SYNTHESES OF [2-^{14}C]PENEM ANTIBACTERIALS; (FCE 22101 and FCE 22891)

Erminia Fontana, Marco Alpegiani, Ettore Perrone and Gian Piero Vicario

Farmitalia Carlo Erba S.p.A.
Chemical Research and Development
Via Giovanni XXIII, 23 - 20014 Nerviano, Milan, Italy

SUMMARY

The synthesis of FCE 22101 (sodium (5R,6S)-6-[(1R)-hydroxyethyl]--2-carbamoyloxymethylpenem-3-carboxylate) labelled with carbon-14 in the 2-position of the penem system ring was performed in eight steps, using sodium salt of [1-^{14}C]glycollic acid 1 as the labelled starting material. The final product, penem [2-^{14}C] FCE 22101 11, was obtained in an overall radiochemical yield of 21%, 98% radiochemically pure and with a specific activity of 641 MBq/mmol (17.3 mCi/mmol). The acetoxymethyl ester FCE 22891 12 was prepared by condensation of 11 with bromomethyl acetate, with a yield of 41%.

INTRODUCTION

Among the β-lactam antibiotics, the bicyclic penem system combines structural features of both penicillins and cephalosporins (1,2). Within this class, the novel compounds FCE 22101, namely sodium (5R,6S)-6-[(1R)-hydroxyethyl]-2-carbamoyloxymethylpenem-3-carboxylate and its acetoxymethyl ester (FCE 22891) showed promising antibacterial activity (3).

In order to perform disposition studies of FCE 22101 and FCE 22891 in laboratory animals, a radiolabelled form of them had to be synthesized. The opportunity to have an *in vivo* stable position of the radiolabel prompted us to consider, as the best choice, to insert the radionuclide in the cyclic system.

Up to now the procedures employed for labelling the β-lactam antibiotics foresaw only the label sites in the side chains functional

Key words : [2-^{14}C]penem system, FCE 22101, FCE 22891, antibacterial.

0362-4803/87/010041-08$05.00

Received January 31, 1986
Revised May 12, 1986

groups of these systems and therefore a new approach to the synthetic route had to be studied. Synthesis with ^{35}S was rejected because of the great number of steps involved, a critical stereoselectivity (4) and the relatively short half-life (87 days) of this radionuclide. On the contrary, the devised procedure, starting from the commercially available [1-^{14}C]glycollic acid (sodium salt) 1, as shown in the Scheme, suited well to our purposes.

RESULTS AND DISCUSSION

Our strategy consisted in the introduction of the radiolabelled atoms as late as possible in the synthetic sequence leading to the target compounds. Immediate precursors of the penem system are phosphorane-thioesters such as 6; they had been previously prepared from a glycollic thioacid by nucleophilic displacement on a 4--acetoxy azetidinone, followed by a three-step building up of the N-appendage (3). On the contrary, we prepared the labelled intermediate 6 in a single step from the O-protected [1-^{14}C]glycollic chloride 4, by reaction with the silver mercaptide 5 (5). The preparation of 4 from the commercial precursor 1 was straightforward, and the radiochemical yield was 45%.

The Wittig ring closure of 6 and selective removal of the primary hydroxyl group (Bu_4NF, 3 h) gave the [2-^{14}C]penem 8 with a radiochemical yield of 72%. The further condensation with trichloroacetyl isocyanate, followed by simultaneous unmasking of the carbamoyl and the secondary hydroxyl group (3) furnished, after chromatographic purification, the allyl ester 10 with a radiochemical yield of 81% from 8. The final conversion to the labelled FCE 22101 11 was accomplished by Pd-catalyzed deallylation (6) of 10 in 78% radiochemical yield. The overall radiochemical yield based on [1-^{14}C]glycollic acid 1 was 21%.

The final aqueous solution of 11 gave a radioactive single spot when mixed with an authentic sample on TLC analysis, but the complete characterization was performed on the free acid, which was obtained, in acetone solution, by treatment of the sodium salt with the cationic ion exchange resin Dowex 50 W-X8. TLC, HPLC and electronic spectrum of 11 showed the compound 98% radiochemically pure with a specific activity of 641 MBq/mmol (17.3 mCi/mmol).

