H), 6.28(s. 0.8x1H), 6.31(s, 0.8x1H), 6.790(s, 0.2x1H), 6.793(s, 0.2x1H), 7.01(s, 0.8x1H), 7.27 ~ 7.36(m, 5H), 7.42(s, 0.2x1H), 7.46(s, 0.8x1H), 7.61(d, 2H, *J* = 8.6 Hz), 8.22(d, 2H, *J* = 8.6 Hz).

Step 4: (5*R*),(6*Z*)-6-(7-Benzyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

(5*R*, 6*RS*)-6-[(*RS*)-Acetoxy(7-benzyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazin-2-yl)

methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl

ester (1.27 g) was dissolved in THF (55 mL) and acetonitrile (25 mL). Freshly activated Zn dust (5.08 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 80 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered through a pad of Celite. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was cooled to 3 °C and 1 *M* NaOH was added to adjust pH to 8.0. The mixture was concentrated

under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (79 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN(1/0 ~ 4/1). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (390 mg, 49%, pH 7.7).

Mp 180 °C (dec); ^{1}H NMR(D_2O) δ 2.84 ~ 2.95(m, 2H), 3.61(d, 2H, *J* = 7.2 Hz), 3.67(s, 2H), 3.96(t, 2H, *J* = 5.7 Hz), 6.43(s, 1H), 6.89(s, 1H), 6.93(s, 1H), 7.28 ~7.37(m, 6H).

Example 21

Preparation of (5R,6Z)-7-oxo-6-{[5-(pyridin-3-ylmethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)]methylene}-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid:

Step 1: 2-Formyl [5-(pyridin-3-ylmethyl)-4,5,6,7-tetrahydrothieno][3,2-c]pyridine:

To a stirred solution of 2-(formyl)-6,7-dihydrothieno[3,2-c]-5(4H)-pyridine (1.05 g, 5.2 mmol) in DMF (20 ml) , 3-picolyl chloride hydrochloride (0.852 g, 5.2 mmol) and N,N-diisopropylethylamine (10 ml, excess) was added at room temperature. The reaction mixture was stirred for 24 hrs and quenched with water. The reaction mixture was extracted with chloroform; washed well with water and dried over anhydrous $MgSO_4$. It was filtered and concentrated. The product was purified by SiO_2 column chromatography by eluting it with ethylacetate. Pale yellow semi-solid. Yield: 800 mg , 59%; M+H 259.

Step 2: 4-Nitrobenzy-6-[(acetyloxy)[5(pyridin-3-ylmethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

2-Formyl [5-(pyridin-3-ylmethyl)-4,5,6,7-tetrahydrothieno][3,2-c]pyridine (516 mg, 2.0 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (772 mg, 2.0 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (390 mg, 1.5 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was

warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 700 mg, 51%; M+H 685 and 687.

Step-3: (5R,6Z)-6-{[5-(pyridin-3-ylmethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)]methylene}-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid:

4-Nitrobenzy-6-[(acetyloxy)[5(pyridin-3-ylmethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (686 mg, 1.0 mmol) was dissolved in THF (20 mL) and acetonitrile (10 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 0.1 *M* NaOH was added to adjust pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 lits) and latter with 10% CAN: Water. The fractions containing the product were collected and concentrated at reduced pressure at room temperature. The yellow solid was washed with acetone and filtered. Dried. Yield: 50 mg, 12%; as yellow crystals; mp. 134-136^{0}C; (M+H) 412 .

^{1}H NMR (DMSO-d_6)δ d 2.8 (m, 2H), 2.92 (bm, 2H), 3.6 (m, 2H), 3.86 (s, 2H), 6.3 (s, 1H), 6.41 (s, 1H), 7.17 (s, 1H), 7.29 (s, 1H), 7.35 (m, 1H), 7.7 (m, 1H), 8.48 (d,1H), 8.54 (s, 1H).

Example 22

Preparation of (5R,6Z)-7-oxo-6-{[5-(pyridin-3-ylcarbonyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)]methylene}-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid:

Step 1: 2-Formyl [5-(pyridin-3-ylcarbonyl)-4,5,6,7-tetrahydrothieno][3,2-c]pyridine:

To a stirred solution of 2-(formyl)-6,7-dihydrothieno[3,2-c]-5(4H)-pyridine (606 mg, 3.0 mmol) in DMF (20 ml) , nicotinoyl chloride hydrochloride (531 mg, 3.0 mmol) and N,N-diisopropylethylamine (10 ml, excess) was added at room temperature. The reaction mixture was stirred for 24 hrs and quenched with water. The reaction mixture was extracted with chloroform; washed well with water and dried over anhydrous $MgSO_4$. It was filtered and concentrated. The product was purified by SiO_2 column chromatography by eluting it with ethylacetate. Pale yellow semi-solid. Yield: 600mg , 73%; M+H 273.

Step 2: 4-Nitrobenzy-6-[(acetyloxy)[5(pyridin-3-ylcarbonyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)carbonyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

2-Formyl [5-(pyridin-3-ylcarbonyl)-4,5,6,7-tetrahydrothieno][3,2-c]pyridine (400 mg, 1.4 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (772 mg, 2.0 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (619 mg, 2.4 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 300 mg, 30%; M.pt. 71^{0}C; M+H 701.

Step-3: (5R,6Z)-6-{[5-(pyridin-3-ylcarbonyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)]methylene}-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt:

4-Nitrobenzy-6-[(acetyloxy)[5(pyridin-3-ylcarbonyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (800 mg, 1.14 mmol) was dissolved in THF (20 mL) and acetonitrile (10 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 0.1 *M* NaOH was added to adjust pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 lits) and latter with 10% CAN: Water. The fractions containing the product were collected and concentrated at reduced pressure at room temperature. The yellow solid was washed with acetone and filtered. Dried. Yield: 50 mg, 12%; as yellow crystals; mp. 195 ^{0}C; (M+H) 426.

Example 23

Preparation of (5R,6Z)-7-oxo-6-{[5-(phenylacetyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)]methylene}-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid:

Step 1: 2-Formyl [5-(phenylacetyl)-4,5,6,7-tetrahydrothieno][3,2-c]pyridine:

To a stirred solution of 2-(formyl)-6,7-dihydrothieno[3,2-c]-5(4H)-pyridine (0.41 mg, 2 mmol) in DMF (20 ml) , phenyl acetyl chloride (0.35 mg, 2.2 mmol) and N,N-diisopropylethylamine (10 ml, excess) was added at room temperature. The reaction mixture was stirred for 24 hrs and quenched with water. The reaction mixture was extracted with chloroform; washed well with water and dried over anhydrous $MgSO_4$. It was filtered and concentrated. The product was purified by SiO_2 column chromatography by eluting it with ethylacetate. White solid. Yield: 510 mg , 89%; M+H 286.

Step 2: 4-Nitrobenzy-6-[(acetyloxy)[5(phenylacetyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

2-Formyl [5-(phenylacetyl)-4,5,6,7-tetrahydrothieno][3,2-c]pyridine (340 mg, 1.2 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (390 mg, 1.0 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (310 mg, 1.2 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 360 mg, 50%; M+H 713.

Step-3: (5R,6Z)-6-{[5-(phenylacetyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)]methylene}-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid:

4-Nitrobenzy-6-[(acetyloxy)[5(phenylacetyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (300 mg, 0.4 mmol) was dissolved in THF (50 mL) and 0.5 *M* phosphate buffer (pH 6.5, 28 mL). This was hydrogenated at 40 psi pressure, in the presence of 10% Pd/C (80 mg) for 2 hrs. at the end, reaction mixture was filtered through a pad of celite and concentrated. The separated yellow solid was dissolved in ethyl acetate and washed well with water. The organic layer was dried and concentrated. The separated yellow solid was triturated with diethyl ether and filtered. The yellow solid was washed well with diethyl ether and it was found to be 95% pure compound. Yield: 160 mg, 91%; Yellow solid; mp. 166-169^{0}C; (M+H) 439 .

Example 24

Preparation of (5*R*),(6*Z*)-6-(5,5-Dioxo-4,5,6,7-tetrahydro-5λ^6-pyrazolo[5,1-*c*][1,4]thiazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: 5,5-Dioxo-4,5,6,7-tetrahydro-5λ^6-pyrazolo[5,1-*c*][1,4]thiazine-2-carbaldehyde

m-Chloroperbenzoic acid (cont. 69%) (6.36 g) was added to the CH_2Cl_2 (111 mL) solution of 6,7-dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazine-2-carbaldehyde (1.86 g) at 0 °C. The reaction mixture was stirred for 0.5 h at the same temperature and stirred for 18 h at room temperature. The reaction mixture was concentrated under reduced pressure. The residue was triturated with 10 mL of THF and filtered to obtain crystals. The filtrate was concentrated under reduced pressure. The residue was triturated with 5 mL of THF and filtered to obtain crystals. The combined crystals were dried under reduced pressure to give the titled compound as colorless crystals (1.96 g, 89%).

^{1}H NMR ($CDCl_3$) δ 3.60 (t, 2H, *J* = 6.1 Hz), 4.47 (s, 2H), 4.87 (t, 2H, *J* = 6.1 Hz), 6.71 (s, 1H), 9.94 (s, 1H).

Step 2:(5*R*, 6*RS*)-6-[(*RS*)-Acetoxy-(5,5-dioxo-4,5,6,7-tetrahydro-5λ^6-pyrazolo[5,1-*c*][1,4]thiazin-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester

5,5-Dioxo-4,5,6,7-tetrahydro-5λ^6-pyrazolo[5,1-*c*][1,4]thiazine-2-carbaldehyde (1.95 g) was added to the dry acetonitrile (112 mL) solution of anhydrous $MgBr_2$ (cont. 98%) (5.48 g) under a nitrogen atmosphere at room temperature. The dry THF solution (112 mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (cont. 96.5%) (3.88 g) was added to the mixture, cooled to –20 °C, and Et_3N (cont. 99%) (3.79 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with acetic anhydride (cont. 97%) (3.79 mL) and DMAP (cont. 99%) (120 mg) in one portion. The reaction mixture was warmed to 0 °C and stirred for 16 h at 0 °C. To the reaction mixture was added acetic anhydride (cont. 97%) (0.95 mL) and DMAP (cont. 99%) (120 mg) in one portion. The mixture was diluted with ethyl acetate and washed with 5% citric

acid aqueous solution, saturated sodium hydrogen carbonate and brine. The organic layer was dried ($MgSO_4$), followed by concentration under reduced pressure. The residue was purified with a silica-gel column chromatography ($CHCl_3$: acetone = 19 :1 – 4 : 1) to give the titled compound as a pale brown amorphous solid (diastereo-mixture (8 : 2), 1.35 g, 22%).

^{1}H NMR ($CDCl_3$) δ 2.07 (s, 3H x 0.2), 2.25 (s, 3H x 0.8), 3.45 – 3.60 (m, 2H), 4.39 (d, 1H, *J* = 17.0 Hz), 4.44 (d, 1H, *J* = 17.0 Hz), 4.65 – 4.78 (m, 2H), 5.28 (d, 1H, *J* = 13.5 Hz), 5.43 (d, 1H x 0.8, *J* = 13.5 Hz), 5.44 (d, 1H x 0.2, *J* = 13.5 Hz), 6.05 (s, 1H x 0.8), 6.20 (s, 1H x 0.8), 6.22 (s, 1H x 0.2H), 6.38 (s, 1H x 0.2), 6.39 (s, 1H x 0.2), 6.79 (s, 1H x 0.8), 7.42 (s, 1H x 0.8), 7.44 (s, 1H x 0.2), 7.60 (d, 2H, *J* = 8.7 Hz), 8.24 (d, 2H, *J* = 8.7 Hz).

Step 3: (5*R*),(6*Z*)-6-(5,5-Dioxo-4,5,6,7-tetrahydro-5λ^6-pyrazolo[5,1-*c*][1,4]thiazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

(5*R*, 6*RS*)-6-[(*RS*)-Acetoxy-(5,5-dioxo-4,5,6,7-tetrahydro-5□^{6}-pyrazolo[5,1-*c*][1,4]-thiazin-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (1.33 g) was dissolved in THF (19 mL) and acetonitrile (9 mL). Freshly activated Zn dust (5.32 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 27 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 h at room temperature. The insoluble material was filtered off and was washed with H_2O (27 mL). The filtrate was added H_2O (27 mL) and washed with ethyl acetate (27 mL) and the aqueous layer was cooled to 3 °C and 1 *M* HCl was added to adjust pH to 2.5. The mixture was stirred for 1 d at the same temperature and added H_2O (55 mL), then stirred for 4 d at the same temperature. The mixture was stirred for 10 h at room temperature. The resultant mixture was cooled to 3 °C and 1 *M* NaOH was added to adjust pH to 8. The mixture was concentrated under high vacuum at 35 °C. The concentrate was treated to Diaion HP-21 (80 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN (1/0 – 9/1). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (306 mg, 38%, pH 7.4).

Mp 180 °C (dec); ^{1}H NMR (D_2O) δ 3.83 (t, 2H, *J* = 6.1 Hz), 4.68 (s, 2H), 4.72 (t, 2H, *J* = 6.1 Hz), 6.37 (s, 1H), 6.40 (s, 1H), 6.95 (s, 1H), 6.98 (s, 1H).

Example 25

Preparation of (5*R*),(6*Z*)-7-Oxo-6-(4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-2-ylmethylene)-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Piperazine-2-carboxylic acid, dihydrochloride:

The titled compound was prepared in the same way of M. T. Wu and co-workers (*Bioorg. Med. Chem. Lett.* **1993**, *3*, 2023-2028).

Step 1: Piperazine-1,3-dicarboxylic acid 1-(4-nitrobenzyl) ester

$CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$ (15.8 g) was added to the H_2O (275 mL) solution of piperazine-2-carboxylic acid, dihydrochloride (22.3 g), then the mixture was refluxed and stirred for 10 min. The insoluble material was filtered off and was washed with hot H_2O (165 mL). The filtrate was cooled to room temperature, and $NaHCO_3$ (9.2 g) and 1,4-dioxane (220 mL) was added to the dark blue solution. The mixture was cooled to 0 °C and $NaHCO_3$ (18.5 g) and 50% solution of 4-nitrobenzyl chloroformate in 1,4-dioxane (61.7 g) was added to the mixture for 0.5 h. After stirring for additional 1.5 h at 0 °C, the precipitate was filtered and washed with cold H_2O (140 mL), EtOH (100 mL), acetone (200 mL) and Et_2O (100 mL), then it was allowed to dry under reduced pressure to obtain the pale blue crystals. The crystals were added to the 1 mol/L HCl (330 mL) solution of EDTA·2Na (20.5 g) for 30 min, and stirred for 2 h at room temperature. The suspension was filtered and the filtered material was diluted with EtOH – H_2O (7 : 3, 550 mL) and refluxed for 10 min. The reaction mixture was filtered to obtain the colorless crystals. The recrystallization from the filtrate was carried out 3 times to obtain additional crystals. The combined crystals were dried under reduced pressure to obtain the titled compound (26.25 g, 77%) as colorless crystals.

