SYNTHESIS OF DISODIUM (+)-(Z)-[[[1-(2-AMINO-4-[2-^{14}C]THIAZOLYL)-2-[[(2S,3S)-2-(CARBAMOYLOXYMETHYL)-4-OXO-1-SULFONATO-3-AZETIDINYL]AMINO]-2-OXOETHYLIDENE]AMINO]OXY]ACETATE

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SUMMARY

Disodium (+)-(Z)-[[[1-(2-amino-4-[2-^{14}C]thiazolyl)-2-[[(2S,3S)-2-(carbamoyloxymethyl)-4-oxo-1-sulfonato-3-azetizinyl]amino-2-oxoethylidene]-amino]oxy]acetate ([^{14}C]AMA-1080) was synthesized via [2-(2-chloroacetamido-4-[2-^{14}C]thiazolyl)-(Z)-2-(4-nitrobenzyloxycarbonylmethoxyimino)]acetic acid from [^{14}C]thiourea. Trisodium (+)-(Z)-[[[1-(2-amino-4-[2-^{14}C]thiazolyl)-2-[[(1S,2S)-3-carbamoyloxy-1-carboxylato-2-sulfonatoaminopropyl]amino]-2-oxoethylidene]amino]oxy]acetate ([^{14}C]AMA-1294) as the principal metabolite of [^{14}C]AMA-1080 was also prepared.

Key words: Syntheses of [^{14}C]AMA-1080 and [^{14}C]AMA-1294

INTRODUCTION

During the course of the study on chemical modification of sulfazecin[1] having a novel 1-sulfo-2-azetidinone structure, a new β-lactam antibiotic, disodium (+)-(Z)-[[[1-(2-amino-4-thiazolyl)-2-[[(2S,3S)-2-(carbamoyloxymethyl)-4-oxo-1-sulfonato-3-azetidinyl]-amino]-2-oxoethylidene]amino]oxy]acetate (AMA-1080) was found to exhibit the most interesting antibiotic activity.[2] On the other hand, based on the studies of distribution and biotransformation of AMA-1080 in animals, the principal metabolite has been identified as expected trisodium (+)-(Z)-[[[1-(2-amino-4-thiazolyl)-2-[[(1S,2S)-3-carbamoyloxy-1-carboxylato-2-sulfonatoaminopropyl]amino]-2-oxoethylidene]amino]oxy]acetate[3] (AMA-1294). This paper deals with the syntheses of disodium (+)-(Z)-[[[1-(2-amino-4-[2-^{14}C]thiazolyl)-2-[[2S,3S)-2-(carbamoyloxymethyl)-4-oxo-1-sulfonato-3-azetidinyl]amino]-2-oxoethylidene]-amino]oxy]acetate (VII) and trisodium (+)-(Z)-[[[1-(2-amino-4-[2-^{14}C]thiazolyl)-2-[[(1S,2S)-3-

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carbamoyloxy-1-carboxylato-2-sulfonatoaminopropyl]amino]-2-oxoethylidene]amino]oxy]-acetate (VIII).

RESULTS

The condensation of [^{14}C]thiourea (I) with t-butyl 4-chloro-2-hydroxyiminoacetoacetate led to t-butyl [2-(2-amino-4-[2-^{14}C]thiazolyl)-(Z)-2-hydroxyimino]acetate (II), which was reacted with p-nitrobenzyl bromoacetate to give t-butyl [2-(2-amino-4-[2-^{14}C]-thiazolyl)-(Z)-2-(4-nitrobenzyloxycarbonylmethoxyimino)]acetate (III). It is known that chloroacetyl group is a useful amino-blocking group, because it is easily cleaved by sodium N-methyldithiocarbamate.[4] Then, III was acylated with chloroacetyl chloride to afford t-butyl [2-(2-chloroacetamido-4-[2-^{14}C]thiazolyl)-(Z)-2-(4-nitrobenzyloxycarbonylmethoxyimino)]acetate (IV), and [2-(2-chloroacetamido-4-[2-^{14}C]thiazolyl)-(Z)-2-(4-nitrobenzyloxycarbonylmethoxyimino]acetic acid (V) was obtained from IV by treating with trifluoroacetic acid. The treatment of V with phosphorus pentachloride provided an intermediate, acid chloride of V. The condensation of the acid chloride with (3S,4S)-3-amino-4-carbamoyloxymethyl-2-azetidinone-1-sulfonic acid, followed by elimination of the chloroacetyl group in the presence of sodium N-methyldithiocarbamate, gave p-nitrobenzyl (+)-(Z)-[[[1-(2-amino-4-[2-^{14}C]thiazolyl)-2-[[(2S,3S)-2-(carbamoyloxymethyl)-4-oxo-1-sodiosulfonato-3-azetidinyl]amino]-2-oxoethylidene]amino]oxy]acetate (VI). Catalytic hydrogenolysis of VI gave VII in 20% overall radiochemical yield. For the additional study on metabolic fate of VIII in animals, VII was hydrolyzed with lithium hydroxide to afford VIII in 38% radiochemical yield based on VII.

