Synthesis of (5R,6S)-6-[(1R)-hydroxyethyl]-3-[1-methylpyridinium-2-yl-methanethio]-[3-^{14}C]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate

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SUMMARY

The synthesis of the title compound (4) is described. Treatment of (3S)-[(1R)-hydroxyethyl]-(4R)-[3-(p-nitrobenzyloxy)carbonyl-2-oxo-[2-^{14}C]-3-diazopropan-1-yl]azetidin-2-one[1] with rhodium diacetate achieved ring closure forming (5R,6S)-p-nitrobenzyl-6-[(1R)-hydroxyethyl]-3,7-dioxo-[3-^{14}C]-1-azabicyclo[3.2.0]heptane-2-carboxylate (1). Reaction with diphenylchlorophosphate followed by 2-(mercaptomethyl)pyridine under basic conditions produced (5R,6S)-p-nitrobenzyl-6-[(1R)-hydroxyethyl]-3-(pyridine-2-yl-methanethio)-[3-^{14}C]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (2). Formation of the quaternary compound with methyl fluorosulfonate and deprotecting the acid by hydrogenolysis gave the title compound (4) in 6% overall radiochemical yield.

Key Words

(5R, 6S)-6-[(1R)-hydroxyethyl]-3-[1-methylpyridinium-2-yl-methanethio]-[3-^{14}C]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate, antibacterial, dehydropeptidase, carbapenem.

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INTRODUCTION

The discovery of the naturally occurring carbapenem antibiotics has opened a new and exciting chapter in β-lactam chemistry. A major initial effort in carbapenem research has been directed toward natural product modification with the goals of defining new structure - activity relationships.[2]

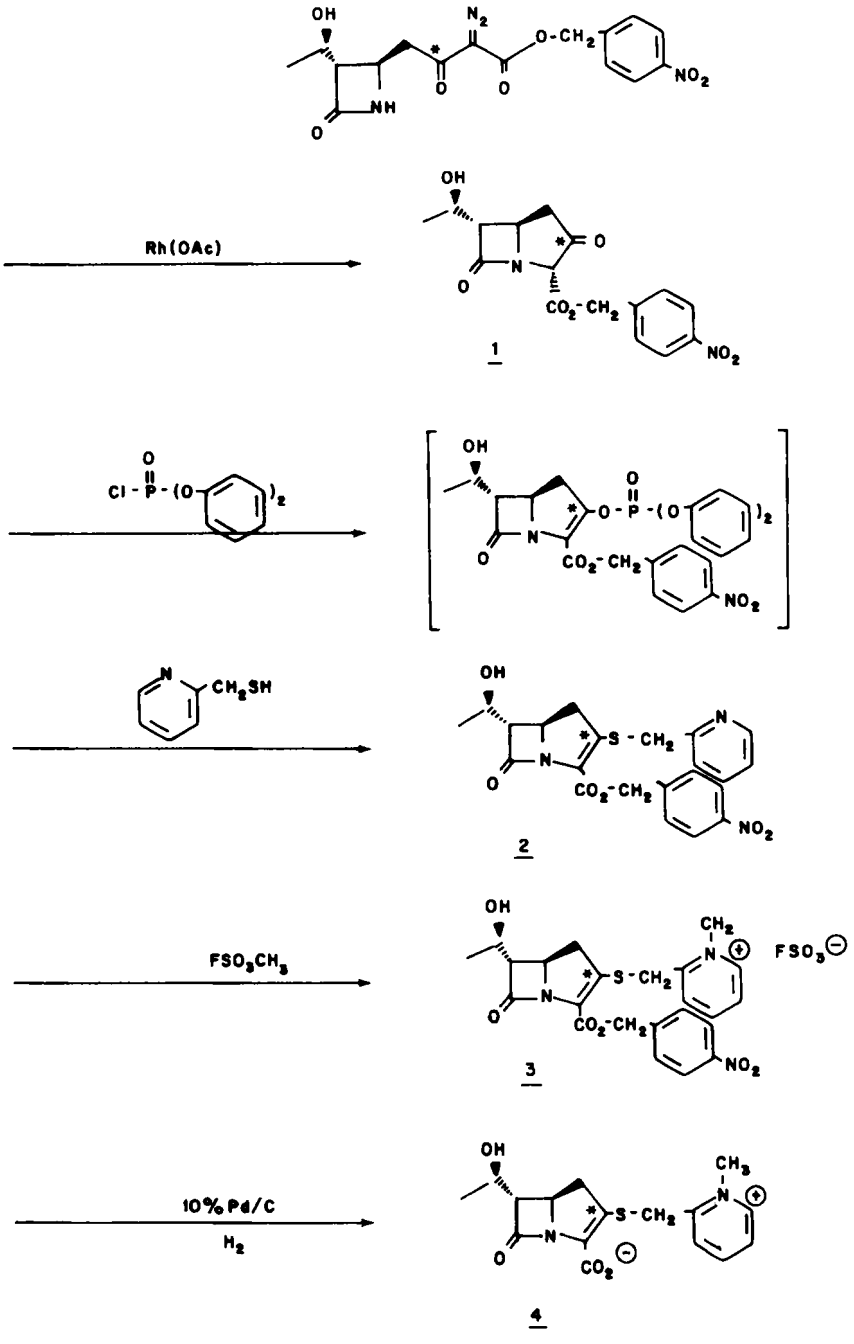
The discovery of N-formimidoyl thienamycin lead to an improved chemical stability and more potent antibacterial activity than thienamycin.[3]