^{1}H NMR (D_2O) δ 2.54 – 2.61 (m, 1H), 2.89 (dt, 2H, *J* = 12.7, 3.4 Hz), 2.97 (br, 1H), 3.13 (br, 1H), 3.62 – 4.04 (m, 2H), 5.16 (s, 2H), 7.49 (d, 2H, *J* = 8.6 Hz), 8.14 (d, 2H, *J* = 8.6 Hz).

Step 2: 5-(4-Nitrobenzyloxycarbonyl)-3-oxo-3a,4,6,7-tetrahydro-3*H*-2-oxa-1,5-diaza-7a-azoniainden-3a-ide

The H_2O (300 mL) solution of $NaNO_2$ (cont. 98.5 %) (6.66 g) was added to the acetic acid (864 mL) solution of piperazine-1,3-dicarboxylic acid 1-(4-nitrobenzyl) ester (26.72 g) under a nitrogen atmosphere at 0 °C for 0.5 h and stirred for 1 h. In addition, the H_2O (132 mL) solution of $NaNO_2$ (cont. 98.5 %) (2.41 g) was added to the solution at 0 °C for 0.5 h and stirred for 1 h. The solution was concentrated under reduced pressure and H_2O (500 mL) was added to the residue. The solution was extracted with AcOEt (5 times) and organic layer was washed with brine. The mixture was dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford crude 4-nitrosopiperazine-1,3-dicarboxylic acid 1-(4-nitrobenzyl) ester as pale brown amorphous (27.83 g (gross), 25.77 g (net), 88.2%).

The THF (10 mL) solution of trifluoroacetic anhydride (24.0 g) was added to the THF (371 mL) solution of crude 4-nitrosopiperazine-1,3-dicarboxylic acid 1-(4-nitrobenzyl) ester under a nitrogen atmosphere at 0 °C for 15 min. The solution was stirred for 1.5 h at 0 °C and for 1h at room temperature. The THF (5 mL) solution of trifluoroacetic anhydride (8.0 g) was added to the solution for 5 min and stirred for 20 h at room temperature. To the solution was added trifluoroacetic anhydride (8.0 g) for 5 min and the solution was stirred for 4h. The precipitate was filtered and washed with THF and Et_2O. The filtrate was concentrated under reduced pressure. The residue was triturated with THF, filtered and washed with Et_2O. These materials were combined and dried under reduced pressure to afford the titled compound as colorless crystals (22.3 g, 91%).

^1H NMR ($CDCl_3$) δ 4.06 (t, 2H, J = 5.4 Hz), 4.37 (t, 2H, J = 5.4 Hz), 4.63 (s, 2H), 5.30 (s, 2H), 7.54 (d, 2H, J = 8.7 Hz), 8.25 (d, 2H, J = 8.7 Hz).

Step 3: 6,7-Dihydro-4*H*-pyrazolo[1,5-*a*]pyrazine-2,5-dicarboxylic acid 2-ethyl ester 5-(4-nitrobenzyl) ester

Ethyl propiolate (cont. 99%)(8.28 g) was added to the *o*-xylene (348 mL) solution of 5-(4-nitrobenzyloxycarbonyl)-3-oxo-3a,4,6,7-tetrahydro-3*H*-2-oxa-1,5-diaza-7a-azoniainden-3a-ide (22.3 g) under a nitrogen atmosphere and refluxed for 16 h. The solution was concentrated under reduced pressure, followed by silica-gel column chromatography 3 times (*n*-hexane /AcOEt = 2/1 – 1/3). The titled compound was obtained as pale yellow crystals (16.78 g, 64%). Besides, 6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrazine-3,5-dicarboxylic acid 3-ethyl ester 5-(4-nitrobenzyl) ester was obtained as pale yellow crystals (6.18 g, 24%).

^{1}H NMR ($CDCl_3$) δ 1.39 (t, 3H, *J* = 7.1 Hz), 4.01 (t, 2H, *J* = 5.5 Hz), 4.31 (t, 2H, *J* = 5.5 Hz), 4.40 (q, 2H, *J* = 7.1 Hz), 4.79 (s, 2H), 5.29 (s, 2H), 6.64 (s, 1H), 7.54 (d, 2H, *J* = 8.6 Hz), 8.24 (d, 2H, *J* = 8.6 Hz).

Step 4: 2-Hydroxymethyl-6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrazine-5-carboxylic acid 4-nitrobenzyl ester

$LiBH_4$ (640 mg) and MeOH (1.2mL) was added to the THF (267 mL) solution of 6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrazine-2,5-dicarboxylic acid 2-ethyl ester 5-(4-nitrobenzyl) ester (10 g) under a nitrogen atmosphere at room temperature and stirred for 3 h at 40 °C. Additional $LiBH_4$ (523 mg) and MeOH (1.0 mL) was added to the solution and stirred for 1 h at 40 °C and 1 h at 50 °C. The mixture was acidified with 3 mol/L HCl to pH 2 and stirred for 1 h at room temperature, then solid K_2CO_3 was added to the solution to adjust pH to 8. The insoluble material was filtered off and the filtrate was extracted with AcOEt. The organic layer was dried (K_2CO_3), and concentrated under reduced pressure. The residue was purified with silica gel column chromatography ($CHCl_3$ / MeOH =49/1 - 19/1) to afford titled compound as pale yellow crystals (8.44 g, 95%).

^{1}H NMR ($CDCl_3$) δ 1.69 (br, 1H), 3.98 (t, 2H, *J* = 5.5 Hz), 4.19 (t, 2H, *J* = 5.5 Hz), 4.65 (s, 2H), 4.75 (s, 2H), 5.28 (s, 2H), 6.08 (s, 1H), 7.53 (d, 2H, *J* = 8.7 Hz), 8.24 (d, 2H, *J* = 8.7 Hz).

Step 5; 2-Formyl-6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrazine-5-carboxylic acid 4-nitrobenzyl ester

MnO_2 (activated) (84.2 g) was added to the $CHCl_3$-MeOH (95 : 5, 253 mL) solution of 2-hydroxymethyl-6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrazine-5-carboxylic acid 4-nitrobenzyl ester (8.42 g), and the mixture was refluxed for 1 h under a nitrogen atmosphere. The reaction mixture was filtered through a pad of Celite. Silica-gel (20 g) was added to the filtrate and the solvent was removed under reduced pressure to give the silica-gel coating with crude reactant. The above silica-gel was adsorbed to silica-gel column chromatography and the column was eluted with $CHCl_3$ - MeOH (49/1 to 19/1). The titled compound was obtained as yellow crystals (2.82 g, 34%).

^{1}H NMR ($CDCl_3$) □4.05 (t, 2H, *J* = 5.5 Hz), 4.32 (t, 2H, *J* = 5.5 Hz), 4.81 (s, 2H), 5.29 (s, 2H), 6.62 (s, 1H), 7.54 (d, 2H, *J* = 8.7 Hz), 8.24 (d, 2H, *J* = 8.7 Hz), 9.93 (s, 1H).

Step 6: 2-{(*RS*)-Acetoxy-[(5*R*, 6*RS*)-6-bromo-2-(4-nitrobenzyloxycarbonyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-en-6-yl]-methyl}-6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrazine-5-carboxylic acid 4-nitrobenzyl ester

2-Formyl-6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrazine-5-carboxylic acid 4-nitrobenzyl ester (2.71 g) was added to the dry acetonitrile (164 mL) solution of anhydrous $MgBr_2$ (cont. 98%) (6.17 g) under a nitrogen atmosphere at room temperature. The dry THF solution (164mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (cont. 96.5%) (3.27 g) was added to the mixture, cooled to –20 °C, and Et_3N (cont. 99%) (9.24 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 1.5 h at –20 °C and treated with acetic anhydride (cont. 97%) (3.19 mL) and DMAP (cont. 99%) (203 mg) in one portion. The reaction mixture was warmed to 0 °C and stirred for 1 h at 0 °C. Acetic anhydride (3.19 mL) was added to the solution and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, water and brine. The organic layer was dried ($MgSO_4$), followed by concentration under reduced pressure. The residue was purified with silica-gel column chromatography three times (*n*-hexane – AcOEt (1/1 to 2/3), $CHCl_3$ – acetone (29/1 to 19/1) and $CHCl_3$ – acetone (29/1)). The titled compound was obtained as yellow amorphous (diastereo-mixture (64 : 36), 3.30 g, 53%).

^{1}H NMR ($CDCl_3$) δ 2.06 (s, 3H x 0.36), 2.26 (s, 3H x 0.64), 3.95 – 4.04 (m, 2H), 4.18 (s, 2H), 4.73 (d, 1H, *J* = 18.2 Hz), 4.78 (d, 1H, *J* = 18.2 Hz), 5.28 (d, 1H, *J* = 13.5 Hz), 5.28 (s, 2H), 5.43 (d, 1H x 0.64, *J* = 13.5 Hz), 5.44 (d, 1H x 0.36), 6.06 (s, 1H x 0.64), 6.08 (s, 1H x 0.64), 6.24 (s, 1H x 0.36), 6.27 (s, 1H x 0.36), 6.41 (s, 1H x 0.36), 6.79 (s, 1H x 0.64), 7.42 (s, 1H x 0.64), 7.44 (s, 1H x 0.36), 7.53 (d, 2H, *J* = 8.6 Hz), 7.60 (d, 2H, *J* = 8.8 Hz), 8.24 (d, 2H, *J* = 8.8 Hz), 8.24 (d, 2H, *J* = 8.6 Hz).

Step 7: (5*R*),(6*Z*)-7-Oxo-6-(4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-2-ylmethylene)-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

To the THF (43 mL) and acetonitrile (20 mL) solution of 2-{(*RS*)-acetoxy-[(5*R*,6*RS*)-6-bromo-2-(4-nitrobenzyloxycarbonyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-en-6-yl]-methyl}-6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrazine-5-carboxylic acid 4-nitrobenzyl ester

was added Zn dust (12.36 g) rapidly with 0.5 *M* phosphate buffer (pH 6.5, 63 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 h at room temperature. The insoluble material was filtered off and was washed with H_2O (63 mL). The filtrate was washed with ethyl acetate (63 mL) and the aqueous layer was cooled to 3 °C and 1 *M* HCl was added to adjust pH to 2.5. The mixture was stirred for 4 h at the same temperature and added H_2O (63 mL) and 1 M HCl to adjust pH to 2.5, then stirred for 17 h at the same temperature. To the mixture was added 1 *M* NaOH to adjust pH to 8. The mixture was concentrated under high vacuum at 35 °C. The concentrate was treated to Diaion HP-21 (124 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN (1/0 – 95/5). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (288 mg, 22%, pH 8.8).

Mp 160 °C (dec); ^{1}H NMR (D_2O) δ 2.94(t, 2H, *J* = 5.6 Hz), 3.67 (d, 1H, *J* = 17.2 Hz), 3.70 (d, 1H, *J* = 17.2 Hz), 3.82 (t, 2H, *J* = 5.6 Hz), 5.84 (s, 1H), 6.03 (s, 1H), 6.65 (s, 1H), 6.67 (s, 1H).

Example 26

Preparation of (5*R*)(6*Z*)-6-(5,5-Dimethyl-4*H*-1,6a-diazapentalen-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt 5,5-Dimethyl-2-piperidone

5-5-Dimethyl-2-piperadinone was prepared in the method of Nagasawa (*J. Med. Chem.*, **20**, 1176 (1977)).

Step 1: 3,3-Dichloro-5,5-dimethyl-2-piperidone

To a cold (0 °C) stirred solution of 5,5-dimethyl-2-piperidone (30.2 g, 0.24 mol) in 475 mL of $CHCl_3$, PCl_5 (57.1 g, 0.26 mol) was added at such a rate that the temperature never exceeded 7 °C. After the addition was complete, stirring was continued for 10 min. Sulfuryl chloride (96.6 g, 0.72 mol) was slowly added and the mixture was heated under reflux for 1 h. The solution was concentrated under reduced pressure. The residue was cooled in ice and diluted with 250 mL of ice-water. The product was then extracted with $CHCl_3$ (6 x 250 mL) and the organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica-gel column chromatography, and then

the column was eluted with $CHCl_3$–MeOH (50 : 1). The titled compound was obtained as a white solid (41.3 g, 88.8 %). (*J. Med. Chem.*, **20**, 1176 (1977))

^1H NMR ($CDCl_3$) δ 1.17 (s, 6H), 2.76 (s, 2H), 3.19 (d, 2H, *J* = 3.0 Hz), 6.82 (brs, 1H).

Step 2: 3-Chloro-5,5-dimethyl-2-piperidone

To 40.8 g (0.21 mol) of 3,3-dichloro-5,5-dimethyl-2-piperidone dissolved in 410 mL of AcOH was added 10% Pd/C (50% wet, 6.2 g) and NaOAc-$3H_2O$ (62.4g, 0.46 mol) and the mixture was hydrogenated at 300 kPa for 20 min. The pressure of hydrogen was adjusted at 300 kPa every 5 min. The catalyst was removed by filtration and the filtrate concentrated under reduced pressure. $CHCl_3$ (400 mL) and water (300 mL) were added to the residue and the aqueous layer was neutralized with 4 mol/L NaOH. The mixture was separated and the aqueous layer was extracted with $CHCl_3$ (5 x 300 mL) and the organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica-gel column chromatography, and then the column was eluted with hexane–AcOEt (1 : 1). The titled compound was obtained as a white solid (20.4 g, 59.9 %). (*J. Med. Chem.*, **20**, 1176 (1977))

^1H NMR ($CDCl_3$) δ 1.10 (s, 3H), 1.12 (s, 3H), 2.02 (dd, 1H, *J* = 10.8, 13.6 Hz), 2.20 (ddd, 1H, *J* = 2.2, 6.7, 13.6 Hz), 2.97 (ddd, 1H, *J* = 2.3, 3.9, 12.1 Hz), 3.22 (d, 1H, *J* = 12.1 Hz), 4.44 (dd, 1H, *J* = 6.8, 10.7 Hz), 6.66 (brs, 1H).