The preparation of the labelled acetoxymethyl ester 12 (FCE 22891) was performed by alkylation of the sodium salt with bromomethyl acetate; a diluted sample of 11 yielded, upon work-up, the expected product 97% radiochemically pure in a yield of 41% and with a specific radioactivity of 169 MBq/mmol (4.56 mCi/mmol).

EXPERIMENTAL

Thin layer chromatography (TLC)

TLC was carried out using Merck silica gel F 254 200 x 50 x 0.25 mm plates. The elutin solvent systems were :

A)	cyclohexane:ethyl acetate	9:1	by volume
B)	cyclohexane:ethyl acetate	2:1	by volume
C)	cyclohexane:ethyl acetate	4:1	by volume
D)	chloroform:methanol:formic acid	95:5	by volume
E)	chloroform:methanol:formic acid	90:20:10	by volume
F)	benzene:acetonitrile:methanol:formic acid	50:50:10:15	by volume
G)	ethyl acetate		

Electronic spectra were determined on a Perkin-Elmer 575 UV/VIS spectrophotometer. Liquid scintillation counting was done with a Packard 300 C liquid scintillation counter using Rialuma (Lumac System A.G.) as liquid scintillation cocktail.

Radiochemical analysis of TLC plates was scanned with a Berthold 2832 automatic TLC linear analyzer. High pressure liquid chromatography (HPLC) was performed using a Perkin-Elmer 2 x 2 solvent delivery system with LC75 UV/VIS detector and Packard TRACE 7130 as radioactivity flow monitor.

[1-^{14}C]Glycollic acid, sodium salt was purchased from Amersham International plc.

t-Butyldiphenylsilyloxy-[1-^{14}C]acetic acid (3)

[1-^{14}C]Glycollic acid (sodium salt) 1 (18.18 mg, 0.181 mmoles, 10 mCi), was dissolved in water (6 ml) and transferred into a 10 ml flask containing "cold" 1 (20.70 mg, 0.313 mmoles). The solution was evaporated to dryness and the residue was dried in vacuo at 180°C for about 8 hours over P_2O_5. Imidazole (74.80 mg, 1.09 mmoles), DMF (1 ml) and 2 (0.13 ml, 0.5 mmoles) were successively added, under stirring, to compound 1.

This reaction mixture was then kept, under stirring, at room temperature for about 5 hours. At the end of the reaction, the mixture was diluted with ethyl acetate (10 ml), washed with 8% HCl (3 ml) and successively three times with water (3 ml). The organic phase, after filtration through a panel of anhydrous Na_2SO_4, was evaporated to dryness in vacuo to yield 7.29 mCi of crude 3. This intermediate, checked by ratio-TLC (system A) was shown to be

sufficiently pure and it was used without further purification in the next step.

t-Butyldiphenylsilyloxy-[1-^{14}C]acetyl chloride (4)

The labelled compound 3 (7.29 mCi) was dissolved in a mixture of dry benzene (1.5 ml) and thionyl chloride (0.1 ml, 1.37 mmoles). The solution was kept at room temperature for about 20 hours. At the end of the reaction the solvent and the remaining thionyl chloride were evaporated in vacuo to give a residue which was used, without further purification, in the next step.