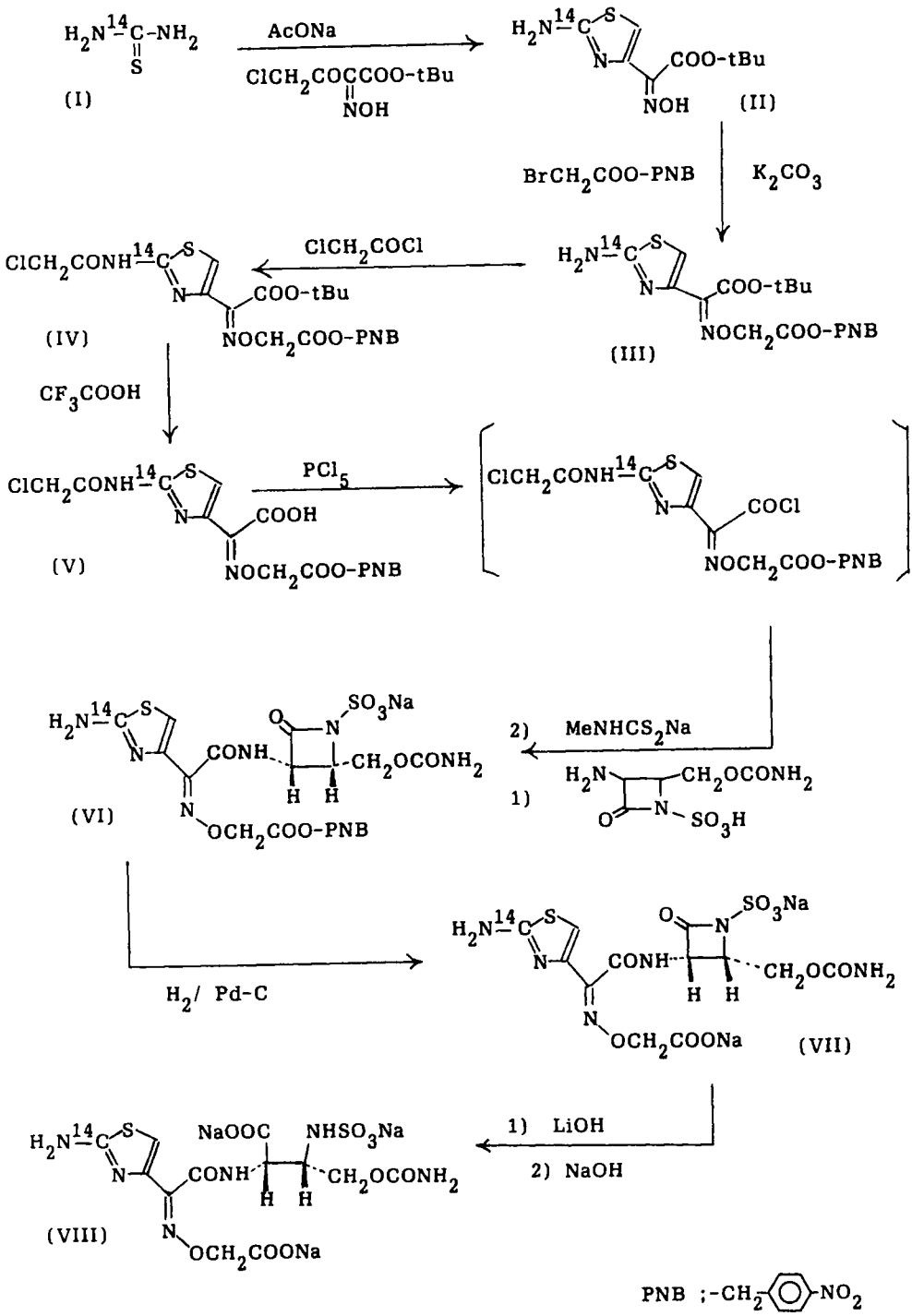
EXPERIMENTAL

t-Butyl [2-(2-amino-4-[2-^{14}C]thiazolyl)-(Z)-2-hydroxyimino]acetate (II)