Step 3: 4,4-Dimethylpyrrolidine-2-carboxylic acid

A suspension of 20.4 g (0.13 mol) of 3-chloro-5,5-dimethyl-2-piperidone and 45.2 g (0.14 mol) of $Ba(OH)_3$-$8H_2O$ in 252 mL of water was heated in a Parr apparatus at 150 °C for 6 h. Then, 18.6 g (0.14 mol) of ammonium sulphate were added. The precipitate was filtered off, and the solution was concentrated under reduced pressure to dryness. Crude 4,4-dimethylpyrrolidine-2-carboxylic acid was obtained as a white solid (37.5 g). (*J. Med. Chem.*, **20**, 1176 (1977), EP 0 447 704 A1, page 17)

^1H NMR (D_2O) δ 1.10 (s, 3H), 1.11 (s, 3H), 1.88 (dd, 1H, *J* = 7.8, 13.2 Hz), 2.21 (dd, 1H, *J* = 9.2, 13.2 Hz), 3.12 (dd, 2H, *J* = 11.5, 23.5 Hz), 4.22 (dd, 1H, *J* = 8.1, 8.9 Hz).

Step 4: 5,5-Dimethyl-3-oxo-3a, 4-dihydro-3*H*, 6*H*-2-oxa-5-1-aza-6a-azonio-3a- pentalenide

To 4.63 g (22.2 mmol) of 5,5-dimethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-2- carboxylic acid ethylester in 222 mL of dry THF was added $LiAlH_4$ (0.85 g, 22.3 mmol) under a nitrogen atmosphere at 0 °C, and then stirred for 1 h. The mixture was quenched with water (5.0 mL) and the precipitate was filtered through a pad of Celite and the pad was washed with water (50 mL) and THF (150 mL). The filtrate was concentrated under reduced pressure, and then water (50 mL) was added. The aqueous layer was extracted with $CHCl_3$ (5 x 100 mL). The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and crude _5,5-Dimethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-2-yl)methanol was obtained as a yellow solid (3.19 g).

To 3.19 g of the crude (5,5-dimethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazol-2-yl) methanol in 222 mL of $CHCl_3$ was added activated MnO_2 (18.5 g) under a nitrogen atmosphere at room temperature, and then refluxed for 1 h. The mixture was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure. The residue was applied to silica-gel column chromatography, and then the column was eluted with hexane-AcOEt (3 : 1). The titled compound was obtained as a brown solid (2.48 g, 68.0 % from the ester).

^{1}H NMR ($CDCl_3$) δ 1.32 (s, 6H), 2.73 (s, 2H), 3.95 (s, 2H), 6.52 (s, 1H), 9.90 (s, 1H).

Step 7: (5*R*)(6*Z*)-6-(5,5-Dimethyl-4*H*-1,6a-diazapentalen-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

The dry acetonitrile (16 mL) solution of 5,5-dimethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*] pyrazole-2-carbaldehyde (2.48 g, 15.1 mmol) was added to the dry acetinitrile (90 mL) solution of $MgBr_2$ (3.07 g, 16.4 mmol) under a nitrogen atmosphere at room temperature, and then the mixture was stirred for 15 min. The dry THF (106 mL) solution of *p*-nitrobenzyl (5*R*, 6*S*)-6-bromopenem-3-carboxylate (5.30 g, 13.8 mmol) was added and the mixture was cooled to –20 °C, and then triethylamine (4.6 mL, 33.0 mmol) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with 4-dimethylamino pyridine (172 mg, 1.4 mmol) and acetic anhydride (2.6 mL, 27.6 mmol) in one portion. The reaction mixture was warmed to 0 °C and stirred for 16 h at 0 °C. Ethyl acetate (420 mL) and 1mol/L citric acid aqueous solution (210 mL) was added to the reaction mixture and separated. The

To a suspension of 37.5 g of the crude 4,4-dimethylpyrrolidine-2-carboxylic acid in 420 mL of AcOH was added a solution of 13.3 g (0.19 mol) of $NaNO_2$ in 210 mL of water over 15 min at room temperature and stirred for 3 h. The solution was concentrated under reduced pressure. Acetone (250 mL) was added to the residue and the precipitate was filtered off, and the solution was concentrated under reduced pressure to dryness and crude 4,4-dimethyl-1-nitrosopyrrolidine-2-carboxylic acid was obtained as brown oil.

To a solution of crude 4,4-dimethyl-1-nitrosopyrrolidine-2-carboxylic acid in 252 mL of dry THF was added trifluoroacetic anhydride (81.3 g, 0.39 mol) under a nitrogen atmosphere at 0 °C and stirred for 6 h at 0 °C. The solution was concentrated under reduced pressure. The residue was applied to silica-gel column chromatography, and then the column was eluted with *n*-hexane - AcOEt (2 : 1). The titled compound was obtained as a brown solid (12.0 g, 61.7 %).

^{1}H NMR ($CDCl_3$) δ 1.38 (s, 6H), 2.71 (s, 2H), 4.12 (s, 2H).

Step 5: 5,5-Dimethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-2-carboxylic acid ethylester

A solution of 5,5-dimethyl-3-oxo-3a, 4-dihydro-3*H*, 6*H*-2-oxa-5-1-aza-6a-azonio-3a- pentalenide (10.8 g, 0.07 mol) and ethyl propiolate (10.8 mL, 0.11 mol) in *o*-xylene (350 mL) was refluxed under a nitrogen atmosphere for 16 h. The solution was cooled to room temperature and concentrated under reduced pressure. The residue was applied to silica gel column chromatography, and then the column was eluted with *n*-hexane - AcOEt (3 : 1). The titled compound was obtained as a pale brown solid (4.63 g, 31.7 %), and 5,5-dimethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-3-carboxylic acid ethylester was obtained as a yellow solid (4.73 g, 32.4 %).

5,5-Dimethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-2-carboxylic acid ethylester: ^{1}H NMR ($CDCl_3$) δ 1,29 (s, 6H), 1.40 (t, 3H, J = 7.1 Hz), 2.71 (s, 2H), 3.93 (s, 2H), 4.39 (q, 2H, J = 7.1 Hz), 6.54 (s, 1H).

5,5-dimethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-3-carboxylic acid ethylester :^{1}H NMR ($CDCl_3$) δ 1,32 (s, 6H), 1.33 (t, 3H, J = 7.1 Hz), 2.89 (s, 2H), 3.90 (s, 2H), 4.26 (q, 2H, J = 7.1 Hz), 7.90 (s, 1H).

Step 6: 5,5-Dimethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-2-carbaldehyde

organic layer was washed with saturated sodium hydrogen carbonate and brine, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and crude (5*R*)-6-[acetoxy-(5,5-dimethyl- 4*H*-1,6a-diazapentalen-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2- carboxylic acid *p*-nitrobenzyl ester was obtained as brown amorphous.

Freshly activated Zn dust (32.0 g) was added rapidly with 0.5 mol/L phosphate buffer (pH 6.5, 167 mL) to the THF (114 mL) and acetonitrile (53 mL) solution of crude (5*R*)-6-[acetoxy-(5,5-dimethyl- 4*H*-1,6a-diazapentalen-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2- carboxylic acid *p*-nitrobenzyl ester. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 h at room temperature. The reaction solution was cooled at 0 °C, and then the pH was adjusted to 8.0. Ethyl acetate (85 mL) was added to the mixture and filtered through a pad of Celite. The pad was washed with water (120 mL). The aqueous layer was separated and then the organic layer was extracted with 0.5 mol/L phosphate buffer (pH 6.5, 2 x 50 mL). The combined aqueous layers were cooled at 0 °C, and then the pH was adjusted to 8.5. The mixture was concentrated to 325 g, and then applied to Diaion HP-21 resin (240 mL, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column was eluted with water (480 mL) and then acetonitrile aqueous solution (10%; 480 mL, 20%; 720 mL). The combined active fractions were concentrated under high vacuum at 35°C and lyophilized to give the titled compound as a yellow amorphous solid (2.00 g, 42.8 %, pH 7.16).

Mp 150 °C (dec); ^{1}H NMR (D_2O) δ 1.19 (s, 6H), 2.67 (s, 2H), 3.85 (s, 2H), 6.15 (s, 1H), 6.45 (s, 1H), 6.96 (s, 1H), 7.03 (s, 1H); IR (KBr) 3422, 1752, 1683, 1598, 1557 cm^{-1}; λ^{max} (H_2O) 296, 198 nm.

Example 27

Preparation of (5*R*),(6*Z*)- 6-(5,6-Dihydro-4*H*-cyclopenta[*b*]furan-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1; 5,6-Dihydro-4*H*-cyclopenta[*b*]furan-2-carboxylic acid methyl ester

The titled compound was prepared according to the procedure of Tim Johnson and co-workers (*Synlett* **2001**, *5*, 646 – 648).

Step 2: (5,6-Dihydro-4*H*-cyclopenta[*b*]furan-2-yl)methanol

5,6-Dihydro-4*H*-cyclopenta[*b*]furan-2-carboxylic acid methyl ester (2.24 g) was added to the THF (59 mL) solution of $LiAlH_4$ (511 mg) under a nitrogen atmosphere at 0 °C and stirred for 1 h at 0 °C. The mixture was quenched with 10 mL of water and filtered. The filtrate was concentrated under reduced pressure and the obtained aqueous solution was extracted with $CHCl_3$. The organic layer was washed with brine and dried over $MgSO_4$ and filtered. The filtrate was concentrated to afford titled compound as yellow oil (1.86 g, quant.).

^1H NMR ($CDCl_3$) δ 1.66 (t, 1H, *J* = 5.9 Hz), 2.38 – 2.46 (m, 2H), 2.50 – 2.55 (m, 2H), 2.65 – 2.70 (m, 2H), 4.54 (d, 2H, *J* = 5.9 Hz), 6.15 (s, 1H).

Step 3: 5,6-Dihydro-4*H*-cyclopenta[*b*]furan-2-carbaldehyde

Activated MnO_2 (9.3 g) was added to the $CHCl_3$ (135 mL) solution of (5,6-dihydro-4*H*-cyclopenta[*b*]furan-2-yl)methanol (1.86 g) and refluxed for 1 h under a nitrogen atmosphere. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (9/1 – 7/1). The titled compound was obtained as yellow crystals (1.51 g, 77%).

^1H NMR ($CDCl_3$) δ 2.47 – 2.57 (m, 2H), 2.63 (t, 2H, *J* = 6.8 Hz), 2.78 (t, 2H, *J* = 7.3 Hz), 7.06 (s, 1H), 9.44 (s, 1H).

Step 4: (5*R*, 6*RS*)-6-[(*RS*)-Acetoxy(5,6-dihydro-4*H*-cyclopenta[*b*]furan-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester

The acetonitrile solution (50 mL) of 5,6-dihydro-4*H*-cyclopenta[*b*]furan-2-

carbaldehyde (1.33 g) was added to the dry acetonitrile (101 mL) solution of anhydrous $MgBr_2$ (cont. 98%) (5.52 g) under a nitrogen atmosphere at room temperature. The dry THF solution (151 mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (cont. 96.5%) (3.91 g) was added to the mixture, cooled to –20 °C, and Et_3N (cont. 99%) (8.28 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (cont. 97%) (4.13 mL) and DMAP (cont. 99%) (121 mg) in one portion. The reaction

mixture was warmed to 0 °C and stirred for 16 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% aqueous solution of citric acid, saturated sodium hydrogen carbonate and brine. The organic layer was dried ($MgSO_4$) then filtered. The filtrate was concentrated under reduced pressure. The residue was purified with a silica-gel column chromatography (*n* - hexane : AcOEt = 4 :1 – 3 : 1) to give the titled compound as a brown amorphous solid (3.34 g, 61%).

^{1}H NMR ($CDCl_3$) δ 2.21 (s, 3H), 2.40 – 2.48 (m, 2H), 2.53 (t, 2H, *J* = 7.0 Hz), 2.69 (t, 2H, *J* = 7.0 Hz), 5.28 (d, 1H, *J* = 13.5 Hz), 5.43 (d, 1H, *J* = 13.5 Hz), 6.00 (s, 1H), 6.37 (s, 1H), 6.71 (s, 1H), 7.41 (s, 1H), 7.60 (d, 2H, *J* = 8.1 Hz), 8.24 (d, 2H, *J* = 8.1 Hz).

Step 5: (5*R*),(6*Z*)- 6-(5,6-Dihydro-4*H*-cyclopenta[*b*]furan-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

(5*R*, 6*RS*)-6-[(*RS*)-Acetoxy(5,6-dihydro-4*H*-cyclopenta[*b*]furan-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (3.28 g) was dissolved in THF (46 mL) and acetonitrile (21 mL). Freshly activated Zn dust (13.12 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 67 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.25 h at room temperature. The reaction mixture was filtered through a pad of Celite. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was cooled to 3 °C and 1 *M* NaOH was added to adjust pH to 8.0. The mixture was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (181 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN (1/0 - 85/15). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the titled crude product (288mg). This was purified by Diaion HP-21 (100 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN (1/0 - 85/15). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the titled compound as a yellow amorphous solid (185 mg, 10%, pH 7.2).

Mp 170 °C (dec); ^{1}H NMR (D_2O) δ 2.24 – 2.30 (m, 2H), 2.37 (t, 2H, *J* = 6.5 Hz), 2.52 – 2.57 (t, 2H, *J* = 7.1 Hz), 6.32 (s, 1H), 6.55 (s, 1H), 6.73 (s, 1H), 6.86 (s, 1H).

Example 28

Preparation of (5*R*)(6*Z*)-6-(4,5-Dihydro-6-thia-1,7a-diazainden-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: *DL*-Tetrahydro-1,3-thiazine-4-carboxylic acid hydrochloride

DL-Tetrahydro-1,3-thiazine-4-carboxylic acid hydrochloride was prepared according to the method of Lewis (*J. Med. Chem.*, **21**, 1070 (1978)).

Step 2: 4,5-Dihydro-3a*H*,7*H*-2-oxa-3-oxo-6-thia-1-aza-7a-azonioinden

To a suspension of *DL*-tetrahydro-1,3-thiazine-4-carboxylic acid hydrochloride (48.6 g, 0.26 mol) in 666 mL of AcOH was added to the solution of 27.4 g (0.40 mol) of $NaNO_2$ in 333 mL of water over 16 min at room temperature and stirred for 3 h. The solution was concentrated under reduced pressure. Acetone (300 mL) was added to the residue and the precipitate was filtered off. The filtrate was concentrated under reduced pressure to dryness and crude 3-nitroso[1,3]thiazinane-4-carboxylic acid was obtained as brown amorphous solid.