The incorporation of a quaternary heterocyclic alkylthio group into the C-2 position of the carbapenem gave rise to a novel class of carbapenem antibiotics. This class of carbapenems retained broad antibacterial activity and had remarkable stability toward dehydropeptidase.[4] The (5R,6S)-6-[(1R)-hydroxyethyl]-3-[1-methyl-pyridinium-2-yl-methanethio]-[3-^{14}C]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate compound is a member of this class. This compound was synthesized with a carbon-14 in the C-3 position for metabolism and pharmacokinetic studies.

RESULTS AND DISCUSSION

Substitution of ethyl [3-^{14}C] acetoacetate along with unlabelled ethyl acetoacetate in the reaction sequence reported by Y. Ueda et al.[1] produced (3S)-[(1R)-hydroxyethyl]-(4R)-[3-(p-nitrobenzyloxy)carbonyl-2-oxo-[2-^{14}C]-3-diazopropan-1-yl]azetidin-2-one in an overall yield of 47%. Reaction with rhodium diacetate in the absence of oxygen caused ring closure at a yield of 91% after purification. This produced (5R,6S)-p-nitrobenzyl-6-[(1R)-hydroxyethyl]-3,7-dioxo-[3-^{14}C]-1-azabicyclo[3.2.0]heptane-2-carboxylate. After the *in situ* activation of the 3-position by the formation of the 3-diphenylphosphoryloxy group, two approaches to form the 1-methylpyridinium methanethio compound (compound 3) were explored. The first approach was the direct incorporation of the quaternary compound as a sodium salt. This approach proved to be undesirable due to low yields (1% - 10%)[5]. The second approach was the incorporation of the pyridine methanethio group followed by quaternization using methyl fluorosulfonate. This method proved to be acceptable in that the yields were increased to 50%. Deprotection of the acid was achieved by the removal of the p-nitrobenzyl group by hydrogenolysis. Lyophilization was necessary to isolate material and to minimize decomposition. Purification was accomplished by high pressure liquid chromatography yielding material with a radiochemical purity of 96% and a specific activity of 18 μCi/mg. This represents a total activity of 1.33 mCi and an overall radiochemical yield of 6%. All experimental conditions were optimized using non-radioactive materials.

SYNTHETIC PATHWAY



1

2

3

4

● Position of radiolabel

EXPERIMENTAL

Materials

Ethyl [3-^{14}C] acetoacetate (100 mCi) was purchased from Amersham Corporation. All chemicals used in the synthesis were purchased commercially and used without any further purification. All other solvents were either redistilled or of analytical reagent quality. Thin layer chromatography plates used were Analtech silica gel GF, scored 10 x 20 cm, 250 microns. Radioactivity was measured by a Beckman LS9000 liquid scintillation counter. All the high pressure liquid chromatography was carried out on Water Associates instrumentation. Nuclear magnetic resonance was measured on a Bruker 360. Weighings were carried out on a Sartorius 200 balance and Mettler Microanalytical M5AS balance.