(3S)-[(1R)-t-Butyldimethylsilyloxyethyl]-(4R)-t-butyldiphenylsilyloxy-[1-^{14}C]acetylthio-1-(1-allyloxycarbonyl-1-triphenylphosphoranylidenemethyl)azetidin-2-one (6)

Compound 5 (362 mg, 0.498 mmoles), dissolved in CH_2Cl_2 (3 ml) was slowly added, under stirring at 0°C, to a CH_2Cl_2 (2 ml) solution of 4. At the end of the addition, the reaction mixture was kept under stirring at room temperature for about 1 hour and then was filtered. The combined filtrates and CH_2Cl_2 washings were evaporated in vacuo to dryness yielding the crude compound 6 which was purified by flash chromatography on silica gel (~3 g) eluting with different mixtures of ethyl acetate-cyclohexane in the ratios 1:4, 1:2, 1:1 and 2:1 (by volume). The fractions containing the pure product (checked by radio-TLC system B; Rf 0.3) were combined, washed with 7% $NaHCO_3$ (5 ml) and water (5 ml). The organic phase, dried over anhydrous Na_2SO_4, yielded, after solvent evaporation, 4.54 mCi of compound 6, 92% radiochemically pure (by radio-TLC system B). The radiochemical yield from 3 to 6 was 62.3%.

Allyl (5R,6S)-6-[(1R)-t-butyldimethylsilyloxyethyl]-2-t-butyldiphenylsilyloxymethyl-[2-^{14}C]penem-3-carboxylate (7)

The compound 6 (4.54 mCi) dissolved in toluene (25 ml) was heated and stirred at 110°C for about 2 hours. At the end of the reaction the solvent was evaporated in vacuo to give the compound 7, 95% radiochemically pure (checked by radio-TLC, system C; Rf 0.73). It was used in the next step without further purification.

Allyl (5R,6S)-6-[(1R)-t-butyldimethylsilyloxyethyl]-2-hydroxymethyl-[2-^{14}C]penem-3-carboxylate (8)

Compound 7 (4.54 mCi), THF (4 ml), glacial acetic acid (0.194 ml,

3.39 mmoles) and TBAF . 3 H_2O (157.25 mg, 0.499 mmoles) were mixed and stirred at room temperature for about 3 hours. This time was sufficient (checked by radio-TLC, system B) to give, after solvent evaporation, the crude compound 8, which was purified by flash chromatography as previously described for compound 6. The fractions containing the expected product were combined to give, after solvent evaporation, 3.28 mCi of 8. Radiochemical purity was 95% (radio-TLC, system B; Rf 0.36). The radiochemical yield from 6 to 8 was 72.2%.

Allyl (5R,6S)-6-[(1R)-hydroxyethyl]-2-carbamoyloxymethyl-[2-^{14}C]penem-3-carboxylate (10)

Trichloroacetyl isocyanate (0.026 ml, 0.219 mmoles) was added under stirring, at -20°C, to a solution of the compound 8 (3.28 mCi) in methylene chloride (2.5 ml). The complete conversion to the intermediate 9 was achieved at the end of the reagent addition (checked by radio-TLC system D). The solvent was evaporated and then THF (1 ml), glacial acetic acid (0.1 ml, 1.75 mmoles) and TBAF. 3 H_2O (263.7 mg, 0.85 mmoles) were added. This mixture was kept at room temperature for 8 hours and, after solvent evaporation in vacuo, yielded the crude 10, which was successively purified by chromatography as described for compounds 6 and 8.

The employed solvents mixtures were: cyclohexane:ethyl acetate (2:1, 1:1 and 1:2 by volume) ethyl acetate, and ethyl acetate-ethanol (95%) 1:1, in that order. The combined fractions containing the pure product (checked by radio-TLC system D), were evaporated to dryness to give 2.68 mCi of 10. The radiochemical purity was shown to be $\geq$ 98% (chromatographed with an authentic sample; radio-TLC system D; Rf 0.15). The radiochemical yield from 8 to 10 was 81.7%.