To a solution of crude 3-nitroso[1,3]thiazinane-4-carboxylic acid in 530 mL of dry THF was added trifluoroacetic anhydride (168.4 g, 0.80 mol) over 60 min under a nitrogen atmosphere at 0 °C and stirred for 5 h at 0 °C. The solution was concentrated under reduced pressure. The residue was applied to silicagel column chromatography, and then the column was eluted with *n*-hexane - AcOEt (1 : 2). The titled compound was obtained as brown powder (28.0 g, 67.0 %).

^{1}H NMR ($CDCl_3$) δ 3.00 (t, 2H, *J* = 5.7 Hz), 3.07 (t, 2H, *J* = 5.7 Hz), 5.16 (s, 2H).

Step 3: 4,5-Dihydro-6-thia-1,7a-diazaindene-2-carboxylic acid ethylester

A solution of 4,5-dihydro-3a*H*,7*H*-2-oxa-3-oxo-6-thia-1-aza-7a-azonioinden (28.0 g, 0.18 mol) and ethyl propiolate (27.0 mL, 0.27 mol) in *o*-xylene (590 mL) was refluxed under a nitrogen atmosphere for 16 h. The solution was cooled to room temperature and concentrated under reduced pressure. The residue was applied to silicagel column chromatography, and then the column was eluted with *n*-hexane - AcOEt (3 : 1). The titled compound was obtained as pale brown needles (22.1 g, 58.7 %), and 4,5-dihydro-6-thia- 1,7a-diazaindene-3-carboxylic acid ethylester was obtained as pale brown crystals (12.7 g, 33.9 %).

4,5-Dihydro-6-thia-1,7a-diazaindene-2-carboxylic acid ethylester ^{1}H NMR ($CDCl_3$) δ 1.39 (t, 3H, *J* = 7.1 Hz), 2.98 (t, 2H, *J* = 6.1 Hz), 3.21 (t, 2H, *J* = 6.1 Hz), 4.40 (q, 2H, *J* = 7.1 Hz), 5.17 (s, 2H), 6.60 (s, 1H).

4,5-dihydro-6-thia- 1,7a-diazaindene-3-carboxylic acid ethylester :^{1}H NMR ($CDCl_3$) δ 1.34 (t, 3H, *J* = 7.1 Hz), 2.99 (t, 2H, *J* = 6.1 Hz), 3.45 (t, 2H, *J* = 6.1 Hz), 4.28 (q, 2H, *J* = 7.1 Hz), 5.11 (s, 2H), 7.85 (s, 1H).

Step 4: 4,5-Dihydro-6-thia-1,7a-diazaindene-2-carbaldehyde

To a 22.1 gram (0.10 mol) of 4,5-dihydro-6-thia-1,7a-diazaindene-2-carboxylic acid ethylester in 520 mL of dry THF was added $LiAlH_4$ (3.95 g, 0.10 mol) under a nitrogen atmosphere at 0 °C, and then stirred for 45 min. The mixture was quenched with water (20 mL) and the precipitate was filtered through a pad of Celite and the pad was washed with water (100 mL) and THF (250 mL). The filtrate was concentrated under reduced pressure, and then water (300 mL) was added. The aqueous layer was extracted with CH_2Cl_2 (6 x 500 mL). The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and crude was obtained as pale yellow crystals (17.2 g).

To a 17.2 gram of the crude (4,5-dihydro-6-thia-1,7a-diazainden-2-yl) methanol in 520 mL of $CHCl_3$ was added activated MnO_2 (88.0 g) under a nitrogen atmosphere at room temperature, and then refluxed for 2 h. The mixture was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure. The residue was applied to silicagel column chromatography, and then the column was eluted with hexane-AcOEt (2 : 1). The titled compound was obtained as yellow crystals (13.0 g, 74.5 %)

^{1}H NMR ($CDCl_3$) δ 3.00 (t, 2H, *J* = 6.0 Hz), 3.23 (t, 2H, *J* = 6.0 Hz), 5.18 (s, 2H), 6.58 (s, 1H), 9.92 (s, 1H).

Step 5: (5*R*)(6*Z*)-6-(4,5-Dihydro-6-thia-1,7a-diazainden-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

The dry acetonitrile (11 mL) solution of 4,5-dihydro-6-thia-1,7a-diazaindene-2- carbaldehyde (1.70 g, 10.1 mmol) was added to the dry acetinitrile (60 mL) solution of $MgBr_2$ (2.03 g, 11.0 mmol) under a nitrogen atmosphere at room temperature, and then the mixture was stirred for 10 min. The dry THF (71 mL) solution of *p*-nitrobenzyl (5*R*, 6*S*)-6-bromopenem-3-carboxylate (3.55 g, 9.2 mmol) was added and the mixture was cooled to –20 °C, and then triethylamine (3.1 mL,

22.2 mmol) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with 4-dimethylamino pyridine (0.11 g, 0.9 mmol) and acetic anhydride (1.8 mL, 18.6 mmol) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. Ethyl acetate (280 mL) and 1mol/L citric acid aqueous solution (140 mL) was added to the reaction mixture and separated. The organic layer was washed with saturated sodium hydrogen carbonate and brine, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and crude (5*R*)-6-[acetoxy-(4,5-dihydro- 6-thia-1,7a-diazainden-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2- carboxylic acid *p*-nitrobenzyl ester was obtained as brown amorphous solid.

Freshly activated Zn dust (21.4 g) was added rapidly with 0.5 mol/L phosphate buffer (pH 6.5, 112 mL) to the THF (76 mL) and acetonitrile (36 mL) solution of crude (5*R*)-6-[acetoxy-(4,5-dihydro- 6-thia-1,7a-diazainden-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2- carboxylic acid *p*-nitrobenzyl ester. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 h at room temperature. The reaction solution was cooled at 0 °C, and then the pH was adjusted to 8.0. Ethyl acetate (56 mL) was added to the mixture and filtered through a pad of Celite. The pad was washed with water (150 mL). The aqueous layer was separated and then the organic layer was extracted with 0.5 mol/L phosphate buffer (pH 6.5, 2 x 30 mL). The combined aqueous layers were cooled at 0 °C, and then the pH was adjusted to 8.0. The mixture was concentrated to 236 g, and then applied to Diaion HP-21 resin (480 mL, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column was eluted with water (960 mL) and then acetonitrile aqueous solution (5%; 960 mL, 10%; 960 mL, 20%; 960 mL). The combined active fractions were concentrated under high vacuum at 35°C and lyophilized to give the titled compound as a yellow amorphous solid (1.28 g, 40.5 %, pH 7.45).

Mp 200 °C (dec); ^{1}H NMR (D_2O) δ 2.95 (t, 2H, J = 6.1 Hz), 3.12 (t, 2H, J = 6.1 Hz), 5.08 (s, 2H), 6.23 (s, 1H), 6.46 (s, 1H), 6.97 (s, 1H), 7.01 (s, 1H); IR (KBr) 3382, 1752, 1684, 1597, 1554 cm^{-1}; λ^{max} (H_2O) 366, 292, 197 nm.

Example 29

Preparation of (5*R*),(6*Z*)-6-(6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrizin-2- ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of 5,5-Dimethyl-2-piperidone

5-5-Dimethyl-2-piperadinone (1) was prepared in the method of Nagasawa (*J. Med. Chem.*, **23**, 1176 (1977)).

Step 2: Preparation of 3,3-Dimethyl-6-methoxy-2,3,4,5-tetrahydropyridine

Trimethyloxonium tetrafluoroborate (97%, 11.9 g, 78 mmol) was added to the dry dichloromethane (156 mL) solution of 5,5-dimethyl-2-piperidone (9.93 g, 78 mmol) at room temperature and stirred for 14 h. The reaction mixture was neutralized with 10% sodium hydrogen carbonate aqueous solution, and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (3 x 120 mL), then the combined organic layer was washed with 10% sodium hydrogen carbonate aqueous solution and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and the titled compound was obtained as pale yellow oil (9.0 g, 82.0 %).

^{1}H NMR ($CDCl_3$) δ 0.92 (s, 6H), 1.49 (t, 2H, *J* = 7.0 Hz), 2.18 (t, 2H, *J* = 7.0 Hz), 3.19 (s, 2H), 3.63 (s, 3H).

Step 3: 5,5-Dimethylpiperidine-2-ylideneamine monohydrochloride

The mixture of 3,3-dimethyl-6-methoxy-2,3,4,5-tetrahydropyridine (9.0 g, 64 mmol) and ammonium chloride (3.4 g, 64 mmol) in dry ethanol (160 mL) was heated to reflux for 2 h. The reaction mixture was then concentrated under reduced pressure and the titled compound was obtained as a white solid (9.9 g, 94.6 %).

^{1}H NMR (DMSO-d^6) δ 0.95 (s, 6H), 1.52 (t, 2H, *J* = 6.9 Hz), 2.55 (t, 2H, *J* = 6.9 Hz), 2.99 (d, 2H, *J* = 2.1 Hz).

Step 4: 6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-2-carbaldehyde &

6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-3-carbaldehyde

The mixture of 2-bromo-3-hydroxypropenal (10.1 g, 67 mmol), p-toluenesulfonic acid monohydrate (0.13 g, 0.6 mmol) and 2-propanol (12.6 mL, 165 mmol) in cyclohexane (100 mL) was azeotroped until the vaper temperature over 80°C. The reaction mixture was concentrated under reduced pressure. The

residue was dissolved in dry EtOH (200 mL). The dry EtOH (350 mL) solution of 5,5-dimethylpiperidine-2-ylideneamine monohydrochloride (9.9 g, 61 mmol) and the dry EtOH (50 mL) solution of NaOMe (28%, 11.7 g, 61 mmol) were added at room temperature. The reaction mixture was stirred at room temperature for 2 h, and then the reaction solution was removed in vacuo. The residue was dissolved in $CHCl_3$ (300 mL) and triethylamine (8.5 mL, 61 mmol) was added, and then the reaction mixture was heated to reflux for 2 h. The reaction mixture was cooled to room temperature, and then the reaction solution was removed in vacuo. The residue was dissolved in CH_2HI_2 (600 mL) and washed with 50% K_2CO_3 aqueous solution (2 x 200 mL). The combined aqueous solution was extracted with CH_2Cl_2 (2 x 200 mL). The combined organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, eluted with $CHCl_3$ – methanol (50 : 1), and the titled compound 6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-2-carbaldehyde (brown solid, 4.4 g, 40.7 %) and 6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-3-carbaldehyde (orange solid, 1.7 g, 15.8 %) were obtained.

6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-2-carbaldehyde: ^{1}H NMR ($CDCl_3$) δ 1.10 (s, 6H), 1.78 (t, 2H, *J* = 6.9 Hz), 2.95 (t, 2H, *J* = 6.9 Hz), 3.71 (s, 2H), 7.46 (s, 1H), 9.83 (s, 1H).

6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-3-carbaldehyde: ^{1}H NMR ($CDCl_3$) δ 1.09 (s, 6H), 1.74 (t, 2H, *J* = 6.8 Hz), 2.97 (t, 2H, *J* = 6.8 Hz), 4.05 (s, 2H), 7.74 (s, 1H), 9.64 (s, 1H).

Step 5: (5*R*),(6*Z*)-6-(6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrizin-2- ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

The dry acetonitrile (28 mL) solution of 6,6-dimethyl-5,6,7,8-tetrahydroimidazo [1,2-*a*]pyridine-2-carbaldehyde (4.55 g, 26 mmol) was added to the dry acetinitrile (152 mL) solution of $MgBr_2$ (5.22 g, 28 mmol) under a nitrogen atmosphere at room temperature, and then the mixture was stirred for 10 min. The dry THF (180 mL) solution of *p*-nitrobenzyl (5R, 6S)-6-bromopenem-3-carboxylate (8.94 g, 23 mmol) was added and the mixture was cooled to –20 °C, and then triethylamine (7.8 mL, 56 mmol) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C

and treated with 4-dimethylamino pyridine (0.29 g, 2.4 mmol) and acetic anhydride (4.4 mL, 47 mmol) in one portion. The reaction mixture was warmed to 0 °C and stirred for 16 h at 0 °C. Ethyl acetate (715 mL) was added to the reaction mixture, and then the organic layer was washed with 1mol/L Citric acid aqueous solution, saturated sodium hydrogen carbonate and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and crude (5*R*)-6-[acetoxy-(6,6-dimethyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia- 1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester was obtained as brown amorphous solid.

Freshly activated Zn dust (53.6 g) was added rapidly with 0.5 mol/L phosphate buffer (pH 6.5, 282 mL) to the THF (192 mL) and acetonitrile (90 mL) solution of (5*R*)-6-[acetoxy-(6,6-dimethyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia- 1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 h at room temperature. The reaction mixture was cooled at 0 °C, and then the pH was adjusted to 7.6. Ethyl acetate (140 mL) was added to the reaction mixture, and then the mixture was filtered through a pad of Celite and the pad was washed with water (200 mL). The aqueous layer was separated and then the organic layer was extracted with 0.5 mol/L phosphate buffer (pH 6.5, 2 x 50 mL). The pH of the combined aqueous layer was adjusted to 8.1 and the mixture was concentrated to 584 g. 1mol/L NaOH was added to adjust pH to 8.2 and applied to Diaion HP-21 resin (420 mL, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column was eluted with 2.5 % (2 bed volume), 5 % (2 bed volume), 10 % (1 bed volume) and 20 % acetonitrile aqueous solution. The combined active fractions were concentrated under high vacuum at 35°C and lyophilized to give the crude (5*R*),(6*Z*)-6-(6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrizin-2- ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt as a yellow amorphous solid (1.19 g).

The crude (5*R*),(6*Z*)-6-(6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrizin-2- ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt was purified by the preparative HPLC (Mightysil RP-18 GP (5 □m), Kanto Chemical Co. Inc., 35 x 250 mm, 0.05 mol/L phosphate buffer (pH 7.2) : CH_3CN = 70 : 30, 20 mL/min.). The purified product was desalted by Diaion HP-21

resin (50 mL) column chromatography and the title compound was obtained 230 mg (2.8 %) as a yellow amorphous solid.

Mp 210°C (dec); ^{1}H NMR (D_2O) δ ; 0.91 (s, 3H), 0.93 (s, 3H), 1.63 (t, 2H, *J* = 6.8 Hz), 2.72 (t, 2H, *J* = 6.8 Hz), 3.60 (s, 2H), 6.44 (s, 1H), 6.90 (s, 1H), 6.91 (s, 1H), 7.19 (s, 1H).