A mixture of 1850 MBq (357 mg) of [^{14}C]thiourea, 1150 mg of t-butyl 4-chloro-2-hydroxyiminoacetoacetate, 816 mg of sodium acetate and 6 ml of ethanol was stirred for 18 h at room temperature. To the mixture was added 15 ml of water. After allowing to stand for 3.5 h in an ice bath, crystals precipitated were filtered, washed successively with water and hexane, and then dried in vacuo to give 1108 mg of II (97% yield).

t-Butyl [2-(2-amino-4-[2-^{14}C]thiazolyl)-(Z)-2-(4-nitrobenzyloxycarbonylmethoxyimino)]-acetate (III)

To a suspension of 1108 mg of II in 30 ml of acetonitrile were added 1500 mg of p-nitrobenzyl bromoacetate, 2700 mg of potassium carbonate and 0.2 ml of water. The mixture was stirred for 2 h at room temperature and then poured into 150 ml of ice water.


AcONa
ClCH2COCCOO-tBu
NOH
(I)
(II)
BrCH2COO-PNB
K2CO3
ClCH2COCl
(III)
(IV)
CF3COOH
PCl5
(V)
COCl
(VI)
2) MeNHCS2Na
1)
H2/ Pd-C
(VII)
1) LiOH
2) NaOH
(VIII)
PNB ;-CH2-C6H4-NO2

The resulting mixture was extracted with ethyl acetate. The extract was washed successively with water and saturated sodium chloride solution, dried over anhydrous sodium sulfate and concentrated under reduced pressure. To the residue was added hexane and the mixture was allowed to stand overnight in a refrigerator. The crystals were filtered, washed with hexane and dried in vacuo to give 1870 mg of III (94% yield).

t-Butyl [2-(2-chloroacetamido-4-[2-^{14}C]thiazolyl)-(Z)-2-(4-nitrobenzyloxycarbonylmethoxyimino)]acetate (IV)

A mixture of 1870 mg of III in 15 ml of N,N-dimethylacetamide and 0.43 ml of chloroacetyl chloride, cooled in an ice bath, was stirred for 2 h. To the mixture was added 0.21 ml of chloroacetyl chloride and stirring was continued for an additional 2 h. The reaction mixture was poured into 100 ml of ice water and extracted with ethyl acetate. The extract was washed successively with sodium hydrogen carbonate solution and water, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was crystallized by addition of hexane. The crystals were collected by filtration and dried in vacuo to give 1866 mg of IV (84.8% yield). The IR spectrum, Rf-value (0.66) on tlc (silica gel 60 F-254, precoated plate, Merck; developing solvent, hexane:ethyl acetate = 1:1, v/v) and melting point (157°C) of IV were identical to those of an authentic sample.

[2-(2-Chloroacetamido-4-[2-^{14}C]thiazolyl)-(Z)—2-(4-nitrobenzyloxycarbonylmethoxyimino)]-acetic acid (V)

A solution of 1835 mg of IV in 45 ml of trifluoroacetic acid was stirred for 2 h at room temperature and then concentrated under reduced pressure. The residue was dissolved in 150 ml of ethyl acetate. The ethyl acetate solution was washed with water, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was crystallized by addition of hexane. The crystals were filtered and dried in vacuo to give 1520 mg of V (93% yield).

p-Nitrobenzyl (+)-(Z)-[[[1-(2-amino-4-[2-^{14}C]thiazolyl)-2-[[(2S,3S)-2-(carbamoyloxymethyl)-4-oxo-1-sodiosulfonato-3-azetidinyl]amino]-2-oxoethylidene]amino]oxy]acetate (VI)

To an ice cooled solution of 1520 mg of V in 32 ml of dried dichloromethane was added portionwise 1040 mg of phosphorus pentachloride over a period of 10 min and the mixture was stirred for 1 h. To the mixture was added 100 ml of dried hexane and stirring was continued for an additional 10 min. The crystalline acid chloride of V was collected by filtration, washed with dried hexane and kept in a desiccator. To an ice cooled solution of 1100 mg of (3S,4S)-3-amino-4-carbamoyloxymethyl-2-azetidinone-1-sulfonic acid in a

mixture of 17 ml of water and 17 ml of tetrahydrofuran was added 1120 mg of sodium hydrogen carbonate. The solution was vigorously stirred and the acid chloride of V was added portionwise over a period of 15 min. After allowing to stir for 1 h, 530 mg of sodium N-methyldithiocarbamate was added portionwise over a period of 10 min with stirring at room temperature. The pH of the solution was adjusted between 4 and 5 with 1N hydrogen chloride. In addition, 750 mg of sodium N-methyldithiocarbamate was added portionwise to the reaction mixture and the mixture was stirred for 20 min. The pH of the solution was adjusted between 4 and 5 with 1N hydrogen chloride and then the solution was kept overnight in a refrigerator. After the pH of the solution was adjusted between 6 and 7 with saturated sodium hydrogen carbonate solution, the resulting solution was concentrated to ca. 10 ml volume in vacuo at room temperature. The concentrated solution was charged on a column of Amberlite XAD-2 and eluted successively with 1.5 l of water, 1.0 l of 2% ethanol and 1.5 l of 5% ethanol. The product fractions were combined and freeze-dried to give 1371 mg of VI (66.1% yield).