Sodium (5R,6S)-6-[(1R)-hydroxyethyl]-2-carbamoyloxymethyl-[2-^{14}C]penem-3-carboxylate (11); [2-^{14}C]FCE 22101

A mixture of compound 10 (2.68 mCi), dry THF (1 ml), PPh_3 (9.15 mg, 34.88 µmoles) and $Pd(PPh_3)_4$ (7.55 mg, 6.52 moles) was stirred at room temperature and, after the solution was complete, sodium 2-ethylhexanoate (37 mg, 0.22 moles) in THF/methylene chloride (0.68 ml, 1:1 by volume) was added. The solution, kept at room temperature under stirring for about 30 minutes, afforded an abundant precipitate of crude 11 (checked by radio-TLC system E; Rf 0.12). The solid was filtered, washed with methylene chloride (5 ml) and dried in vacuo. The residue, taken up in water (3 ml) was chromatographed on a Lichroprep RP-C18 ($\sim$3 g) column using water as eluting solvent. The fractions containing the pure compound

were combined to yield 2.131 mCi of 11, 98% radiochemically pure (radio-TCL system F; Rf 0.47). The specific activity was measured on 11-free acid, which was prepared starting from a small sample of 11 (85 μCi) suspended in acetone (2 ml) added with 10 mg of Dowex 50 W-X8 resin. The mixture after shaking for about 2 hours was filtered, concentrated and TLC chromatographed (system F). The band corresponding to 11-free acid was extracted from silica gel with acetonitrile (40 ml). The recovered compound had a specific activity of 17.34 mCi/mmol (641 MBq/mmol). The UV absorption in acetonitrile at 317 nm ($E^{1\%}_{1cm}$ = 214) was in agreement to that of an authentic sample. For both 11 and 11-free acid radiochemical purity was >98% [radio-TLC system F; radio HPLC: retention time 10.6 min.; Merck RP 8 (10 μ); 4.6 mm ID x 25 cm; MeCN:KH_2PO_4 buffer 0.02 M, pH 2.5 (10:90) flow rate 1 ml/min.; UV detection 315 nm]. The overall radiochemical yield from 1 was 21.3%.

Acetoxymethyl (5R,6S)-6-[(1R)-hydroxyethyl]-2-carbamoyloxymethyl-[2-^{14}C]penem-3-carboxylate (12); [2-^{14}C]FCE 22891

Bromomethyl acetate (0.02 ml; 19.62 mg; 0.128 mmoles) was added under stirring at 0°C to a mixture of 1.5 M sodium 2-ethylhexanoate (0.049 ml, 10.9 mg; 0.065 mmol) in ethyl acetate (0.044 ml) and DMF (1 ml). The resulting solution, after 1 hour of stirring at 0°C, was added, at 0°C, to a solution of 479 μCi of 11 diluted with the same "cold" compound (20.4 mg, 0.066 mml) in DMF (1 ml). The reaction mixture was then kept at 0-5°C for 12 hours and finally ethyl acetate (10 ml) was added. This solution was washed with 1% $NaHCO_3$ (3 ml) and the organic layer was collected. The aqueous phase was salted with NaCl and exhaustively extracted with ethyl acetate (30 ml). The combined organic fractions were dried over sodium sulphate and evaporated under reduced pressure to give 336 μCi of compound 12 (83% radiochemically pure by radio-TLC system G). The residue was purified by preparative TLC using ethyl acetate as chromatographic eluent. The chromatographic band corresponding to 12 was removed and the product was extracted with acetone (20 ml). The combined extracts were filtered and evaporated in vacuo to dryness to yield 195 Ci of the pure compound 12, which migrates with an Rf identical to that of an authentic unlabelled sample when chromatographed beside or mixed with the labelled one. No chemical impurities (detection by UV light) were found and the radiochemical purity, as checked by radio-TLC (system G; Rf 0.31) was shown to be ≥97%. Specific radioactivity was found to be equal to 4.56 mCi/mmol and UV absorption in ethanolat 326 nm ($E^{1\%}_{1cm}$ = 215) was in agreement

to that of an authentic sample. The radiochemical yield from 11 was found to be 41%.