Example 30

Preparation of (5*R*), (6*Z*)-6-(5,6-Dihydro-8-*H*-imidazo[2,1-c][1,4]thiazin-3-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

The dry acetonitrile (40 mL) solution of 5,6-dihydro-8*H*-imidazo[2,1-c][1,4]thiazine-3-carbardehyde (813 mg) was added to the dry acetinitrile (40 mL) solution of $MgBr_2$ (2.2 g) under a nitrogen atmosphere at room temperature then the mixture was stirred for 10 min. The dry THF (80 mL) solution of *p*-nitrobenzyl (5R, 6S)-6-bromopenem-3-carboxylate (2.1 g) was added, the mixture was cooled to –20 °C then triethylamine (1.7 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3.5 h at –20 °C and treated with 4,4-dimethylamino pyridine (64 mg) and acetic anhydride (0.9 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 14 h at 0 °C. 10% Citric acid aqueous solution (500 mL) was added to the reaction mixture and the aqueous layer was extracted with ethyl acetate (3 x 200 mL). The organic layer was washed with water, saturated sodium hydrogen carbonate and brine, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography and eluted with CH_2Cl_2 – acetone (20 : 1) to obtain crude (5*R*)-6-[acetoxy-(5,6-dihydro-8*H*-imidazo[2,1-c][1,4]thiazin-3-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester as a brown solid.

The solid obtained above chromatography was dissolved in THF (11 mL). Freshly activated Zn dust (1.4 g) was added rapidly with 0.5 mol/L phosphate buffer (pH 6.5, 11 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction solution was filterd through a pad of Celite and the pad was washed with water (26 mL) and *n*-butanol (26 mL). The aqueous layer was separated and then the organic layer was extracted with 0.5 mol/L phosphate buffer (pH 6.5, 2 x 5 mL). The combined aqueous layer was concentrated to 18 g, 1mol/L NaOH was added to

adjust pH to 7.3 and applied to Diaion HP-21 resin (20 mL, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column was eluted with water and then 5% acetonitrile aqueous solution. The combined active fractions was concentrated under high vacuum at 35°C and lyophilized to give the title compound as a yellow amorphous solid (81 mg).

Mp 145°C (dec); ^{1}H NMR (D_2O) δ 3.05-3.08 (m, 1H), 3.83 (s, 1H), 4.13-4.16 (m, 1H), 6.37 (s, 1H), 6.91 (s, 1H), 7.01 (s, 1H), 7.04 (s, 1H); IR (KBr) 3371, 1770, 1672, 1613 cm^{-1}; λ^{max} (H_2O) 314 nm.

Example 31

Preparation of (5*R*)(6*Z*)-6-(2,3-Dihydropyrazolo[5,1-*b*]thiazol-6-ylmethylene)-7-oxo-4-thia-1- azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of 3-Oxo-3a, 4-dihydro-3*H*, 6*H*-2-oxa-4-thia-1-aza-6a-azonio-3a-pentalenide

To a suspension of thiazolidine-2-carboxylic acid (39.9 g, 0.30 mol) in 1,000 ml of acetic acid was added a solution of 31.0 g (0.45 mol) of sodium nitrite in 500 ml of water over 13 minutes at room temperature and stirred for 5 hours. The reaction solution was concentrated under reduced pressure. Acetone (500 ml) was added to the residue and the precipitate was filtered through a pad of Celite. The pad was washed with acetone (500 ml). The filtrate was concentrated under reduced pressure to dryness and crude 3-nitrosothiazolidin-2- carboxylic acid was obtained as a yellow solid.

To a solution of crude 3-nitrosothiazolidin-2- carboxylic acid in 600 ml of dry tetrahydrofuran was added trifluoroacetic anhydride (189.6 g, 0.90 mol) over 20 minutes under a nitrogen atmosphere at 0 °C and stirred for 19 hours at 0 °C. The solution was concentrated under reduced pressure. The residue was applied to a silica-gel column chromatography, and then the column was eluted with *n*-hexane – ethyl acetate (1 : 1). The titled compound was obtained as a pale brown crystal (19.2 g, 44.5 %).

^{1}H NMR ($CDCl_3$) δ 3.98 (t, 2H, J = 7.7 Hz), 4.65 (t, 2H, J = 7.7 Hz).

Step 2: Preparation of 2,3-Dihydropyrazolo[5,1-*b*]thiazol-6-carboxylic acid ethyl ester and 2,3- dihydropyrazolo[5,1-*b*]thiazol-7-carboxylic acid ethyl ester

Ethyl propiolate (20.3 ml, 0.20 mol) was added to an *o*-xylene (600 ml) solution of 3-oxo-3a, 4-dihydro-3*H*, 6*H*-2-oxa-4-thia-1-aza-6a-azonio-3a-pentalenide (19.2 g, 0.13 mol) under a nitrogen atmosphere and refluxed for 21 hours. The solution was cooled to room temperature and concentrated under reduced pressure. The residue was applied to a silica gel column chromatography, and then the column was eluted with *n*-hexane – ethyl acetate (2 :21 to 1 : 1). The mixture of 2,3-Dihydropyrazolo[5,1-*b*]thiazol-6-carboxylic acid ethyl ester and 2,3-dihydropyrazolo[5,1-*b*]thiazol-7-carboxylic acid ethyl ester was obtained as a brown oil in the ratio of 1:1.5 respectively. (21.2 g, Yield: 80.0 %).

2,3-Dihydropyrazolo[5,1-*b*]thiazol-6-carboxylic acid ethyl ester; ^{1}H NMR ($CDCl_3$) δ 1.39 (t, 3H, *J* = 7.1 Hz), 3.82 (t, 2H, *J* = 7.5 Hz), 4.39 (q, 2H, *J* = 7.1 Hz), 4.42 (t, 2H, *J* = 7.5 Hz), 6.52 (s, 1H).

2,3- dihydropyrazolo[5,1-*b*]thiazol-7-carboxylic acid ethyl ester; ^{1}H NMR ($CDCl_3$) δ 1.34 (t, 3H, *J* = 7.1 Hz), 3.85 (t, 2H, *J* = 7.8 Hz), 4.28 (q, 2H, *J* = 7.1 Hz), 4.39 (t, 2H, *J* = 7.8 Hz), 7.87 (s, 1H).

Step 3: 2,3-Dihydropyrazolo[5,1-*b*]thiazol-6-carbaldehyde and 2,3-dihydro- pyrazolo[5,1-*b*]thiazol-7-carbaldehyde

To the mixture [21.2 g (0.11 mol)] of 2,3-dihydropyrazolo[5,1-*b*]thiazol-6-carboxylic acid ethyl ester and 2,3-dihydropyrazolo[5,1-*b*]thiazol-7-carboxylic acid ethyl ester in 540 ml of dry tetrahydrofuran was added $LiAlH_4$ (4.05 g, 0.11 mol) under a nitrogen atmosphere at 0 °C, and then stirred for 2.5 hours at room temperature. The mixture was quenched with water (15 ml) and the precipitate was filtered through a pad of Celite. The pad was washed with water (100 ml) and tetrahydrofuran (500 ml). The filtrate was concentrated under reduced pressure, and then water (150 ml) was added. The aqueous layer was extracted with dichloromethane (15 x 250 ml). The combined organic layers were dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and a mixture of (2,3-dihydropyrazolo[5,1-*b*]thiazol-6-yl) methanol and (2,3-dihydropyrazolo[5,1-*b*]thiazol-7-yl) methanol was obtained as pale brown oil (15.5 g).

To the mixture [15.5 g (0.10 mol)] of (2,3-dihydropyrazolo[5,1-*b*]thiazol-6-yl) methanol and (2,3-dihydropyrazolo[5,1-*b*]thiazol-7-yl) methanol in 500 ml of chloroform was added activated MnO_2 (77.7 g) under a nitrogen atmosphere at room temperature, and then refluxed for 3 hours. The mixture was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure. The residue was applied to a silica gel column chromatography, and then the column was eluted with hexane-ethyl acetate (2 : 1 to 1 : 1). The titled compound 2,3-Dihydropyrazolo[5,1-*b*]thiazol-6-carbaldehyde was obtained as a yellow crystal (2.50 g, 15.2 %) and _2,3-dihydro- pyrazolo[5,1-*b*]thiazol-7-carbaldehyde was obtained as a pale brown solid (5.57 g, 33.8)

2,3-Dihydropyrazolo[5,1-*b*]thiazol-6-carbaldehyde; ^{1}H NMR ($CDCl_3$) δ 3.86 (t, 2H, *J* = 7.5 Hz), 4.45 (t, 2H, *J* = 7.5 Hz), 6.50 (s, 1H), 9.83 (s, 1H).

2,3-dihydro- pyrazolo[5,1-*b*]thiazol-7-carbaldehyde_; ^{1}H NMR ($CDCl_3$) δ 3.92 (t, 2H, *J* = 7.9 Hz), 4.40 (t, 2H, *J* = 7.9 Hz), 7.91 (s, 1H), 9.76 (s, 1H).

Step 4: Preparation of (5*R*)(6*Z*)-6-(2,3-Dihydropyrazolo[5,1-*b*]thiazol-6-ylmethylene)-7-oxo-4-thia-1- azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

A dry acetonitrile (19 ml) solution of 2,3-dihydropyrazolo[5,1-*b*]thiazol-6- carbaldehyde (2.50 g, 16.2 mmol) was added to a dry acetinitrile (106 ml) solution of $MgBr_2$ (3.67 g, 19.9 mmol) under a nitrogen atmosphere at room temperature then the mixture was stirred for 10 minutes. A dry tetrahydrofuran (125 ml) solution of *p*-nitrobenzyl (5R, 6S)-6-bromopenem-3- carboxylate (6.23 g, 16.2 mmol) was added and the mixture was cooled to –20 °C then triethylamine (5.4 ml, 38.7 mmol) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 hours at –20 °C and treated with 4-dimethylamino pyridine (198 mg, 1.62 mmol) and acetic anhydride (3.1 ml, 32.9 mmol) in one portion. The reaction mixture was warmed to 0 °C and stirred for 16 hours at 0 °C. Ethyl acetate (500 ml) was added to the reaction mixture and then the organic layer was washed with 1mol/l citric acid aqueous solution, saturated sodium hydrogen carbonate and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and the crude (5*R*)-6-[acetoxy-(2,3-dihydropyrazolo [5,1-*b*]thiazol-6-yl)methyl]-6-bromo-7-oxo-4-thia-1-

azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester was obtained as a brown amorphous solid.

Freshly activated Zn dust (37.4 g) was added rapidly with 0.5 mol/l phosphate buffer (pH 6.5, 196 ml) to tetrahydrofuran (134 ml) and acetonitrile (62 ml) solution of (5*R*)-6-[acetoxy-(2,3-dihydropyrazolo [5,1-*b*]thiazol-6-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 hours at room temperature. The reaction mixture was cooled at 0 °C, and then the pH was adjusted to 8.0. Ethyl acetate (100 ml) was added to the reaction mixture. The mixture was filtered through a pad of Celite and the pad was washed with water (300 ml). The aqueous layer was separated and then the organic layer was extracted with 0.5 mol/l phosphate buffer (pH 6.5, 2 x 50 ml). The pH of the combined aqueous layer was adjusted to 8.0 and the mixture was concentrated to 426 g. The concentrate was adjust pH to 8.0 and applied to Diaion HP-21 resin (540 ml, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column was eluted with water (1 bed volume) and then 5 % (2 bed volume), 10 % (2 bed volume) and 20 % acetonitrile aqueous solution. The combined active fractions were concentrated under high vacuum at 35°C and lyophilized to give the title compound as a orange amorphous solid (2.09 g, 39.2 %, pH 7.10).

Mp 150 °C (dec); ^{1}H NMR (D_2O) δ 3.75 (t, 2H, *J* = 7.5 Hz), 4.27 (t, 2H, *J* = 7.5 Hz), 6.00 (s, 1H), 6.34 (s, 1H), 6.85 (s, 1H), 6.94 (s, 1H); IR (KBr) 3392, 1755, 1596, 1554 cm^{-1}; λ^{max} (H_2O) 290, 223 nm.

Example 32

Preparation of (5*R*)(6*Z*)-6-(2,3-Dihydropyrazolo[5,1-*b*]oxazol-6-ylmethylene)-7-oxo-4-thia-1- azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of ethyl2,3-dihydropyrazolo[5,1-b][1,3]oxazole-6-carboxylate:

To the stirred suspension of ethyl 5-hydroxy-1H-pyrazole-3-carboxylate (10.34 g, 0.66 mol) and 36.62 g of potassium carbonate in 500 ml of acetonitrile was added 13.68 g of 1,2-dibromoethane, and refluxed for 16 hours. The reaction mixture was allowed to cool to room temperature, then filtered, the solid was washed with

acetonitrile. The filtrate was concentrated to an oil. The residue was dissolved in ethyl acetate and extracted with water. The organic phase was dried over $MgSO_4$ and evaporated to dryness. 5.80 g of the desired product was obtained (48%).

Step 2: Preparation of 2,3-dihydropyrazolo[5,1-b][1,3]oxazole-6-methanol:

To the stirred solution of ethyl2,3-dihydropyrazolo[5,1-b][1,3]oxazole-6-carboxylate

(5.47 g, 35 mmol) of in 100 ml of THF was added 1.05 g of lithium borohydride and 1.54 g of methanol. The solution was heated at 40C for 2.5 hour. The reaction was quenched by 1N HCl, and adjusted to pH 1.3 and stirred at room temperature for 1 hour. The reaction mixture was adjusted pH to 8 with k_2CO_3. The reaction mixture was extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, and concentrated to an oil and column chromatographyed to give 2.68 g of the desired product (65%).

Step 3: Preparation of 2,3-dihydropyrazolo[5,1-b][1,3]oxazole-6-carbaldehyde:

To the stirred solution of 2,3-dihydropyrazolo[5,1-b][1,3]oxazole-6-methanol

(2.60 g, 18.5 mmol) in 60 ml of CH_3Cl was added 12.9 g of MnO_2. Th suspension was refluxed for 1.5 hour under a nitrogen atmosphere. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated to give yellow oil. The product was purified by chromatography. 2.15 g of the product was obtained (84.3%).

Step 4: 4-Nitrobenzy (5R)-6-[(acetyloxy)(2,3-dihydropyrazolo[5,1-b][1,3]oxazol-6-yl) -)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

2,3-dihydropyrazolo[5,1-b][1,3]oxazole-6-carbaldehyde (607 mg, 4.3 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.54 g, 4.6 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (2.21 g , 8.5 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate,

and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 1.9 g, 81%; M+H 566.

H-NMR($CDCl_3$) 8.24(2H, d, J= 6.6 Hz), 7.60(2H, d, J= 6.6Hz), 7.44(1H, s), 6.34(1H, s), 6.23(1H, s), 5.56(1H, s), 5.44(1H, d, J=10.2 Hz), 5.27(1H, d J=10.2Hz), 5.04(2H, m), 4.30(2H, m), 2.10(3H, s).