Disodium (+)-(Z)-[[[1-(2-amino-4-[2-^{14}C]thiazolyl)-2-[[(2S,3S)-2-(carbamoyloxymethyl)-4-oxo-1-sulfonato-3-azetidinyl]amino]-2-oxoethylidene]amino]oxy]acetate (VII)

A solution of 1371 mg of VI in a mixture of 60 ml of water and 60 ml of tetrahydrofuran was hydrogenated in the presence of 500 mg of 10% palladium carbon and 200 mg of sodium hydrogen carbonate for 1 h at room temperature. The catalyst was removed by filtration and the filtrate was further hydrogenated with 500 mg of fresh 10% palladium carbon for 1.5 h. The catalyst was removed by filtration and 7 ml of 1N hydrogen chloride was added to the filtrate. The solution was concentrated to ca. 5-10 ml volume in vacuo at room temperature. The concentrated solution was charged on a column of Amberlite XAD-2 and eluted successively with 1.5 l of water and 1.0 l of 3% ethanol. The product fractions were combined and freeze-dried to give the free acid of VII. The free acid of VII and 192 mg of sodium hydrogen carbonate were dissolved in 2.3 ml of water with stirring. To the solution was added dropwise 9 ml of ethanol and allowed to stand for 1 h. In addition, 7 ml of ethanol was added dropwise. The crystals separated out were filtered, dried and allowed to stand for 7 h in a desiccator which was evacuated at 20 mmHg in the presence of a sheet of wet filter paper (diameter, 7 cm). The water content in the crystals was measured by the Karl Fisher method. VII (515 mg) having equimolecular amount of water was obtained in 20.8% overall chemical yield. The specific activity of VII and the

overall radiochemical yield were 381 MBq/mmol and 20.1%, respectively. The radiochemical purity was determined by the HPLC method and found to be 99%. The Rf-value (0.46) of VII on tlc (precoated plate, silica gel 60 F-254, Merck; developing solvent, acetonitrile:water:acetic acid = 10:3:1, v/v) and the retention time (9.71 min) of HPLC of VII (column, 4 x 150 mm, Nucleosil 5C18; mobile phase, 0.1% ammonium sulfate:methanol: acetic acid = 97:2:1, v/v; flow rate, 0.8 ml/min) were identical to those of an authentic sample.

Trisodium (+)-(Z)-[[[1-(2-amino-4-[2-^{14}C]thiazolyl)-2-[[(1S,2S)-3-carbamoyloxy-1-carboxylato-2-sulfonatoaminopropyl]amino]-2-oxoethylidene]amino]oxy]acetate (VIII)

To a solution of 290 mg of VII in a mixture of 4 ml of water and 14 ml of ethanol, maintained at -20°C, was added dropwise 0.63 ml of 1N lithium hydroxide with stirring. The solution was allowed to stand in a freezer (-15°C) for 20 h and then added to 6 ml of Dowex 50W (H^+-form, 100-200 mesh). The mixture was stirred for 1 h at room temperature, then charged on a column packed with 10 ml of Dowex 50W (H^+-form) and eluted with 150 ml of water. The effluent was concentrated to ca. 10 ml volume in vacuo at room temperature. The concentrated solution was charged on a column packed with 400 ml of Mitsubishi Diaion CHP20P and eluted with water. The product fractions were combined, neutralized with 7.1 ml of 0.1N sodium hydroxide and freeze-dried to give VIII which was further dried in a vacuum desiccator. VIII with a specific activity of 399 MBq/mmol was obtained in 38% radiochemical yield. The radiochemical purity was determined by the HPLC method and found to be 97.8%. Identity of VIII was established by comparison of the Rf-value (0.17) on tlc (precoated plate, silica gel 60 F-254, Merck; developing solvent, acetonitrile:water:acetic acid = 10:3:1, v/v) and the retention time (10.16 min) of HPLC (column, 4.6 x 250 mm YMC-gel (ODS); mobile phase, 0.1% ammonium sulfate:methanol:acetic acid = 95:4:1, v/v; flow rate, 0.9 ml/min) with those of an authentic sample.

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