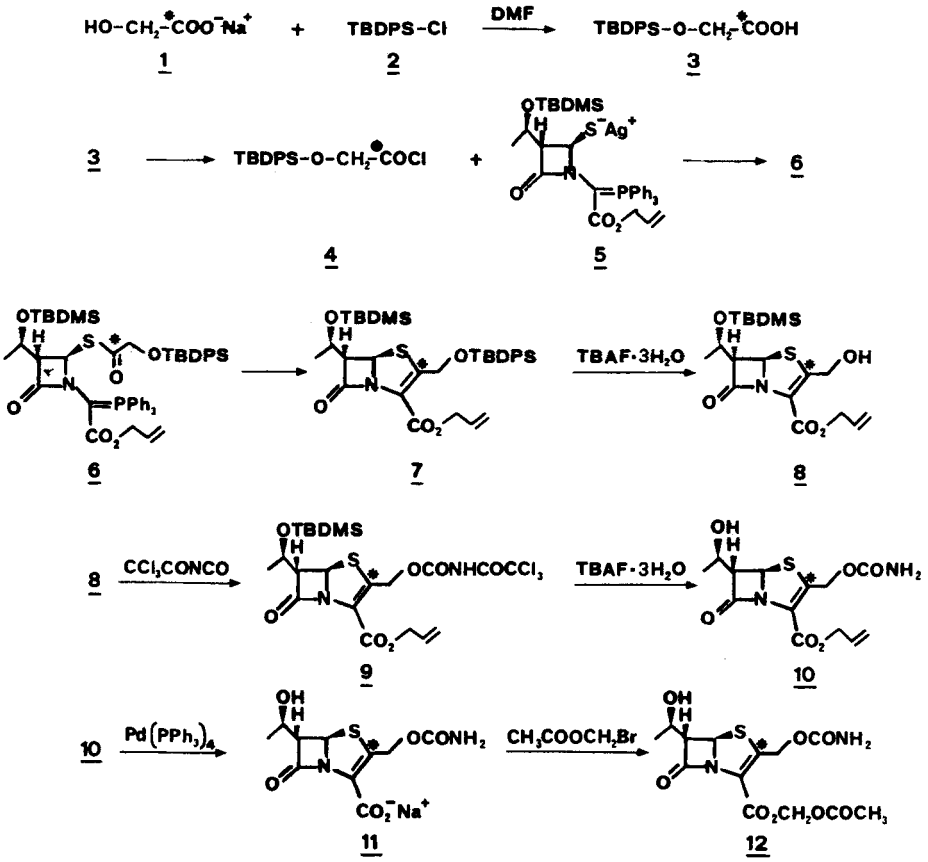
ACKNOWLEDGMENTS

The authors thanks Mrs. M.R. Tessera for helpful technical assistance.

REFERENCES

1. Woodward R.B. - Acta Pharm. Suec. 14 (Suppl.):23,(1977).
2. Ernst I., Gosteli J., Greengrass C.W., Holick W., Jackman D.E., Pfaendler H.R. and Woodward R.B. - J. Amer. Chem. Soc. 100:8214, (1978).
3. Franceschi G., Foglio M., Alpegiani M., Battistini C., Bedeschi A., Perrone E., Zarini F., Arcamone F., Della Bruna C. and Sanfilippo A. - J. Antibiotics 36:938, (1983).
4. Perrone E., Alpegiani M., Battaglia R., Bedeschi A. and Franceschi G. - in "Recent Advances in the Chemistry of β-Lactam Antibiotics" - Brown A.G. and Roberts S.M. Eds., The Royal Society of Chemistry, Spec. Publ. n. 52, London 1985, pp 361-365.
5. The mercaptide 5 was obtained by silver-assisted ($AgNO_3$) methanolysis of the corresponding tritylthioazetidinone, according to minor modifications of a published procedure : Martel A., Dextraze P., Daris J.P., Saintonge R., Lapointe P., Conway T.T., Monkovic I., Kavadias G., Ueda Y., Elie P., Patil S., Caron G., Douglas J.L., Menard M. and Belleau B. - Can. J. Chem. 60:942,(1982).
6. Jeffrey P.D. and McCombie S.W. - J. Org. Chem. 47:587, (1982).

SCHEME



TBDPS = t-butyldiphenylsilyl
TBDMS = t-butyldimethylsilyl
TBAF = tetrabutylammonium fluoride
[*] = ^{14}C