Anal.Calcd. for $C_{21}H_{17}BrN_4O_8S$: C, 44.61, H, 3.03, N, 9.91

Found: C, 45.00, H, 3.14, N, 9.53

Step-5: (5R,6Z)-6-(2,3-dihydropyrazolo[5,1-b][1,3]oxazol-6-ylmethylene}-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt:

4-Nitrobenzy-6-[(acetyloxy)(2,3-dihydropyrazolo[5,1-b][1,3]oxazol-6-yl) -)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (700 mg, 1.2 mmol) was dissolved in THF (20 mL), acetonitrile (10 mL) and 0.5 *M* phosphate buffer (pH 6.5, 28 mL) and hydrogenated over 10% Pd/C at 40 psi pressure. After 4 hrs the reaction mixture was filtered, cooled to 3 °C, and 0.1 *M* NaOH was added to adjust pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 lits) and latter with 10% acetonitrile: Water. The fractions containing the product were collected and concentrated at reduced pressure at room temperature. The yellow solid was washed with acetone and filtered. Dried. Yield: 276 mg, 73%; as yellow amorphous solid; (M+H+Na)314. .

^{1}H-NMR(D_2O); δ 6.97(1H, s), 6.95(1H, s), 6.46(1H, s), 5.56(1H, s) 5.07(2H, d, J= 6.3 Hz), 4.30(2H, t, J=6.3 Hz).

Example 33

Preparation of (5R,6Z)-6-[(5-acetyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methylene]-oxo-4-thia-1-azabicyclo[3.2.0.]hept-2-ene-2-carboxylic acid (E+Z Isomers mixture,Sodium salt)

Step 1: 5-acetyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridine-2-carbaldehyde:

To a cold (0^0 C) suspension of 1.5 g.(7.4 mmol) of 4,5,6,7-tetrahydrothieno[3,2-c]pyridine-2-carbaldehyde hydrochloride in 50 mL methylene chloride, under N_2 atm., dry conditions, was added dropwise under stirring 2.6 mL (2.5 eqs) of triethylamine. RM stirred for 30 min at 0^0 C. and a solution of 0.7 g.(8.1 mmol,1.1 eqs) of acetyl chloride in 15 mL methylene chloride was dropwise added, RM allowed to reach RT and stirred for 3 hours. Filtered trough a celite pad, filtrate washed with 3x 50 mL water, dried, evaporated, gave 1.1g.(71.4 %) of the title compound, viscous oil, $(M+H)^+$ 210.3.

Step 2: Preparation of 4-nitrobenzyl(5R)-6-[(acetyloxy)(5-acetyl-4,5,6,7-tetrahydrotieno[3,2-c] pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0.]hept-2-ene-2carboxylate

5-acetyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridine-2-carbaldehyde (540 mg, 2.57 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (950 mg, 2.5 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (2.21 g, 8.5 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 870 mg, 53%; m.p. 46- 48°C; $(M+H)^+$ 637.6.

1HNMR(CDCl3): δ 2.15(t,6H);2.8-3.0(m,2H);3.7-3.9(m,2H);4.58-4.68(m,2H);5.30-5.45(dd,2H);5.85(d,1H);6.71(s,1H);6.95(s,1H);7.35-7.45(d,1H); 7.60(dd,2H); 8.25(dd,2H).

Step 3: (5R,6Z)-6-[(5-acetyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methylene]-oxo-4-thia-1-azabicyclo[3.2.0.]hept-2-ene-2-carboxylic acid (E+Z

Isomers mixture,Sodium salt)

A solution of 0.77g.(1.21 mmol, 4-nitrobenzyl(5R)-6-[(acetyloxy)(5-acetyl-4,5,6,7-tetrahydrotieno[3,2-c] pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0.]hept-2-ene-2carboxylate in 40 mL THF and 40 mL phosphate buffer solution (pH=6.36) was hydrogenated at 40 psi for 3 hours in the presence of 0.4g. Palladium on Carbon 10% catalyst. Reaction mixture was filtrated through celite pad, filtrate adjusted to pH=8.0, concentrated in vacuo, residue purified on a reverse-phase column (amberlite), using 5%..10% ACN/water mixture as solvent, gave 0.107g.(23%) of the title compound, reddish crystals, m.p.362.4^0 C, $(M+H)^+$ 409.5. 1H NMR:δ 2.08 (s,3H);2.80-2.95 (m,1H);3.74(m,2H);3.98-4.06(d,2H)6.32-6.42 (s,1H); 6.50-6.60(s,1H);6.98-7.20 (s,1H);7.30-7.40 (s,1H).

Example 34

Preparation of (5*R*,6*Z*)-6-(6,7-dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step 1: 4-Nitrosomorpholine-3-carboxylic acid

To a solution of morpholine-3-carboxylic acid (6.96 g, 52 mmol) in water (20 ml), at 0 °C under nitrogen, was added concentrated hydrochloric acid (4 ml), followed by sodium nitrite (5.0 g, 72 mmol) in small portions. The mixture was stirred at 0 °C for 1 hr, and then concentrated under vacuum at 30 to 35 °C. The residue was stirred with 200 ml of acetone and filtered. The filtrate was evaporated and the residue treated with 50 ml of THF and concentrated. The process was repeated with 2x50 ml of THF to give 11.87 g of light yellow foam; MS (ESI) *m/z* 159.2 (M-H).

Step 2: 6,7-Dihydro-4*H*-[1,2,3]oxadiazolo[4,3-c][1,4]oxazin-8-ium-3-olate

The crude 4-nitrosomorpholine-3-carboxylic acid (11.0 g) from step 1 was dissolved in THF (250 ml) and cooled to 0 °C. A solution of trifluoroacetic anhydride (7.4 ml, 52 mmol) in THF (20 ml) was added with stirring over 10 min. The resulting mixture was stirred at 0 °C for 5 hr, and warmed to room temperature for 16 hr. The solvent was evaporated and the residue was diluted with 250 ml of ethyl acetate and stirred with 30 g of anhydrous potassium carbonate. The mixture was filtered through a pad of silica gel and the filtrate evaporated. The residue was washed with a mixture of ethyl acetate-ether to give 3.80 g of a white solid; mp 132-133 °C; MS (ESI) *m/z* 143.1 (M+H).

Step 3: Ethyl 6,7-dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazine-2-carboxylate

To a partial solution of 6,7-dihydro-4*H*-[1,2,3]oxadiazolo[4,3-c][1,4]oxazin-8-ium-3-olate (3.41 g, 24 mmol) in o-xylene (80 ml), was added ethyl propiolate (2.7 ml, 26 mmol). The mixture was stirred at 140 °C for 3 hr. An additional 2.0 ml (19 mmol) of ethyl propiolate was then added and the mixture was stirred at reflux for 18 hr. The final solution was evaporated under vacuum, and the residue was dissolved in a mixture of methylene chloride and hexanes (1:5). The solution was passed through a pad of silica gel and the filter pad was eluted with methylene chloride-hexanes, followed by ethyl acetate. The ethyl acetate eluent was evaporated and the residue washed with hexanes to give 4.10 g of a white solid; mp 63 °C; MS (ESI) *m/z* 197.1 (M+H).

Step 4: 6,7-Dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazin-2-ylmethanol

To a solution of ethyl 6,7-dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazine-2-carboxylate (1.57 g, 8.0 mmol) in methylene chloride (30 ml) was added 24 ml of a 1.0 M solution of diisobutylaluminum hydride in methylene chloride at 0 °C, under nitrogen. After stirring for 0.5 hr at 0 °C, the mixture was warmed to room temperature for 2 hr. It was then treated with 30 ml of saturated ammonium chloride solution and extracted with ethyl acetate. The organic solution was washed with brine, dried over anhydrous sodium sulfate, filtered and evaporated to give 1.27 g of a colorless oil; MS (ESI) *m/z* 155.3 (M+H).

Step 5: 6,7-Dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazine-2-carbaldehyde

To a solution of 6,7-dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazin-2-ylmethanol (1.08 g, 7.0 mmol) in 1,2-dichloroethane (30 ml) was added 5.4 g of activated manganese dioxide at room temperature with stirring. The mixture was heated to 60 °C for 1 hr and then stirred at room temperature for 16 hr. The final mixture was filtered through a column of silica gel topped with celite. The filter pad was eluted with methylene chloride, followed by ethyl acetate. The ethyl acetate eluent was evaporated and the residue triturated with to give 0.81 g of a white solid; mp 91 °C; MS (ESI) *m/z* 153.2 (M+H).

Step 6: 4-Nitrobenzyl (5*R*)-6-[(acetyloxy)(6,7-dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate

To a solution of $MgBr_2$ (0.94 g, 5.1 mmol) in acetonitrile (25 ml) under nitrogen was added 6,7-dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazine-2-carbaldehyde (0.26 g, 1.7 mmol) at room temperature with stirring. A solution of (5R,6S)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (0.58 g, 1.5 mmol) in THF (25 ml) was then added, and the mixture was cooled to –20 °C. Triethylamine (0.71 ml, 5.1 mmol) was introduced, and the mixture was stirred at –20 °C in the dark for 5 hr. It was then treated with acetic anhydride (0.6 ml, 6.0 mmol), and 4-N,N-dimethylaminopyridine (24 mg, 0.2 mmol), and kept at 0 °C for 18 hr. The mixture was concentrated and the residue was dissolved in ethyl acetate. The ethyl acetate solution was washed with 5% citric acid, saturated sodium bicarbonate solution, and brine, dried over anhydrous sodium sulfate, and evaporated. The crude material was chromatographed with silica gel (EtOAc-CH2Cl2/1:5) to give 0.77 g of a white foam; MS (ESI) *m/z* 578.9 (M+H).

Step 7: (5*R*,6*Z*)-6-(6,7-dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

To a solution of 4-nitrobenzyl (5*R*)-6-[(acetyloxy)(6,7-dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (0.35 g, 0.6 mmol) in THF (20 ml), under nitrogen, was added 20 ml of a phosphate buffer solution (0.5M, pH 6.5), and 120 mg of 10% Pd/C. The mixture was hydrogenated at 40-50 psi for3 hr, and then filtered through Celite. The filter pad was washed with THF, and the filtrate was extracted with ethyl acetate. The organic extract was dried over anhydrous magnesium sulfate and evaporated. The residue was washed with ether to give 0.09 g of a yellow solid; HRMS: calcd for $C_{13}H_{11}N_3O_4S$, 305.0470; found (ESI+), 306.05434; ^1H NMR (DMSO-d_6) δ 4.07-4.09 (t, 2H), 4.13-4.17 (t, 2H), 4.82 (s, 2H), 6.36 (s, 1H), 6.55 (s, 1H), 7.17 (s, 1H), 7.55 (s, 1H), 12.80 (bs, 1H).

Example 35

Preparation of (5*R*)(6*Z*)-6-(6,7-5H--Dihydropyrazolo[5,1-*b*]oxazin-2-ylmethylene)-7-oxo-4-thia-1- azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of ethyl 6,7-dihydro-5H-pyrazolo[5,1-b][1,3]oxazine-2-carboxylate:

To the stirred suspension of ethyl 5-hydroxy-1H-pyrazole-3-carboxylate (10.34 g, 0.66 mol) and 36.62 g of potassium carbonate in 500 ml of acetonitrile was added 14.7 g of 1,3-dibromopropane, and refluxed for 16 hours. The reaction mixture was allowed to cool to room temperature, then filtered, the solid was washed with acetonitrile. The filtrate was concentrated to an oil. The residue was dissolved in ethyl acetate and extracted with water. The organic phase was dried over $MgSO_4$ and evaporated to dryness. 8.80 g of the desired product was obtained (68%), m.p. 44-46^{0}C $(M+H)^+$ 197.1.

Step 2: Preparation of 2,3-dihydro-5H-pyrazolo[5,1-b][1,3]oxazin-2-yl-methanol:

To the stirred solution of 6,7-dihydro-5H-pyrazolo[5,1-b][1,3]oxazine-2-carboxylate:

(4.0 g, 20 mmol) of in 100 ml of THF was added 0.71 g of lithium borohydride and 1.03 g of methanol. The solution was heated at 40C for 2.5 hour. The reaction was quenched by 1N HCl, and adjusted to pH 1.3 and stirred at room temperature for 1 hour. The reaction mixture was adjusted pH to 8 with k_2CO_3. The reaction mixture was extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, and concentrated to an oil and column chromatographyed to give 2.08 g of the desired product (67%); (M+H) 155.

Step 3: Preparation of 6,7-dihydro-5H-pyrazolo[5,1-b][1,3]oxazine-2-carbaldehyde:

To the stirred solution of 2,3-dihydro-5H-pyrazolo[5,1-b][1,3]oxazin-2-yl-methanol

(2.08 g, 13.5 mmol) in 60 ml of CH_3Cl was added 9.38 g of MnO_2. Th suspension was refluxed for 2 hour under a nitrogen atmosphere. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated to give yellow oil. The product was purified by chromatography. 2.15 g of the product was obtained (78%).

Step 4: 4-Nitrobenzy(5R)-6-[(acetyloxy)(6,7-dihydro-5H-pyrazolo[5,1-b][1,3]oxazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

6,7-dihydro-5H-pyrazolo[5,1-b][1,3]oxazine-2-carbaldehyde (330 mg, 2 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (0.794 g, 2.2 mmol)

were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (1.2 g)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 0.76 g, 65%; M+H 579.

Step-5: (5*R*)(6*Z*)-6-(6,7-5H--Dihydropyrazolo[5,1-*b*]oxazin-2-ylmethylene)-7-oxo-4-thia-1- azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

4-Nitrobenzy(5R)-6-[(acetyloxy)(6,7-dihydro-5H-pyrazolo[5,1-b][1,3]oxazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (350 mg, 0.6 mmol) was dissolved in THF (20 mL), acetonitrile (10 mL) and 0.5 *M* phosphate buffer (pH 6.5, 28 mL) and hydrogenated over 10% Pd/C at 40 psi pressure. After 4 hrs the reaction mixture was filtered, cooled to 3 °C, and 0.1 *M* NaOH was added to adjust pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 lits) and latter with 10% acetonitrile: Water. The fractions containing the product were collected and concentrated at reduced pressure at room temperature. The yellow solid was washed with acetone and filtered. Dried. Yield: 103 mg, 52%; as yellow amorphous solid; (M+H+Na)327.

^{1}H-NMR(D_2O); δ 6.97(1H, s), 6.93(1H, s), 6.47(1H, s), 5.65(1H, s) 4.28(2H, m), 4.10(2H,m), 2.21 (2H,m).