(5R,6S)-p-Nitrobenzyl-6-[(1R)-hydroxyethyl]-3,7-dioxo-[3^{14}C]-1-azabicyclo[3.2.0]heptane-2-carboxylate (1).

Through a suspension of (3S)-[(1R)-hydroxyethyl]-(4R)-[3-(p-nitrobenzyloxy)carbonyl-2-oxo-[2-^{14}C]-2-diazopropan-1-yl]azetidin-2-one[1] (3.44 g) in ethyl acetate (67 ml) was bubbled nitrogen gas for 10 min. To this was added rhodium diacetate (7 mg) and the mixture heated under reflux for 0.5 hr. The reaction was concentrated to an oil, which crystallized under reduced pressure. Material was purified by column chromatography over silica gel (Woelm) using methylene chloride followed by ethyl acetate. Wt = 2.88 g; yield = 91%.

Thin Layer Chromatography

Eluent - ethyl acetate; plates - Analtech silica gel; visualization - ultraviolet light; compound - Rf = 0.48.

(5R,6S)-p-Nitrobenzyl-6-[(1R)-hydroxyethyl]-3-(pyridine-2-yl-methanethio)-[3-^{14}C]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (2).

To a solution of (1) (2.88 g) in acetonitrile (30 ml), at 4°C, under a nitrogen atmosphere, was added diphenylchlorophosphate (1.92 ml) followed by ethyl diisopropylamine (1.62 ml) and stirred in the cold for 0.5 hr. To the mixture was added ethyl diisopropylamine (1.62 ml) followed by 2-(mercaptomethyl)pyridine (1.19 g) and stirred for 2 hr. The resulting crystalline solid was removed by filtration and dried. Wt = 2.63 g; yield = 64%. NMR (DMSO-d6) 1.26(3H,d,J=7.0Hz), 2.7-3.5(4H,m), 3.9-4.3(2H, m), 4.2(2H,s), 5.42(2H,ABq,J=14.4Hz) and 7.2-8.8(8H,m).

Thin Layer Chromatography

Eluent - acetone 30%, methylene chloride 70%; plates - Analtech silica gel; visualization - ultraviolet light; compound - Rf = 0.23.

(5R,6S)-p-Nitrobenzyl-6-[(1R)-hydroxyethyl]-3-[1-methylpyridinium-2-yl-methanethiofluorosulfonate]-[3-^{14}C]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (3).

To a solution of (2) (2.63 g) in methylene chloride (180 ml), under a nitrogen atmosphere, was added methyl fluorosulfonate (0.85 ml) and the mixture stirred at room temperature for 2 hr. The resulting crystalline solid was removed by filtration and dried. Wt = 2.94 g; yield = 82%. NMR (DMSO-d6) 1.26(3H,d,J=7.0Hz) 3.9-4.2(2H,m), 4,4(3H,s), 4.78(2H,s), 5.2(1H,d,J=3.9Hz) 5.50 (2H,Abq,J=14Hz) and 7.8-9.4(8H,m)

(5R,6S)-6-[(1R)-Hydroxyethyl]-3-[1-methylpyridinium-2-yl-methanethio]-[3-^{14}C]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (4).

To a mixture of tetrahydrofuran (90 ml) and pH 7 phosphate buffer (90 ml) was added compound (3) (980 mg) followed by 10% palladium on charcoal (980 mg) and then diethyl ether (90 ml). This was hydrogenated at 48 psi for 35 min at room temperature. The diethyl ether was separated from the aqueous phase and the aqueous phase filtered through a pad of diatomaceous earth, frozen and then lyophilized. The lyophilite was purified by high pressure liquid chromatography. Wt = 74 mg. This material had a radiochemical purity of 96% as determined by high pressure liquid chromatography and a specific activity of 18 μCi/mg. Yield = 13%. NMR (D_2O) 1.30(3H,d,J=6.2Hz), 3.2(2H,q,J=9.0Hz,3.6Hz), 3.46(1H,q,J=6.0Hz,2.7Hz), 4.1-4.6(3H,m), 4.60(3H,s) and 7.9-8.9(4H,m).

High Pressure Liquid Chromatography

Waters Associates instrumentation was used with the following parameters: Eluent - 5% acetonitrile in water; Flow rate - 20 ml/min; Detector - ultraviolet at 254 nm; Temperature - 22.5°C; Column - Whatman Mag 20 C-18; Retention time - 35 min.

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