Example 36

Preparation of (5R),(6Z)-6-[5-(3-carboxypropionyl)-4,5,6,7-

tetrahydropyrazolo[1,5-a]pyrazin-2-ylmethylene]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-carboxylic acid,disodium salt

The above mentioned compound was prepared by the procedures outlined in all the above examples. Starting from (5R),(6Z)-6-{5-[3-(4-itrobenzyloxycarbonyl)propionyl]-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-2-ylmethylene}-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt (467 mg) and hydrogenating it over Pd/C (10%), 276 mg of (74%) of (5R),(6Z)-6-[5-(3-carboxypropionyl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-2-ylmethylene]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-carboxylic acid,disodium salt was isolated as yellow amorphous solid. Mp. 180°C (Dec); ^{1}H NMR (D_2O) □ 2.41 (t, 2H), 2.42 (t, 2H), 2.67 (t, 2H), 2.72 (t, 2H), 3.95-4.09 (m, 2H), 4.18 (t, 2H), 4.28 (t, 2H), 4.75 (s, 2H), 4.87 (s, 2H), 6.33 (s,1H), 6.34 (s, 1H), 6.53 (s, 1H), 7.00 (s, 1H), 7.09 (s, 1H).

Example 37

Preparation of (5R),(6Z)-6-[5-(2-methoxyacetyl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-2-ylmethylene]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-carboxylic acid,sodium salt

(5*R*),(6*Z*)-7-Oxo-6-(4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-2-ylmethylene)-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt (Example 25) To the THF (64 mL) and H_2O (64 mL) solution of (5*R*),(6*Z*)-7-Oxo-6-(4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-2-ylmethylene)-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt (Example 25) (638 mg) was added 0.1 M NaOH aq slowly to adjust pH to 12.5 at 0 °C. To the mixture was added methoxyacetyl -chloride (0.28 mL) over 5 min. The mixture was stirred for 0.5 h at 0 °C and methoxyacetylchloride (0.09 mL) was added to the mixture. After stirring the mixture for 0.5 h at the same temperature, 0.1 M NaOH aq was added to adjust pH to 8.05. The mixture was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (78 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN (1:0 to 9:1). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (509 mg, 65%, pH 7.58).

Mp 170 °C (dec); ^{1}H NMR (D_2O) □ 3.28 (s, 3H x 1/2), 3.29 (s, 3H x 1/2), 3.78 (t, 2H x 1/2, J = 5.4 Hz), 3.89 – 3.93 (m, 2H x 1/2), 4.09 (t, 2H x 1/2, J = 5.4 Hz), 4.14

(t, 2H x 1/2, J = 5.4 Hz), 4.20 (s, 2H x 1/2), 4.25 (s, 2H x 1/2), 4.61 (s, 2H x 1/2), 4.66 (s, 2H x 1/2), 6.19 (s, 1H x 1/2), 6.22 (s, 1H x 1/2), 6.37 (s, 1H x 1/2), 6.372 (s, 1H x 1/2), 6.87 (s, 1H), 6.93 (s, 1H)

Example 38

Preparation of (5*R*), (6*Z*)-6-[5-(4,5-Dihydrothiazol-2-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin- 2-ylmethylene]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt

(4,5,6,7-Tetrahydropyrazolo[1,5-a]pyrazin-2-yl)-methanol

Methanol (150 ml) was added to the mixture of 2-hydroxymethyl-6,7-dihydro-4H-pyrazolo[1,5-a]pyrazine-5-carboxylic acid 4-nitrobenzyl ester (Example 25) (2.38 g) and 10% Pd-C (50% wet, 1.19 g). The reaction mixture was stirred for 2 hours under a hydrogen atmosphere. The mixture was filtered and concentrated under reduced pressure. The residue was applied to silica-gel column chromatography, then the column was eluted with 50% methanol in chloroform. The titled compound was obtained as a white solid (1.08 g, 98 %).

^{1}H NMR (400 MHz, CD_3OD) □3.22 – 3.25 (m, 2H), 3.99 (s, 2H), 4.03 – 4.06 (m, 2H), 4.52 (s, 2H), 6.06 (s, 1H).

[5-(4,5-Dihydrothiazol-2-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-2-yl]-methanol

Hydrogen chloride (2 mol/l) solution in diethyl ether (0.7 ml) was added to the methanol (20 ml) solution of (4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-2-yl)-methanol (1.08 g) and 2-methylsulfanyl-4,5-dihydrothiazole (1.03 g). The reaction mixture was refluxed for 4 days. The mixture was quenched with small amount of saturated potassium carbonate solution, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica-gel column chromatography, then the column was eluted with 10% methanol in chloroform. The titled compound was obtained as a white solid (1.49 g, 89 %).

^{1}H NMR (400 MHz, $CDCl_3$) □ 2.04 (brs, 1H), 3.39 (t, 2H, J = 7.5 Hz), 3.90 (t, 2H, J = 5.3 Hz), 4.06 (t, 2H, J = 7.5 Hz), 4.21 (t, 2H, J = 5.3 Hz), 4.66 (s, 2H), 4.69 (s, 2H), 6.07 (s, 1H).

(5*R*), (6*Z*)-6-[5-(4,5-Dihydrothiazol-2-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin- 2-ylmethylene]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt

The activated manganese (IV) oxide (16.75 g) was added to the mixture of chloroform (180 ml) solution of [5-(4,5-dihydrothiazol-2-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-2-yl]-methanol (3.35 g) at room temperature. The reaction mixture was refluxed for 1 hour. After refluxing, the mixture was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure. The residue was dried in vacuo and the crude 5-(4,5-dihydrothiazol-2-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine-2-carbaldehyde was obtained as a colorless solid. The crude aldehyde thus obtained(2.56 g) was added to a dry acetonitrile (200 ml) solution of $MgBr_2$ (7.36 g) under a nitrogen atmosphere at room temperature then the mixture was stirred for 10 minutes. A dry THF (200 ml) solution of WLJ 20,014 (4.16 g) was added and the mixture was cooled to –20 °C. Then triethylamine (11.3 ml) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 1.5 hours at –20 °C and treated with 4-dimethylaminopyridine (132 mg) and acetic anhydride (4.2 ml) in one portion. The reaction mixture was warmed to 0 °C and stirred for 20 hours at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then eluted with *n*-hexane-AcOEt (1:2) and chloroform - methanol (9:1). The (5*R*,6*RS*)-6-{(*RS*)-acetoxy-[5-(4,5-dihydrothiazol-2-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-2-yl]-methyl}-6-bromo-7-oxo-4-thia-1- azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester was obtained (5.41 g, 75.4%).

The (5*R*,6*RS*)-6-{(*RS*)-acetoxy-[5-(4,5- dihydrothiazol-2-yl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-2-yl]-methyl}-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (5.41 g) was dissolved in THF (76 ml) and acetonitrile (35 ml). Freshly activated Zn dust (21.6 g) and 0.5 mol/l phosphate buffer (pH 6.5, 111 ml) were added to the mixture. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 hours at 30 to 35 °C. The reaction mixture was cooled at 0 °C, and then the pH was adjusted to 7.6. Ethyl acetate was added to the reaction mixture and filtered through a pad of Celite. The pad was washed with water and the aqueous layer was separated. The aqueous layer was concentrated under high

vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (170 ml, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with water and then with 5% to 15% acetonitrile aqueous solution. The combined active fractions was concentrated under high vacuum at 35 °C and lyophilized to give the titled compound as a crude yellow amorphous solid (1.60 g).

The crude compound was purified by preparative HPLC (Mightysil RP-18GP, KANTO CHEMICAL CO., INC., 35 x 250 mm, 0.05 mol/l phosphate buffer (pH 7.1) : acetonitrile = 80 : 20, 25 ml/min) followed by desaltation on Diaion HP-21 resin (150 ml, Mitsubishi Kasei Co. Ltd.) to give the titled compound as a yellow amorphous solid (1.06 g, y. 31.5 %, pH 8.33).

Mp 100 °C (dec); ^{1}H NMR (D_2O) □ 3.18 (t, 2H, *J* = 7.6 Hz), 3.60 (t, 2H, *J* = 5.3 Hz), 3.73 (t, 2H, *J* = 7.6 Hz), 3.94 (t, 2H, *J* = 5.3 Hz), 4.37 (s, 2H), 6.01 (s, 1H), 6.21 (s, 1H), 6.77 (s, 1H), 6.78 (s, 1H); IR (KBr) 3381, 1752, 1606 cm^{-1}; □^{max} (H_2O) 369, 291, 208 nm.

Example 39

Preparation of (5*R*,6*Z*)-6-{[6-(ethoxycarbonyl)-4,5,6,7-tetrahydrofuro[2,3-*c*]pyridin-2-yl]methylene}-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Preparation of Ethyl 2-[(acetyloxy)((5*R*)-6-bromo-2-{[(4-nitrobenzyl)oxy]carbonyl}-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-en-6-yl)methyl]-4,7-dihydrofuro[2,3-*c*]pyridine-6(5*H*)-carboxylate

The titled compound was prepared from 0.669 grams of methyl 2-formyl-4,7-dihydrofuro[2,3-*c*]pyridine-6(5*H*)-carboxylate and 1.155 grams of 4-nitrobenzyl (5*R*)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate yielded 1.65 grams of product (84%), which was used directly for the next step. MS: 652.2(M+H)

Preparation of (5*R*,6*Z*)-6-{[6-(ethoxycarbonyl)-4,5,6,7-tetrahydrofuro[2,3-*c*]pyridin-2-yl]methylene}-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

The title compound was prepared from1.65 g of ethyl 2-[(acetyloxy)((5*r*)-6-bromo-2-{[(4-nitrobenzyl)oxy]carbonyl}-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-en-6-yl)methyl]-4,7-dihydrofuro[2,3-*c*]pyridine-6(5*h*)-carboxylate, yielded 0.386 grams of product (41%). Mp: decomposed at 175°c. Ms: 375.0 (m-h). H-nmr(d2o): □ 6.91 (s, 1h),

6.84(s, 1h), 6.62(s, 1h), 6.39(s, 1h), 4.41(b, 2h), 4.04 (q, 2h, j=5hz), 3.52(b, 2h), 2.42(b, 2h), 1.14 (t, 3h, j=5hz),

Brief Description of Biological Test Procedure(s) and Text Summary of Results.

Antimicrobial susceptibility testing. The in vitro activities of the antibiotic, piperacillin in this case, against resistant pathogens expressing class-D enzymes were determined by the microbroth dilution method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS). (**NCCLS.** 2000. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standards: M7-A5, vol. 19. National Committee for Clinical Laboratory Standards, Villanova, PA). Mueller-Hinton II broth (MHBII)(BBL Cockeysville, MD), was used for the testing procedure. Microtiter plates containing 50 µL per well of two-fold serial dilutions of piperacillin combined with a constant amount (4ug/ml) of a B-lactamase inhibitor were inoculated with 50 µL of inoculum to yield the appropriate density (10^5 CFU/mL) in 100 µL final volume. The plates were incubated for 18 - 22 hours at 35°C in ambient air. The minimal inhibitory concentration (MIC_{50}) for all isolates was defined as the lowest concentration of antimicrobial agent that completely inhibits the growth of the organism as detected by the unaided eye. The MIC_{50} data obtained by the above said procedure are enlisted in Table 1. As a control piperacillin has an MIC_{50} value of >64 µg/mL. Both OXA-10 and PSE-2 are class D β-lactamases. (Bush, K. Jacoby, G.A., Medeiros, A.A. *Antimicrob. Agents Chemother.*, **1995**, 39, 1211.)

Table 1: Minimal Inhibitory Concentration (MIC_{50}) (µg/mL) Data: Inc: 35°C for 18 hours

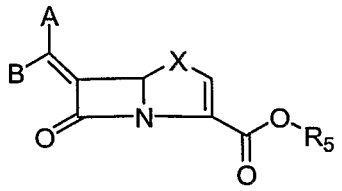
Against class-D producing organism E. coli GC 2883 (OXA-10+PSE-2)

Example	MIC_{50} Data
1	4
2	4
3	2
4	4
5	2
6	2
7	4
8	4

9	1
10	8
11	2
12	2
13	2
14	1
15	4
16	8
17	4
18	4
19	2
20	16
21	8
22	16
23	8
24	4
25	4
26	4
27	2
28	4
29	4
30	4
31	2
32	2
33	16
34	2
35	2
36	4
37	2
38	2
39	4

What is claimed is:

1. A method of inhibiting class D enzymes in the treatment of bacterial infection in a patient in need thereof which comprises providing to said patient an effective amount of a compound of formula I:



I

wherein:

one of A and B is hydrogen and the other is an optionally substituted fused bicyclic heteroaryl group;

X is O or S;

R_5 is H, C1 –C6 alkyl, C5 – C6 cycloalkyl, CHR_3OCOC1-C6alkyl; and

R_3 is hydrogen, C1-C6 alkyl, C5 – C6 cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl;

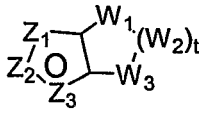
or a pharmaceutically acceptable salt thereof.

2. The method according to claim 1 wherein the compound is co-administered with a β-lactam antibiotic.

3. The method according to claim 2 wherein the ratio of β-lactam antibiotic to the compound is in a range from about 1:1 to 100:1.

4. The method according to claim 3 wherein the ratio of the β-lactam antibiotic to the compound is less than 10:1.

5. The method according to claim 1 wherein the bicyclic heteroaryl group is



1-A

wherein

Z1, Z2 and Z3 are independently CR_2, N, O, S or N-R_1 provided one of Z1 – Z3 is carbon and is bonded to the remainder of the molecule;

W_1, W_2 and W_3 are independently CR_4R_4, S, SO, SO_2, O, or N-R_1; with the proviso that no S-S or O-O or S-O bond formation can occur to form the saturated ring system;

t= 1 to 4;

R_1 is H; optionally substituted C1-C6 alkyl, optionally substituted aryl, optionally substituted heteroaryl or mono or bicyclic saturated heterocycles, optionally substituted C5-C7 cycloalkyl, optionally substituted C3-C6 alkenyl, optionally substituted C3-C6 alkynyl with the proviso that neither the double bond nor the triple bond should be present at the carbon atom which is directly linked to N; optionally substituted C1-C6 perfluoroalkyl,

$-S(O)_p$ optionally substituted alkyl or aryl where p is 0-2, optionally substituted -C=Oheteroaryl, optionally substituted -C=Oaryl, optionally substituted -C=O (C1-C6) alkyl, optionally substituted -C=O(C5-C6)cycloalkyl, optionally substituted -C=O mono or bicyclic saturated heterocycles, optionally substituted C1-C6 alkylaryl, optionally substituted C1-C6 alkyl heteroaryl, optionally substituted aryl-C1-C6 alkyl, optionally substituted heteroaryl-C1-C6 alkyl, optionally substituted C1-C6 alkyl mono or bicyclic saturated heterocycles, optionally substituted arylalkenyl of 8 to 16 carbon atoms, $-CONR_6R_7$, $-SO_2NR_6R_7$, optionally substituted arylalkyloxyalkyl, optionally substituted -alkyl-O-alkyl-aryl, optionally substituted -alkyl-O-alkyl-heteroaryl, optionally substituted aryloxyalkyl, optionally substituted heteroaryloxyalkyl, optionally substituted aryloxyaryl, optionally substituted aryloxyheteroaryl, optionally substituted C1-C6alkylaryloxyaryl, optionally substituted C1-C6 alkylaryloxyheteroaryl, optionally substituted alkylaryloxyalkylamines, optionally substituted alkoxycarbonyl, optionally substituted aryloxycarbonyl, or optionally substituted heteroaryloxy carbonyl;

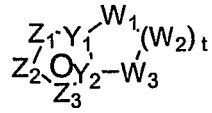
R_2 is hydrogen, optionally substituted C1-C6 alkyl, optionally substituted C2-C6 alkenyl, optionally substituted C2-C6 alkynyl, halogen, cyano, N-R_6R_7, optionally

substituted C1-C6 alkoxy, hydroxy; optionally substituted aryl, optionally substituted heteroaryl, $COOR_6$, optionally substituted alkyl aryloxy alkylamines, optionally substituted aryloxy, optionally substituted heteroaryloxy, optionally substituted C3-C6 alkenyloxy, optionally substituted C3 –C6 alkynyloxy, C1-C6 alkylamino-C1-C6 alkoxy, alkylene dioxy, optionally substituted aryloxy-C1-C6 alkyl amine, C1-C6 perfluoro alkyl, $S(O)_q$-optionally substituted C1-C6 akyl, $S(O)_q$- optionally substituted aryl where q is 0, 1 or 2, $CONR_6R_7$, guanidino or cyclic guanidino, optionally substituted C1-C6 alkylaryl, optionally substituted arylalkyl, optionally substituted C1-C6 alkylheteroaryl, optionally substituted heteroaryl-C1-C6 alkyl, optionally substituted C1-C6 alkyl mono or bicyclic saturated heterocycles, optionally substituted arylalkenyl of 8 to 16 carbon atoms, $SO_2NR_6R_7$, optionally substituted arylalkyloxyalkyl, optionally substituted aryloxyalkyl, optionally substituted heteroaryloxyalkyl, optionally substituted aryloxyaryl, optionally substituted aryloxyheteroaryl, substituted heteroaryloxyaryl, optionally substituted C1-C6alkyl aryloxyaryl, optionally substituted C1-C6 alkylaryloxyheteroaryl , optionally substituted aryloxyalkyl, optionally substituted heteroaryloxyalkyl, or optionally substituted alkylaryloxyalkylamine;

R_4 is H, optionally substituted C1-C6 alkyl, one of R_4 is OH, C1-C6 alkoxy, -S-C1-C6 alkyl, $COOR_6$, $-NR_6R_7$, $-CONR_6R_7$; or R_4R_4 may together be =O or R_4R_4 together with the carbon to which they are attached may form a spiro system of five to eight members with or without the presence of heteroatoms selected from N, O, S=(O)n (where n =0 to 2), and N-R_1; and

R_6 and R_7 are independently H, optionally substituted C1-C6 alkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted C1-C6 alkylaryl, optionally substituted arylalkyl, optionally substituted heteroarylalkyl, optionally substituted C1-C6 alkylheteroaryl, or R_6 and R_7 can be together to form a 3-7 membered saturated ring system optionally having one or two heteroatoms selected from N, O, or S.

6. The method according to claim 1 wherein the bicyclic heteroaryl group is



1-B

wherein

Z1, Z2 and Z3 are independently CR_2, N, O, S or N-R_1 provided one of Z1 – Z3 is carbon and is bonded to the remainder of the molecule;

W_1, W_2 and W_3 are independently CR_4R_4, S, SO, SO_2, O, or N-R_1;

t= 1 to 4;

Y_1 and Y_2 are independently N or C; with the proviso that if the aromatic ring portion of the bicyclic heteroaryl group is imidazole, the nonaromatic ring portion may not contain a S adjacent to the bridgehead carbon;

R_1 is H, optionally substituted C1-C6 alkyl, optionally substituted aryl, optionally substituted heteroaryl or mono or bicyclic saturated heterocycles, optionally substituted C5-C7 cycloalkyl, optionally substituted C3-C6 alkenyl, optionally substituted C3-C6 alkynyl with the proviso that neither the double bond nor the triple bond should be present at the carbon atom which is directly linked to N; optionally substituted C1-C6 perfluoroalkyl,

-$S(O)_p$ optionally substituted alkyl or aryl where p is 0-2, optionally substituted -C=Oheteroaryl, optionally substituted -C=Oaryl, optionally substituted -C=O (C1-C6) alkyl, optionally substituted -C=O(C5-C6)cycloalkyl, optionally substituted -C=O mono or bicyclic saturated heterocycles, optionally substituted C1-C6 alkylaryl, optionally substituted C1-C6 alkyl heteroaryl, optionally substituted aryl-C1-C6 alkyl, optionally substituted heteroaryl-C1-C6 alkyl, optionally substituted C1-C6 alkyl mono or bicyclic saturated heterocycles, optionally substituted arylalkenyl of 8 to 16 carbon atoms, -$CONR_6R_7$, -$SO_2NR_6R_7$, optionally substituted arylalkyloxyalkyl, optionally substituted -alkyl-O-alkyl-aryl, optionally substituted -alkyl-O-alkyl-heteroaryl, optionally substituted aryloxyalkyl, optionally substituted heteroaryloxyalkyl, optionally substituted aryloxyaryl, optionally substituted aryloxyheteroaryl, optionally substituted C1-C6alkylaryloxyaryl, optionally substituted C1-C6 alkylaryloxyheteroaryl, optionally

substituted alkylaryloxyalkylamines, optionally substituted alkoxycarbonyl, optionally substituted aryloxycarbonyl, or optionally substituted heteroaryloxy carbonyl;

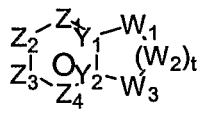
R_2 is hydrogen, optionally substituted C1-C6 alkyl, optionally substituted C2-C6 alkenyl, optionally substituted C2-C6 alkynyl, halogen, cyano, N-R_6R_7, optionally substituted C1-C6 alkoxy, hydroxy; optionally substituted aryl, optionally substituted heteroaryl, $COOR_6$, optionally substituted alkyl aryloxy alkylamines, optionally substituted aryloxy, optionally substituted heteroaryloxy, optionally substituted C3-C6 alkenyloxy, optionally substituted C3 –C6 alkynyloxy, C1-C6 alkylamino-C1-C6 alkoxy, alkylene dioxy, optionally substituted aryloxy-C1-C6 alkyl amine, C1-C6 perfluoro alkyl, $S(O)_q$-optionally substituted C1-C6 akyl, $S(O)_q$- optionally substituted aryl where q is 0, 1 or 2, $CONR_6R_7$, guanidino or cyclic guanidino, optionally substituted C1-C6 alkylaryl, optionally substituted arylalkyl, optionally substituted C1-C6 alkylheteroaryl, optionally substituted heteroaryl-C1-C6 alkyl, optionally substituted C1-C6 alkyl mono or bicyclic saturated heterocycles, optionally substituted arylalkenyl of 8 to 16 carbon atoms, $SO_2NR_6R_7$, optionally substituted arylalkyloxyalkyl, optionally substituted aryloxyalkyl, optionally substituted heteroaryloxyalkyl, optionally substituted aryloxyaryl, optionally substituted aryloxyheteroaryl, substituted heteroaryloxyaryl, optionally substituted C1-C6alkyl aryloxyaryl, optionally substituted C1-C6 alkylaryloxyheteroaryl , optionally substituted aryloxyalkyl, optionally substituted heteroaryloxyalkyl, or optionally substituted alkylaryloxyalkylamine;

R_4 is H, optionally substituted C1-C6 alkyl, one of R_4 is OH, C1-C6 alkoxy, -S-C1-C6 alkyl, $COOR_6$, -NR_6R_7, -$CONR_6R_7$; or R_4R_4 may together be =O or R_4R_4 together with the carbon to which they are attached may form a spiro system of five to eight members with or without the presence of heteroatoms selected from N, O, S=(O)n (where n =0 to 2), and N-R_1; and

R_6 and R_7 are independently H, optionally substituted C1-C6 alkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted C1-C6 alkylaryl, optionally substituted arylalkyl, optionally substituted heteroarylalkyl, optionally substituted C1-C6 alkylheteroaryl, or R_6 and R_7 can be together to form a

3-7 membered saturated ring system optionally having one or two heteroatoms selected from N, O, or S.

7. The method according to claim 1 wherein the bicyclic heteroaryl group is



1-C

wherein

Z1, Z2, Z3 and Z4 are independently CR_2 or N provided one of Z1 –Z4 is carbon and is bonded to the remainder of the molecule;

W_1, W_2 and W_3 are independently CR_4R_4, S, SO, SO_2, O, or N-R_1; with the proviso that no S-S or O-O or S-O bond formation can occur to form the saturated ring system;

t= 1 to 4;

Y_1 and Y_2 are independently C or N;

R_1 is H, optionally substituted C1-C6 alkyl, optionally substituted aryl, optionally substituted heteroaryl or mono or bicyclic saturated heterocycles, optionally substituted C5-C7 cycloalkyl, optionally substituted C3-C6 alkenyl, optionally substituted C3-C6 alkynyl with the proviso that neither the double bond nor the triple bond should be present at the carbon atom which is directly linked to N; optionally substituted C1-C6 perfluoroalkyl,

-$S(O)_p$ optionally substituted alkyl or aryl where p is 0-2, optionally substituted -C=Oheteroaryl, optionally substituted -C=Oaryl, optionally substituted -C=O (C1-C6) alkyl, optionally substituted -C=O(C5-C6)cycloalkyl, optionally substituted -C=O mono or bicyclic saturated heterocycles, optionally substituted C1-C6 alkylaryl, optionally substituted C1-C6 alkyl heteroaryl, optionally substituted aryl-C1-C6 alkyl, optionally substituted heteroaryl-C1-C6 alkyl, optionally substituted C1-C6 alkyl mono or bicyclic saturated heterocycles, optionally substituted arylalkenyl of 8 to 16 carbon atoms, -$CONR_6R_7$, -$SO_2NR_6R_7$, optionally substituted arylalkyloxyalkyl, optionally

substituted -alkyl-O-alkyl-aryl, optionally substituted -alkyl-O-alkyl-heteroaryl, optionally substituted aryloxyalkyl, optionally substituted heteroaryloxyalkyl, optionally substituted aryloxyaryl, optionally substituted aryloxyheteroaryl, optionally substituted C1-C6alkylaryloxyaryl, optionally substituted C1-C6 alkylaryloxyheteroaryl, optionally substituted alkylaryloxyalkylamines, optionally substituted alkoxycarbonyl, optionally substituted aryloxycarbonyl, or optionally substituted heteroaryloxy carbonyl;

R_2 is hydrogen, optionally substituted C1-C6 alkyl, optionally substituted C2-C6 alkenyl, optionally substituted C2-C6 alkynyl, halogen, cyano, N-R_6R_7, optionally substituted C1-C6 alkoxy, hydroxy; optionally substituted aryl, optionally substituted heteroaryl, $COOR_6$, optionally substituted alkyl aryloxy alkylamines, optionally substituted aryloxy, optionally substituted heteroaryloxy, optionally substituted C3-C6 alkenyloxy, optionally substituted C3 –C6 alkynyloxy, C1-C6 alkylamino-C1-C6 alkoxy, alkylene dioxy, optionally substituted aryloxy-C1-C6 alkyl amine, C1-C6 perfluoro alkyl, $S(O)_q$-optionally substituted C1-C6 akyl, $S(O)_q$- optionally substituted aryl where q is 0, 1 or 2, $CONR_6R_7$, guanidino or cyclic guanidino, optionally substituted C1-C6 alkylaryl, optionally substituted arylalkyl, optionally substituted C1-C6 alkylheteroaryl, optionally substituted heteroaryl-C1-C6 alkyl, optionally substituted C1-C6 alkyl mono or bicyclic saturated heterocycles, optionally substituted arylalkenyl of 8 to 16 carbon atoms, $SO_2NR_6R_7$, optionally substituted arylalkyloxyalkyl, optionally substituted aryloxyalkyl, optionally substituted heteroaryloxyalkyl, optionally substituted aryloxyaryl, optionally substituted aryloxyheteroaryl, substituted heteroaryloxyaryl, optionally substituted C1-C6alkyl aryloxyaryl, optionally substituted C1-C6 alkylaryloxyheteroaryl , optionally substituted aryloxyalkyl, optionally substituted heteroaryloxyalkyl, or optionally substituted alkylaryloxyalkylamine;

R_4 is H, optionally substituted C1-C6 alkyl, one of R_4 is OH, C1-C6 alkoxy, -S-C1-C6 alkyl, $COOR_6$, $-NR_6R_7$, $-CONR_6R_7$; or R_4R_4 may together be =O or R_4R_4 together with the carbon to which they are attached may form a spiro system of five to eight members with or without the presence of heteroatoms selected from N, O, S=(O)n (where n =0 to 2), and N-R_1; and

R_6 and R_7 are independently H, optionally substituted C1-C6 alkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted C1-C6 alkylaryl, optionally substituted arylalkyl, optionally substituted heteroarylalkyl, optionally substituted C1-C6 alkylheteroaryl, or R_6 and R_7 can be together to form a 3-7 membered saturated ring system optionally having one or two heteroatoms selected from N, O, or S.

8. The method according to claim 2 wherein the β-lactam antibiotic is selected from the group consisting of a penicillin antibiotic, a carbapenem antibiotic, and a cephalosporin antibiotic.

9. The method according to claim 8 wherein the β-lactam antibiotic is selected from thegroup consisting of piperacillin, amoxycillin, ticarcillin, benzylpenicillins, ampicillin, sulbenicillin, cefatrizine, cephaloridine, cephalothin, cefazolin, cephalexin, cephradine, aztreonam, and latamoxef.

10. The method according to claim 9 wherein the β-lactam antibiotic is piperacillin or amoxycillin.

11. The method according to claim 10 wherein the β-lactam antibiotic is piperacillin and is provided to the patient intravenously.

12. The method according to claim 10 wherein the β-lactam antibiotic is amoxycillin and is provided to the patient orally.